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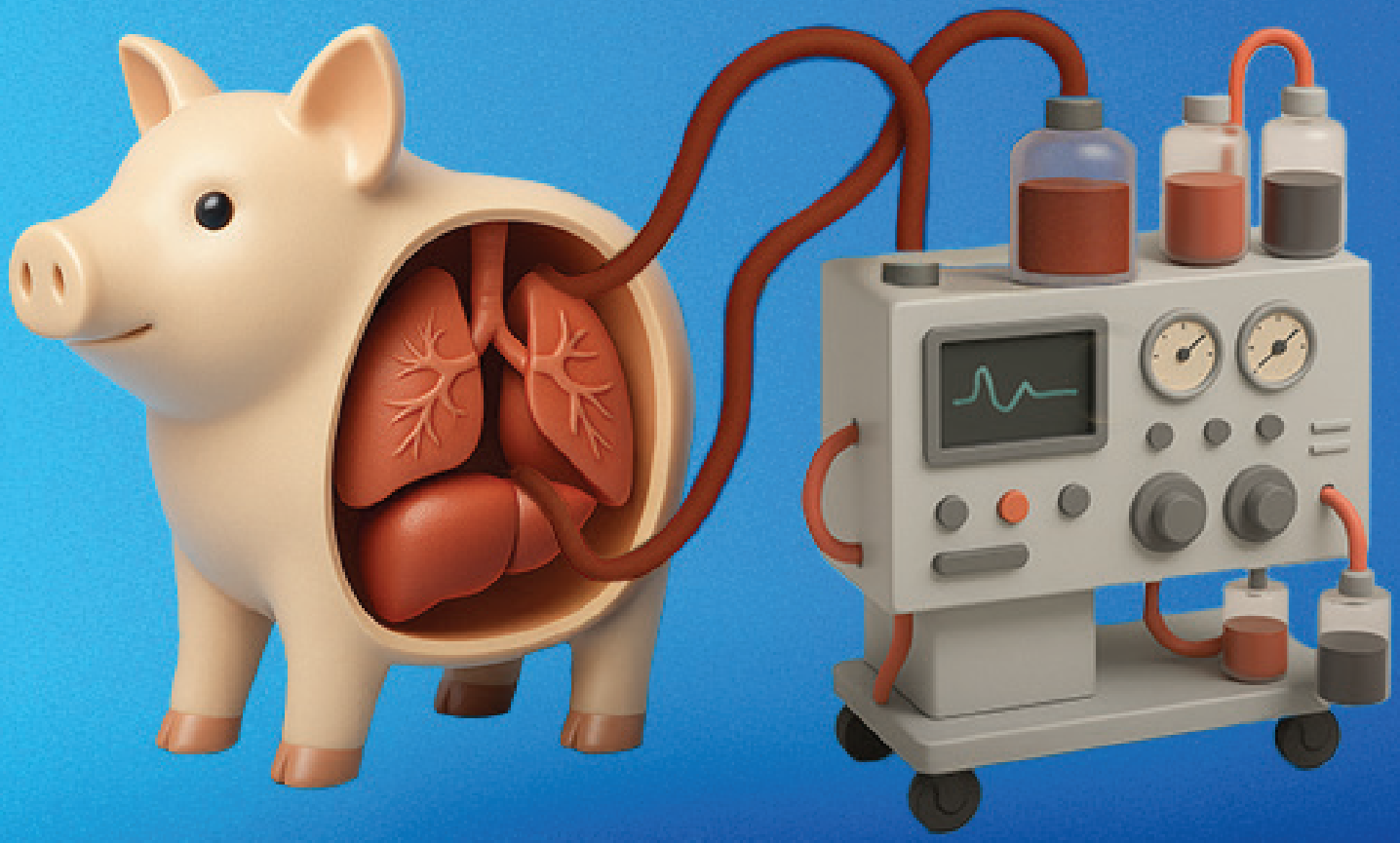

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TRANSPLANT TRIAL WATCH
published: 26 March 2025
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Transplant Trial Watch

John O'Callaghan[1,2]* *and John Fallon*[1]*

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Keywords: randomised controlled trial, liver transplantation, lung transplantation, extracorporeal photopheresis, survival

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

Multicenter Randomised Controlled Trial of Single Versus Double Venous Outflow Reconstruction in Right Lobe Living Donor Liver Transplantation- Venous Outflow in Liver Transplantation (VOLT) Trial.

by Reddy, M. S., et al. Annals of Surgery 2024 [record in progress].

Aims

The authors aim to compare early patency of the reconstructed anterior sector vein (neoMHV) and clinical outcomes between a single outflow technique (SOT) and double outflow technique (DOT) in right lobe living donor liver transplantation (RtLDLT).

Interventions

One arm received the double outflow technique (DOT): Separate anastomoses of the right hepatic vein (RHV) and the prosthetic neo-middle hepatic vein (neoMHV) to the recipient inferior vena cava (two openings). The other arm received the single outflow technique (SOT): Conjoint venoplasty on the back table, creating a single common outflow orifice (RHV + neoMHV together) that is then anastomosed *en bloc* to the recipient vena cava (one opening).

Participants

219 adult patients undergoing right lobe LDLT who required prosthetic anterior sector vein (ASV) reconstruction. To be included grafts needing at least one reconstructed ASV (>4 mm). Prosthetic grafts (PTFE or Dacron) used. Key exclusion criteria were: retransplant, graft with middle hepatic vein included, non-prosthetic reconstructions, contraindication to contrast imaging.

Outcomes

The primary outcome was NeoMHV (anterior sector vein) patency at multiple time points (2, 4, and 6 weeks post-transplant) evaluated by Doppler ultrasound and cross-sectional imaging. The secondary outcomes were: Intraoperative metrics (cold ischemia time, graft implantation time, blood loss, etc.), postoperative complications (e.g., vascular/biliary events, Clavien-Dindo classification, Comprehensive Complications Index), early allograft dysfunction, ICU/hospital length of stay & In-hospital, 90-day, and 1-year patient survival.

Transplant Trial Watch

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Follow-Up
Primary patency assessments up to 6 weeks post-transplant. Additional postoperative outcomes (including survival) tracked up to 1 year (median survival data reported).

CET Conclusion

by John Fallon

The authors conducted a well-designed and blinded Multicentre, randomised controlled trial at 5 LDLT centres in India. 219 recipients were included in the study with 110 undergoing SOT and 109 DOT. They demonstrated NeoMHV Patency was significantly better at 2 weeks (92.5% vs. 82.9%, p = 0.032) and 4 weeks (84% vs. 69%, p = 0.011) in SOT compared to DOT, but at 6 weeks, the difference was not statistically significant (69.5% vs. 59.2%, p = 0.124). Cox proportional hazards analysis identified DOT and Dacron graft use as independent predictors of early neoMHV thrombosis. With regards their clinical Outcomes SOT had slightly shorter graft implantation time (41 min vs. 49 min, p = 0.002). In-hospital mortality was lower in SOT (2.7% vs. 9.2%, p = 0.044), but no difference in 1-year survival. NeoMHV thrombosis before 4 weeks was associated with worse morbidity and early mortality, underscoring the importance of early outflow patency. Overall this is a good quality study on a very specialised procedure within LDLT, they constructed a multicentre RCT design with reasonably balanced groups. They recognise the potential limitations of potential centre-specific protocol variations and short-to-medium follow-up for patency. In right lobe LDLT requiring anterior sector venous reconstruction, single outflow technique in the correct hands appears to achieve better early venous patency and may confer a survival advantage during the initial postoperative period. Further long-term data are required to evaluate late outcomes.

Jadad Score
3.

Data Analysis
Strict intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
CTRI Number - REF/2021/08/046152.

Funding Source
No funding received.

RANDOMISED CONTROLLED TRIAL 2

Extracorporeal Photopheresis for the prevention of rejection after lung transplantation - a prospective randomized controlled trial.

by Benazzo, A., et al. European Respiratory Journal 2024 [record in progress].

Aims
This study aimed to examine whether extracorporeal photopheresis (ECP) was effective as a prophylactic treatment for preventing acute cellular rejection (ACR), incidence of (CMV) infections as well as for reducing the risk of chronic lung allograft dysfunction (CLAD), in lung transplant recipients.

Interventions
Participants were randomly assigned to receive either ECP plus standard triple-drug immunosuppression or standard triple-drug immunosuppressive treatment alone.

Participants
31 lung transplant recipients.

Outcomes
The primary outcome was a composite of high-grade ACR, CMV infection or CLAD. The secondary outcomes included ACR and lymphocytic bronchiolitis frequency, patient survival, graft survival, immune cell phenotyping, detection of plasma CMV DNA, number of antibody-mediated rejection (AMR) episodes, use of antilymphocyte globulin, and the incidence of clinically treated infections, *de novo* donor specific antibodies (dnDSAs), CLAD and serious adverse events (SAE).

Follow-Up
24 months.

CET Conclusion

by John O'Callaghan

This is a well-written report of a very interesting study in lung transplantation. The results are significant, showing a considerable and statistically significant reduction in acute rejection when extracorproeal photophoresis (ECP) was used in addition to standard immune suppression. This treatment was also associated with a significant reduction in infectious complications and chronic lung allograft dysfunction at 24 months, and lower hospital admissions. The group allocation could not be blinded, due to the nature of the ECP treatment, but the primary outcome is robust and the randomisation method reliable. Over 77% of patients in the ECP group received 90% of ECP treatment sessions. There was no significant difference in patient survival, however the study is likely to be underpowered for that outcome. The study only included patients transplanted for COPD, affecting generalizability to other indications for lung transplant.

Jadad Score
3.

Data Analysis
Strict intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
ClinicalTrials.gov - NCT05721079.

Funding Source
Industry funded.

CLINICAL IMPACT SUMMARY

by John O'Callaghan

This paper reports on a study that has the potential to influence clinical treatment protocols. The trial was conducted in clinical lung transplantation for COPD. Extracorporeal photopheresis was incorporated into a standard immune suppression regime and the primary outcome of interest was a composite outcome defined as incidence of high-grade acute cellular rejection, CMV infection or chronic lung-allograft dysfunction within 24 months after transplantation. The photopheresis system requires 1,500 mL of the patient's blood, separated to isolate white blood cells, which are treated with a photosensitizing agent (like methoxsalen) and exposed to ultraviolet light. The modified "immunomodulated" white blood cells are then reinfused back into the patient. All patients received PCP and CMV prophylaxis as well as a protocol bronchoscopy with bronchiolar lavage and transbronchial biopsy at weeks 2, 4, 8, 12, 24 and 52. Additional bronchoscopies were performed if clinically indicated.

The treatment was associated with a significant reduction in the primary outcome: Freedom from the primary composite endpoint was 93% at 1 year and 76% at 2 years (compared to 52% and 45% in the control arm). The treatment was also associated with a significant reduction in high grade acute cellular rejection. It is very interesting that the intervention was associated with a reduction in rejection as well as a reduction in infections. There was a significantly higher incidence of SAEs in the control group, particularly infections, but not CMV.

The mechanism through which ECP modulates immune cell activity is not fully understood, and this study showed no shift in subpopulations between the control and study groups during the trial. This trial has shown some very promising results that warrant a multicentre study to follow-up.

Clinical Impact
4/5.

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.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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ORIGINAL RESEARCH
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Advances in Xenotransplantation: Evaluation of αGal-KO Porcine Livers and Lungs Using Normothermic Machine Perfusion in a Collaborative Perfusion Hub

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Recently, initial clinical experience has been gained with the xenotransplantation of pig organs such as heart and kidney into terminally ill human patients in an effort to overcoming organ shortage. Here, we investigated the use of normothermic machine perfusion (NMP) to advance xenotransplantation research and develop bridging therapies for acute organ failure such as the use of pig livers as a liver dialysis system. We simultaneously analyzed livers and lungs from genetically modified pigs, carrying a knock-out of the GGTA1 gene, which is essential for xenoreactive αGal-KO-epitopes, by applying clinically established normothermic perfusion systems, solutions and human blood. Experiments involved perfusing organs with cell-free solutions as well as human erythrocyte concentrates for up to six hours, analyzing organ quality using invasive and non-invasive methods, and the isolation and analysis of immune cells from the perfusate. The results obtained show stable flow characteristics with physiological perfusion and oxygenation levels of the organs, and a largely intact organ architecture, confirmed by histological sections before and after perfusion. Overall, this study demonstrates the feasibility of normothermic machine perfusion of xenogeneic organs by an interdisciplinary team, thus paving the way for clinical applications of porcine xenografts involving NMP.

Keywords: xenotransplantation, liver, normothermic machine perfusion, genetically modified pigs, EVLP, perfusion hub, bridging therapy

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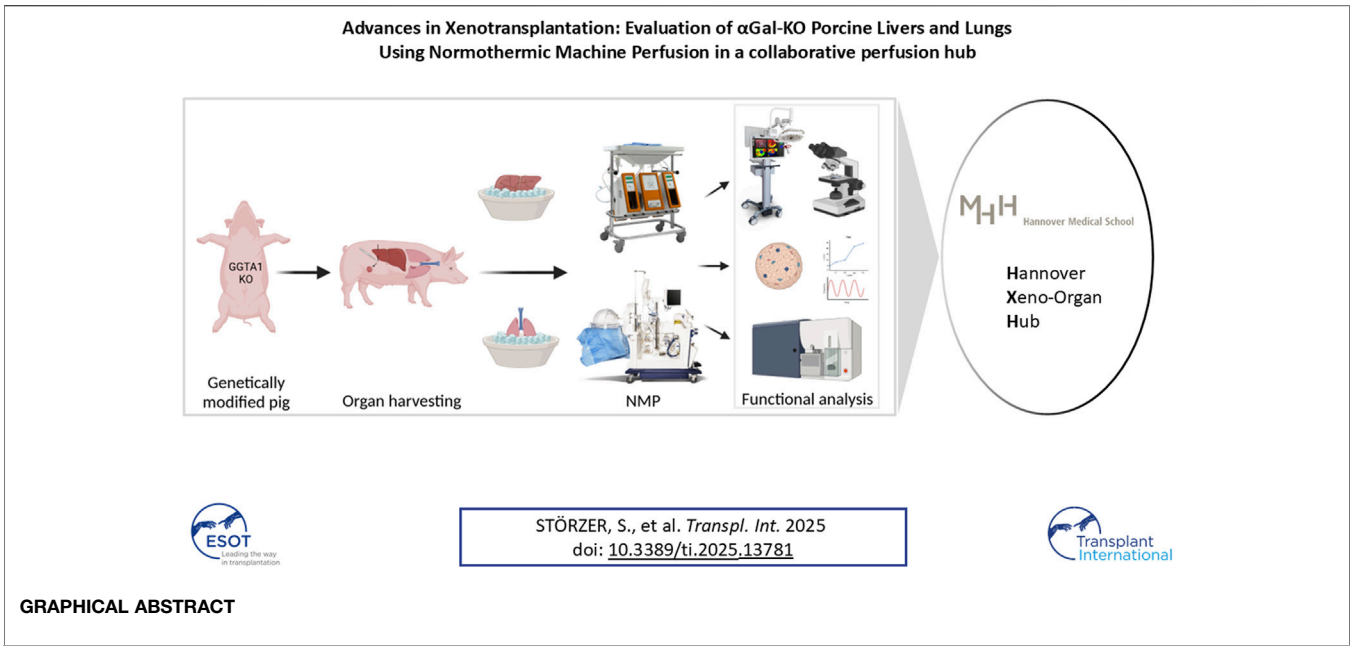
INTRODUCTION

The critical shortage of donor organs poses a significant barrier to organ transplantation in patients with end-stage organ disease. Even using organs from aged and already diseased donors (extended criteria donors) the utilization rate for donor lungs in the US remains relatively low, at approximately 30% [1]. In addition, the increasing donor age, fibrosis and Metabolic dysfunction-associated



GRAPHICAL ABSTRACT

steatohepatitis (MASH) in liver grafts results in diminished graft acceptance. Consequently, this trend in donor organ quality leads to extended waiting lists and unacceptably high mortality rates among patients awaiting a transplantation [2]. Therefore, new innovative sources for donor organs are needed to close the gap between available and required organs.

One promising approach to overcome critical organ shortage is the use of porcine organs for human transplantation approaches (xenotransplantation). Due to the high degree of anatomical and physiological similarity, porcine liver and lung represent a promising source for this experimental approach and have great potential for clinical application.

However, a direct transplantation of porcine grafts into humans is generally prohibited due to the immediate activation of the immune system or incompatibilities in the proteins that regulate complement formation or blood coagulation (reviewed in [3]).

Therefore, the decent aim of ongoing xenotransplantation research is to implement genetic modifications into the genome of donor pigs (reviewed in [4]) to enhance the compatibility of the porcine organs with the human immune system (reviewed in [5]).

Based on this approach, several highly specialized research centers have produced pigs with multiple knockouts and several different human transgenes. By implementing these modifications into pigs, special attention was paid on controlling the human complement system, to provide anticoagulation and ultimately to prevent death of the porcine tissue and cells (reviewed in [6]).

All these developments have already contributed to first promising results of xenotransplantation in preclinical and clinical settings. Especially by transplanting porcine hearts and kidneys in non-human primates (NHP) and first human patients, postoperative survival of 6–7 weeks were already reported [7, 8].

In contrast, xenotransplantation of porcine lung and liver has not yet reached these long survival periods. The first transplantations of these organs in NHP have demonstrated short-term organ survival rates in preclinical trials (reviewed in [9]).

To improve these first results and to extend the survival time following xenotransplantation, a pre-transplantation testing of genetically modified liver and lung is required. By using human blood components, the modified organs can be tested for functional, immunological and coagulation aspects of the grafts prior to the transplantation process.

Next to hypothermic perfusion systems [10] normothermic machine perfusion (NMP) has emerged as a very useful tool to evaluate and preserve organs prior to transplantation [11]. Both, the Liver Assist™ (XVIVO) system and the XPS system with multiple options of adapting the physical perfusion settings (perfusion or ventilation pressure, perfusion temperature) and improved analysis options, provide nearly optimal conditions for translating the xenotransplantation approach into the clinic.

However, the development of genetically modified animals as well as the functional evaluation of the grafts prior to the transplantation process requires a high level of expertise that can only be provided by a centralized xenotransplantation hub.

To support the establishment of such a center for xenotransplantation, we evaluated the challenges associated with xenoperfusion using human blood in a preclinical setting. Using pigs genetically engineered to lack αGal expression together with clinically certified Liver Assist™ (XVIVO) and XPS™ (XVIVO) systems, viability of explanted porcine grafts in such a centralized hub have been as assessed.

MATERIALS AND METHODS

Study Design

Extracorporeal liver and lung perfusion was performed with organs from four genetically modified pigs. To enable the

perfusion, pigs lacking the major xenoantigen αGal (αGal-KO), that were created by knocking out the GGTA1 gene were used for this study [12]. Animals deficient for GGTA1 were generated by breeding of parental lines carrying a homozygous or heterozygous knockout of the GGTA1 gene. Offspring was tested by PCR using saliva or tissue biopsy. The knockout was confirmed on porcine peripheral blood mononuclear cells (PBMCs) by flow cytometry analysis prior to organ procurement. Organ procurement was performed according to German animal welfare guidelines using a non-heart-beating donor model at the Friedrich-Loeffler Institute of Farm Animal Genetics in Mariensee (Neustadt). During the procedure, liver and lung were perfused with PERFADEX® Plus solution containing 2,000 I.U. Heparin in preparation for subsequent extracorporeal machine perfusion. For perfusion, the organs were transported on ice to the surgical research lab, at Hannover Medical School (MHH). The extracorporeal machine perfusion of lung and liver was performed on XVIVO XPS System (Serial number: XPS0132), and the XVIVO Liver Assist™ for up to 6 h. Tissue and perfusate samples were taken to monitor machine perfusion and organ-specific functions on a regular basis. Additionally, hyperspectral images (HSI) were captured prior to and during perfusion in the respective organs. Perfusion success, defined as maintained organ perfusion over time, was evaluated in consideration of the respective tissue, perfusate, bile and HSI data.

Surgical Procedures

Without premedication, animals were electrically stunned and killed according to standard procedure. After animals were exsanguinated, the abdominal cavity was opened immediately and the abdominal aorta and vena cava were identified.

Following cannulation of both vessels, perfusion of the organs was started with 2 L of ice-cold PERFADEX® Plus solution containing 2,000 I.U. Heparin. Simultaneously, the abdominal aorta was ligated shortly behind passing through the diaphragm. During *in situ* perfusion, a sternotomy was performed, and lung and liver were explanted according to the German guidelines of organ procurement [13]. Following explantation, both organs were perfused again at the side of retrieval with 2 L of ice-cold PERFADEX® Plus containing 2,000 I.U. Heparin (lungs) or with Histidine-tryptophan-ketoglutarate/Custadiol® (HTK, plus 2,000 I.U. Heparin) (liver) in preparation for extracorporeal organ perfusion.

Extracorporeal Liver Perfusion Using the XVIVO Liver Assist™

Following liver procurement procedure and 2 h of transport period to the MHH at +4°C, the explanted liver was prepared for extracorporeal organ perfusion. Back table, the portal vein and hepatic artery were cannulated using a 24 Fr portal vain and 3,33 Fr hepatic artery cannula. A representative liver tissue sample from the median liver lobe was taken for subsequent histological analysis. Furthermore, the common bile duct was drained by inserting a 10 CH suction tube (Asid Bonz, Herrenberg, Germany). Simultaneously, the XVIVO Liver Assist™ was primed with two bags of human erythrocytes of type 0 Rh^+ and Gelafundin to reach a total perfusion volume of 2 L. Heparin (25,000 I.U.), Ilomedin (20 μg/mL (1:10 dilution); 2 mL/h), Insulin (1 mL/h minimum), 2 L/min of oxygen and 0.5–1 L/min of CO_2 were added continuously for maintaining physiological perfusion conditions. The graft was connected to the XVIVO Liver Assist™ to perfuse the liver under physiological conditions with a portal vain flow of 550 mL to 450 mL/h and an arterial flow of 0.1–0.2 L/min for up to six hours. Hourly blood gas analysis was performed throughout the perfusion period to monitor the composition of the perfusion solution.

Ex Vivo Lung Perfusion (EVLP)

After approximately two hours of cold ischemia during transport to the MHH, EVLP was performed according to the Toronto protocol [14]. The system was first primed with 1.5 L of STEEN Solution™ (XVIVO Perfusion AB, Moelndal, Sweden) and a total of 10,000 I.U. of heparin. The flow rate was set at approximately 40% of the equivalent cardiac output and the temperature was set at 34°C. Next to priming the device, the lung was prepared for the perfusion by inserting a endotracheal tube with 9 mm inner diameter (Ruesch, Rommelshausen, Germany), and usage of the XVIVO Lung Canulla Set™ for the pulmonary artery and right atrium. A standard retrograde flush with 1 L PERFADEX® Plus was performed in the grafts prior to connecting the organ to the EVLP. Then, recruitment was performed at least one time per run, with positive end expiratory pressure (PEEP) continuously increased to approximately 10 and maintained at 100% FIO_2 for a total of 10 min. Within the first run, recruitment was conducted for longer times. EVLP runs were proceeded from 60 up to 160 min and a maximum of 1 L STEEN Solution™ was added during procedure.

In two EVLP runs (2nd & 4th), additional simulation of extracorporeal perfusion using human blood was achieved by using one bag of human erythrocytes of type 0 Rh+ added to the circulation at the end of the respective perfusion time. This modified perfusion setting was conducted for up to 15 min.

Perfusion Monitoring During Machine Perfusion

During extracorporeal perfusion, the physical perfusion parameters were monitored continuously for both organs. For liver graft perfusion, additional samples of the perfusate were taken prior to perfusion and in two-hour intervals to monitor the metabolic function of the grafts (urea, creatinine, AST, ALT, GLDH, alkaline phosphatase, gamma-GT, total bilirubin, and ammonia). The sampling was conducted in our clinical laboratory. Additionally, a complete blood count was conducted prior to perfusion and again at 3 h and 6 h after initiating the perfusion to monitor any hematological changes.

Hyperspectral Imaging During Liver and Lung Perfusion

HSI, using TIVITA® 2.0 (TIVITA® Tissue System, Diaspective Vision GmbH, Am Salzhaff, Germany), was conducted hourly during the perfusion. In addition, HSI was used during one exemplary run of EVLP prior to the perfusion and after each

recruitment during perfusion STEEN Solution™ as well as alongside with the hemoperfusion.

The parameters hemoglobin oxygen saturation (StO_2; 1 mm deep, 500–600 nm), tissue hemoglobin index (THI, 500–600 nm), near-infrared (NIR; 4–6 mm, 700–1,000 nm) perfusion index and tissue water index (TWI, 900–980 nm) were recorded.

Assessment of αGal Epitopes in Porcine PBMCs

PBMCs were isolated from the blood of αGal-KO pigs, which were used as donors for perfusion experiments. PBMCs from a wildtype (wt) pig served as control. αGal epitopes were detected by staining with FITC conjugated *Griffonia simplicifolia* isolectin B4 (IB4-FITC). Analysis was performed on a FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA, United States) and data were processed using FCS Express 7 (*De Novo* software, Pasadena, CA, United States).

Flow Cytometry Analysis

Flow cytometry was employed to analyze perfusate samples during liver NMP to detect and quantify the release of porcine immune cells into the perfusate. Specific porcine antibodies detecting CD45 (Clone K252.1E4, Acris), CD3 (Clone BB23-8E6-8C8, BDBiosciences) CD21 (Clone LT21, Origene), CD4 (Clone 74-12-4, Acris), CD8 (Clone 76-2-11, Acris), CD14 (Clone MIL2, Serotec) and CD56 (Clone MEM-188, Biolegend) were used, allowing for identification and characterization of cellular components in the perfusate. For flow cytometry analysis, we used the BD FACSCalibur with CellQuest™ Pro software and FCS Express 7.

Histology

Histological examination was performed in both organs, on different time points. During lung perfusion, tissue samples (2 cm × 2 cm) of each lobe were taken before and at the end of the perfusion interval. From liver grafts, samples (2 cm × 2 cm) were taken prior to connecting the organ to the extracorporeal system as well as at the end of the perfusion interval. All histological samples were fixed in 10% methanol buffered formalin for 24 h at room temperature prior to further processing.

Dehydration using increasing concentrations of ethanol and embedding in Paraffin was performed according to standard procedure. Sections of 2 μm thickness were cut using a microtome and stained applying a standard Hematoxylin and Eosin (H&E) protocol.

Data Export and Statistical Analysis

Data export from the XVIVO XPS and Liver Assist™ systems was performed after each run. Perfusion data were then manually organized and processed. Statistical analysis was performed using appropriate methods for data validation and analysis to ensure reproducibility and significance of results. EVLP-Data were analyzed using R version 4.2.3 (2023-03-15), utilizing the ggpubr package to examine pulmonary arterial systolic pressure and left atrium oxygen partial pressure over the time during EVLP. Continuous variables were summarized using median values with interquartile ranges [IQR].

RESULTS

General Animal Data

In total, four female genetically modified pigs (chronological order of experiments #1529, #1544, #1421, #1396), and aged between 20 and 45 months, with a weight range of 250 kg–350 kg, were used for the extracorporeal organ perfusion experiments. Prior to organ procurement, the flow cytometry analysis of peripheral blood mononuclear cells (PBMCs) verified the absence of αGal epitopes, in three of four animals. Despite a confirmed bi-allelic knockout of the GGTA1-gene (deletions of one base (Δ1) and five bases (Δ5) within the coding from of GGTA1), PBMCs of pig #1396 stained positive for αGal (**Figure 1**). Further genetic analysis revealed a third, most likely functional, copy of the GGTA1-gene (not shown, probably reflecting a copy number variation for this locus). Due to the size of the animals, the liver weight was between 2,800 and 3,200 g, weight of the lungs was not determined. No evidence of intrahepatic clotting or pulmonary edema prior to the perfusion was observed in any of the organs.

Extracorporeal Liver Perfusion Data

Extracorporeal liver perfusion could be maintained for up to 252.5 ± 86.7 min. The extracorporeal liver perfusion was conducted under physiological conditions, with the following portal venous and hepatic artery flows: #1529, arterial flow 483.3 ± 169.4 mL/min, portal vein flow 0.518 ± 0.178 L/min, #1396, arterial flow 130.4 ± 70.4 mL/min, portal vein flow 0.503 ± 0.024 L/min, #1544, arterial flow 13.3 ± 1.3 mL/min, portal vein flow 0.492 ± 0.031 L/min (**Figures 2A, B**). The vascular resistance of liver #1544 in the portal venous system branches was already high at the beginning of perfusion (VR of 4.65 vs. 0.3 in liver #1529) and increased by more than 25% in the first hour of liver perfusion. In consideration of the decreasing hemoglobin and hematocrit values found in the blood gas analysis, perfusion of this particular organ was terminated after 3 h. The liver of #1421 was discarded, due to initial perfusion problems during graft procurement after DCD.

Clinical Chemistry Data of the Perfused Livers

Analysis of ALT and AST levels in three pigs over time during perfusion was performed (**Figures 3A, B**). ALT and AST levels increased over time in all three pigs, with pig #1544 showing the highest ALT and AST levels. The slope for pig #1544 is the steepest, indicating a faster increase in ALT and AST levels compared to the other pigs. ALT and AST levels increases during perfusion suggest liver stress or damage over time in all three pigs.

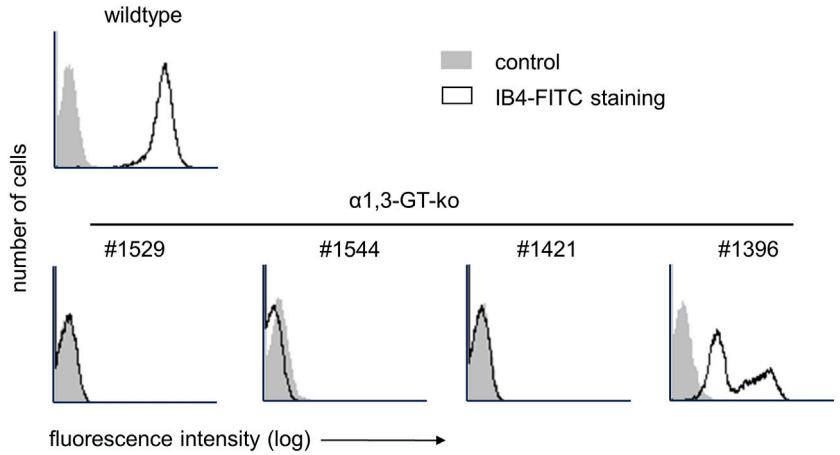


FIGURE 1 | Flow cytometry analysis of αGal expression on PBMCs of genetically modified pigs with IB4-FITC. In the top row, staining on PBMC of a wild type pig is shown. The bottom row shows αGal expression on the four genetically modified pigs used for liver and lung machine perfusion.

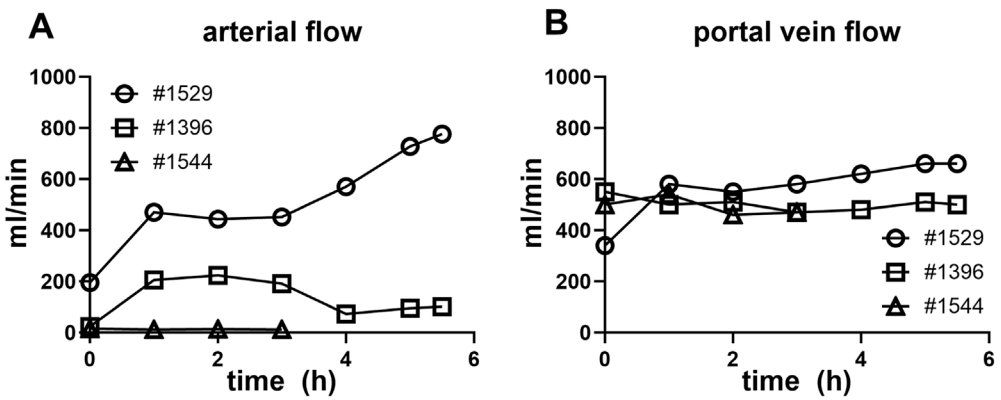


FIGURE 2 | Liver NMP perfusate flow characteristics. **(A)** Arterial vein flow in mL/min over the duration of the NMP (conclusion at 3–6 h). **(B)** Portal vein flow in mL/min is shown over the duration of the normothermic liver perfusion (conclusion at 3–6 h).

Also, a continuous increase in lactate levels was observed by blood gas analysis in all three perfusions. This rise in lactate suggests a shift towards anaerobic metabolism. Possible causes include hypoxia, insufficient perfusion into capillary structures or cellular stress or damage (**Figure 3C**).

The decline in hemoglobin levels, which were below the measurable threshold at termination of all three perfusions, is indicative of a substantial loss of erythrocytes.

Accelerated hemolysis could be due to mechanical stress of erythrocytes passing the perfusion pump or through contact with non-biocompatible surfaces within the perfusion apparatus. Additionally, the presence of porcine immune cells or the lack of human plasma in the perfusate could also lead to increased hemolysis.

The accompanying figure (**Figure 3D**), clearly shows a steady decline, ultimately reaching unmeasurable hemoglobin levels during perfusion. All measured clinical parameters underscore the physiological challenges encountered during the perfusion process.

EVLP Perfusion Data

Extracorporeal lung perfusion was maintained with STEEN Solution™ in all organs as planned for up to two hours. A significant increase of oxygen saturation in the left atrium (pO_2(LA)) was observed in each run during recruitment maneuvers (100% FiO_2) (**Figures 4A–D**). However, the pO_2(LA) returned to its initial level following these maneuvers and did not improve further after repeated recruitments (**Figures 4A–D**).

According to the Toronto protocol, left atrium pressure (PLA) was intended to be between 3 and 5 mmHg. Although there were some brief periods in runs 1–3 where the pressure exceeded 5 mmHg, a median left atrium pressure of 4 mmHg was maintained across all runs (first run: 4 (4–5); second run: 4

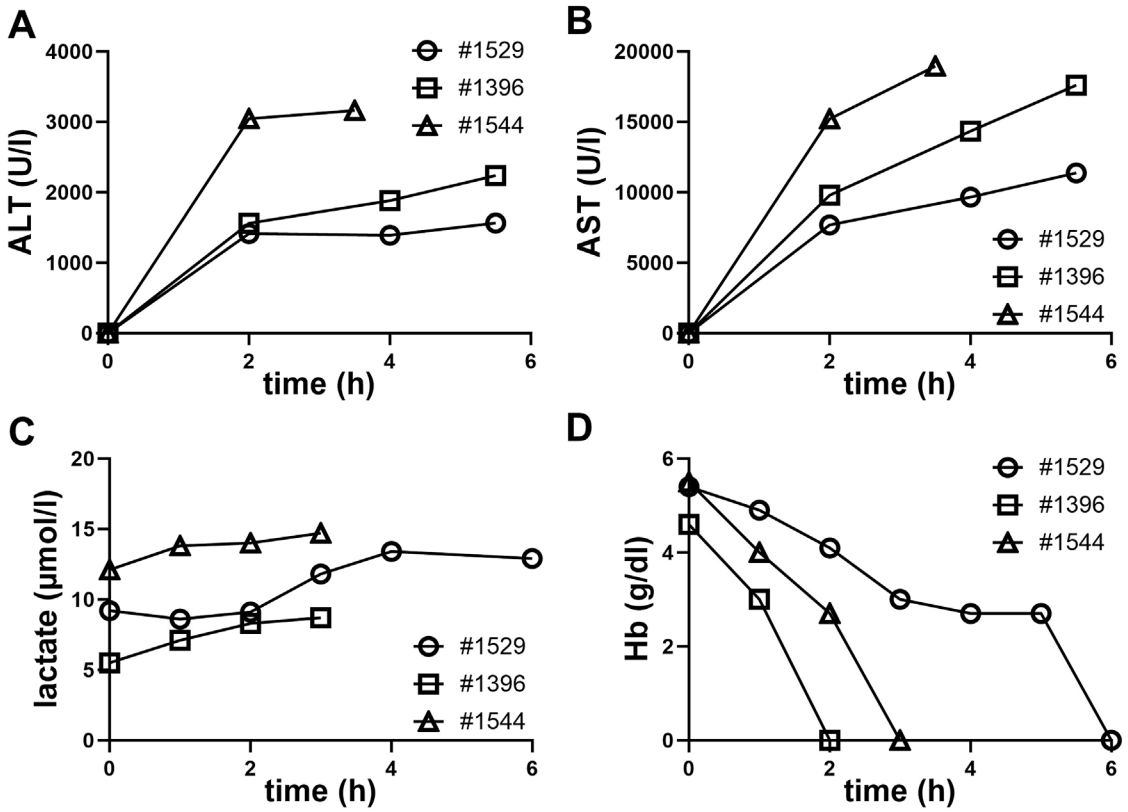


FIGURE 3 | Clinical chemistry from NMP perfusates during the perfusion period. **(A)** Alanine aminotransferase (ALT), **(B)** aspartate aminotransferase (AST), **(C)** lactate, **(D)** hemoglobin (Hb).

(4–5), third run: 4 (3–4; fourth run: 4 (4–5); $p < 0.001$) (**Figure 4E**).

During regular perfusion with STEEN Solution™, pulmonary arterial pressure (PAP) should remain below a cut-off of 20 mmHg. With the exception of the initial phase of the first run and after erythrocyte administration in the fourth run, pressure was maintained within the targeted range (**Figure 4F**). The median PAP across all runs was 10 (9–14) mmHg (first run: 12 (11–12); second run: 16 (15–17), third run: 9 (8–10; fourth run: 9 (9–10); $p < 0.001$) (**Figure 4F**). The addition of human erythrocytes in run 4 (pig #1396) caused an increase in PAP to 30 mmHg, which subsequently declined (**Figure 4F**). In congruence with PAP, pulmonary vascular resistance (PVR) increased from 100 to 1,500 dyn/s/cm^{-5} within 5 min (Data not shown) immediately after addition of one erythrocyte concentrate at the end of run 4 (**Figure 4F**). Simultaneously, the Horowitz-index decreased from 400 to 160 mmHg within 3 min, and remained steady over 15 min and subsequently increased to 300 mmHg (not shown). Within 15 min after reaching peak values, PAP and PVR decreased to normal levels.

Flow Cytometry

Flow cytometry analysis demonstrated the presence of specific porcine immune cell populations, including NK cells (CD56), monocytes (CD14), T cells (CD4/CD8), and B cells (CD21)) in the perfusate during perfusion. FSC, SSC dot blots show no immune cells in the perfusate before perfusion, but significant amounts at 5 min and 3 h into perfusion (**Figure 5A**). The analysis indicated an enrichment of B and T cells and a decrease in monocytes after 3 h NMP compared to the 5 min time point (**Figure 5B**). Only few NK cells were detectable at both time points with no significant difference in NK cell numbers between the 5 min and 3 h time point (**Figure 5B**). The persistence of immune cells in the perfusate until late into the NMP suggests active immune cell release from the liver graft during the perfusion period.

Hyperspectral Imaging

HSI provided additional insights into the tissue condition during perfusion (**Figures 6A–Y**). The NIR index started at a baseline value indicating undisturbed perfusion of the liver. During the first 2 h, there was a noticeable increase in NIR values (**Figure 6H**). From the third hour onwards, NIR values decreased and plateaued at a low level at the end of the 6 h perfusion (**Figure 6M**). Initial THI measurements suggested a normal distribution of hemoglobin within the liver tissue. Over the 6-h period, the THI values remained within a consistent range, showing no signs of significant hemoglobin depletion or concentration (**Figures 6D, I, N**). Perfusion heat maps displayed uniform perfusion across superficial liver tissue, with high

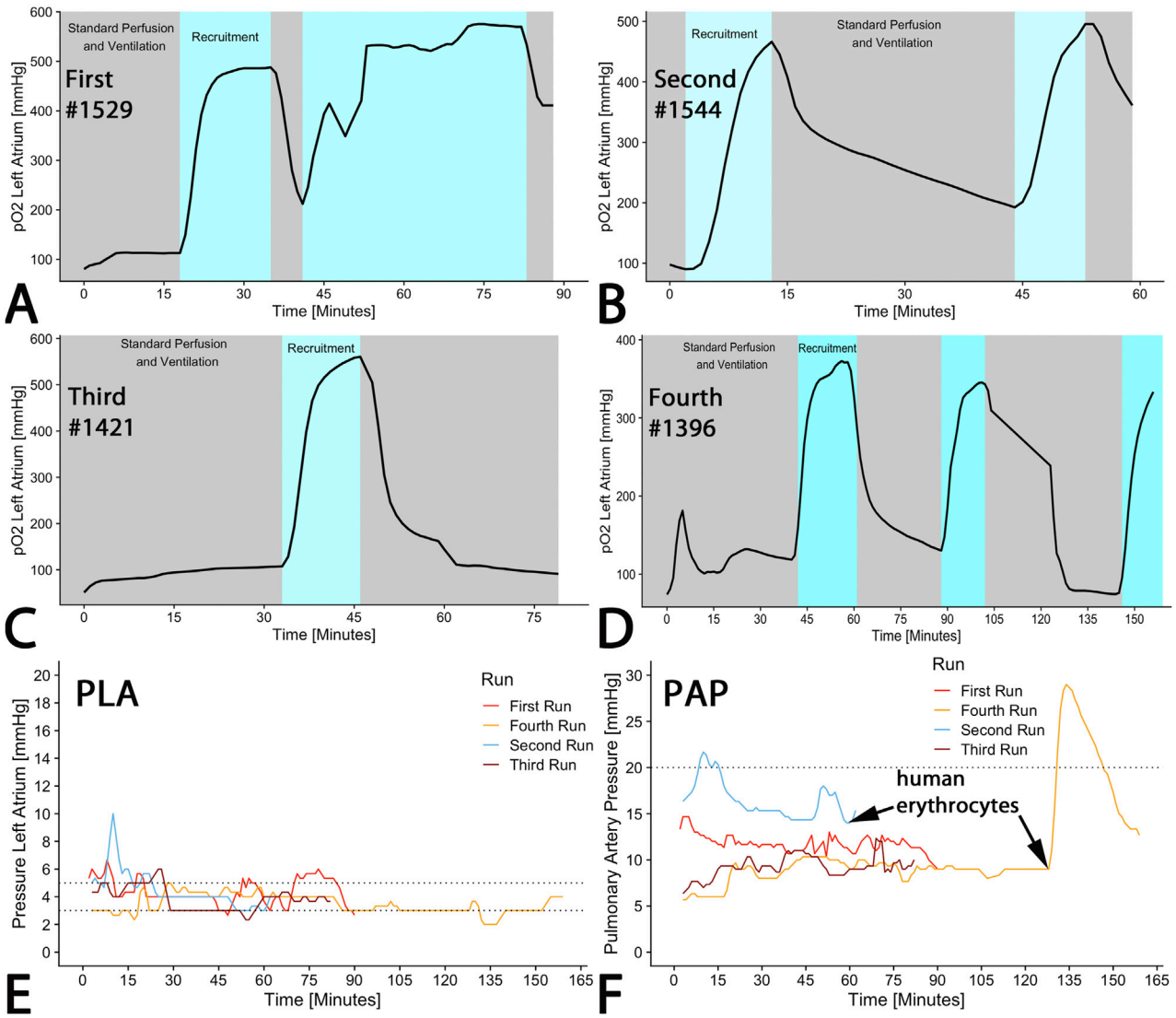


FIGURE 4 | EVLP perfusion parameter. **(A–D)** Oxygenation of the perfusate was measured in the left atrium showing increase due to recruitment applying 100% oxygen as ventilation gas. **(E)** A recommended pressure of 5 mmHg at the outflow from the left atrium (PLA) was targeted. **(F)** Inflow pressure at the pulmonary artery (PAP) was measured, exhibiting a dramatic increase upon addition of human erythrocytes. The curves represent online measurements of each run.

perfusion areas correlating with high oxygenation levels. When comparing StO_2 values at the start of the perfusion (0 h) with measurements at 2 h, an increase can be observed (0 h: central 41%/periphery 0%; 2 h: 49%/42%), at the peripheral measurement point. After 6 h, we observed decreased StO_2 values (35%/29%). NIR values at 2 h (37/26) versus 6 h NMP (5/0) also show a significant decrease. The same decrease is seen for TWI (2 h: 44/38 vs. 6 h: 36/32), but not for THI (2 h: 98/99 vs. 6 h: 93/100).

During EVLP, substantial differences caused by the addition of human erythrocytes to the cell-free STEEN Solution have been observed applying HSI. After addition of the erythrocyte concentrate, oxygenation and THI increased compared to only STEEN Solution™ (**Figures 6Q, S, V, X**). In contrast, TWI decreased in most areas (**Figures 6T, Y**). Area of venous congestion (*) could be identified exhibiting low StO_2 and high THI in lungs perfused with erythrocytes (**Figures 6V, X**).

Histological Results

The histological examination of peripheral liver tissue samples taken prior to perfusion (**Figures 7A–C**) and at the end of perfusion (**Figures 7D–F**), revealed shrunken periportal fields and partly collapsed sinusoidal space at 6 h perfusion. No significant necrotic areas were detected. Nuclear staining remained nearly unchanged as most cell nuclei were well-defined (**Figures 7B, C, E, F**). The imperfect perfusion described above, did not seem to cause extensive cell death or tissue damage in the liver.

Lung tissue exhibited intact lung architecture before and after perfusion with cell-free STEEN Solution™ (**Figures 7G–N**).

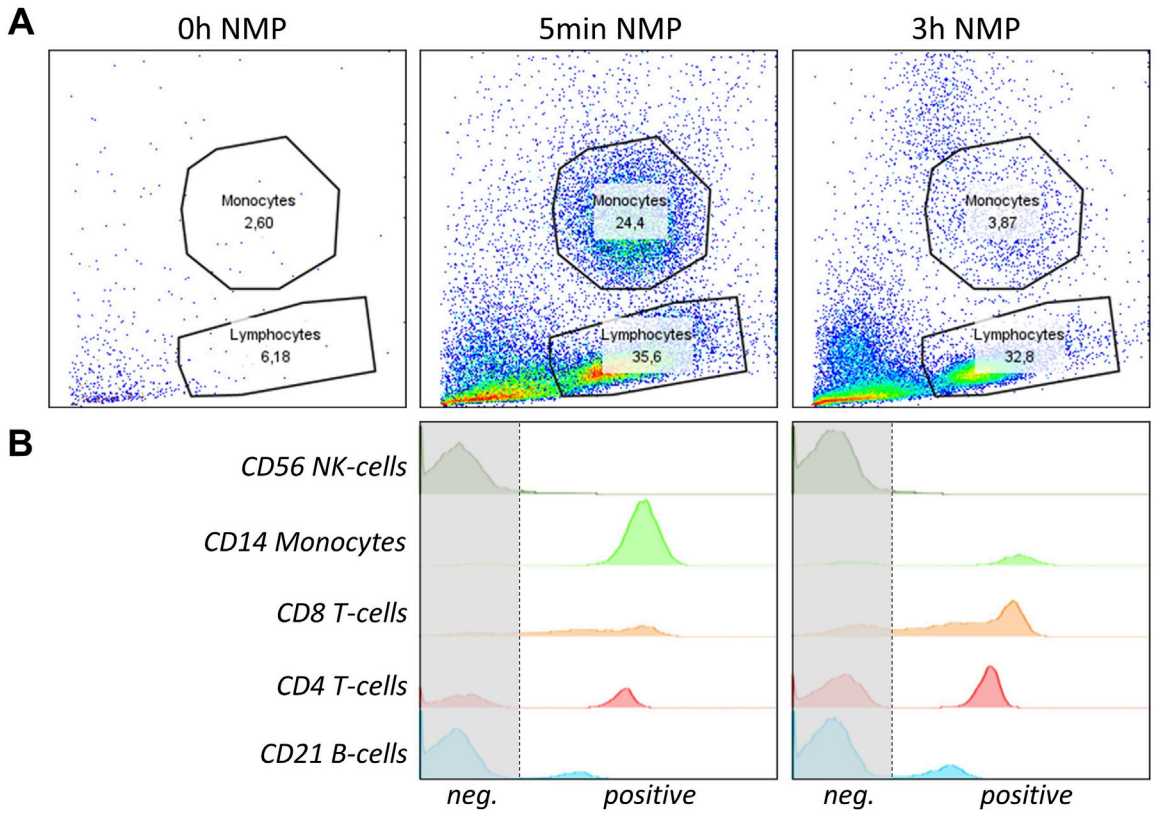


FIGURE 5 | NMP perfusate immune cell analysis of a representative pig liver is shown. **(A)** FSC, SSC dot blots with lymphocyte and monocyte gating of liver perfusate before perfusion (0 h), at 5 min and 3 h into perfusion are shown. **(B)** Flow cytometry histograms of specific immune cell populations isolated from the perfusate of NMP after 5 min and 3 h of perfusion are shown. Cells were gated to be lymphocytes or monocytes and to be $CD45^+$. From top to bottom, anti-human CD56 (NK-cells), CD14 (Monocytes), CD8 (cytotoxic T-cells), CD4 (T-helper cells) and CD21 (B-cells) were used to identify specific populations. Graphs represent single measurements.

Compared to lungs directly after harvesting, vessels and capillaries were free of erythrocytes (**Figures 7K–N**). Edema formation could not be observed. After addition of human erythrocytes, extravasation of erythrocytes into the airspace, (alveoli as well as bronchi) could be observed, indicating a loss of barrier function (**Figures 7O–R**).

DISCUSSION

This study presents initial results of our extracorporeal machine perfusion of lungs and livers isolated from αGal-KO pigs, demonstrating the feasibility of xenogeneic perfusion using clinically approved devices in a realistic setting. In combination with the also established first structures of a xenotransplantation hub including administration and analytic pathways. All these achievements will be the first steps towards building an interdisciplinary xenoperfusion hub, for implementing xenotransplantation in a clinical application.

Extracorporeal Liver Perfusion

In this study, we utilized genetically modified pigs that do not express αGal, aiming to reduce immunogenicity, a key target in xenotransplantation [15]. This approach of genetic modification, particularly the knockout of xenoantigens such as αGal, Neu5Gc and Sda, alongside the introduction of immunoprotective human transgenes, is well documented in the literature. For instance, Detelich et al. noted maximum liver perfusion times of 5–7 h in wild-type pigs, 4–6 h in αGal-KO/hCD55 pigs, and 8–14 h in pigs with multiple knockouts and transgenes. Here, we achieved satisfactory perfusion parameters in 3 out of 4 normothermic liver perfusions. We stopped perfusion at 6 h at the latest, but we achieved comparable perfusion times as in the αGal-KO/hCD55 group reported by Detelich et al. [15, 16].

Histological analysis revealed intact tissue, lacking major necrotic areas and well-defined hepatocyte nuclei after 6 h of perfusion, indicating that the liver's structural integrity had been preserved. These encouraging results suggests that the present NMP method is capable of maintaining liver structures over extended periods.

Hyperspectral imaging demonstrated consistent perfusion during the initial hours. Despite a constant THI at the end of the perfusion period, StO_2, NIR and TWI decreased, indicating hypoxia or ischemia. A drop in StO_2 values has also been observed in other studies, often attributed to reduced oxygen delivery or increased oxygen consumption [17, 18].

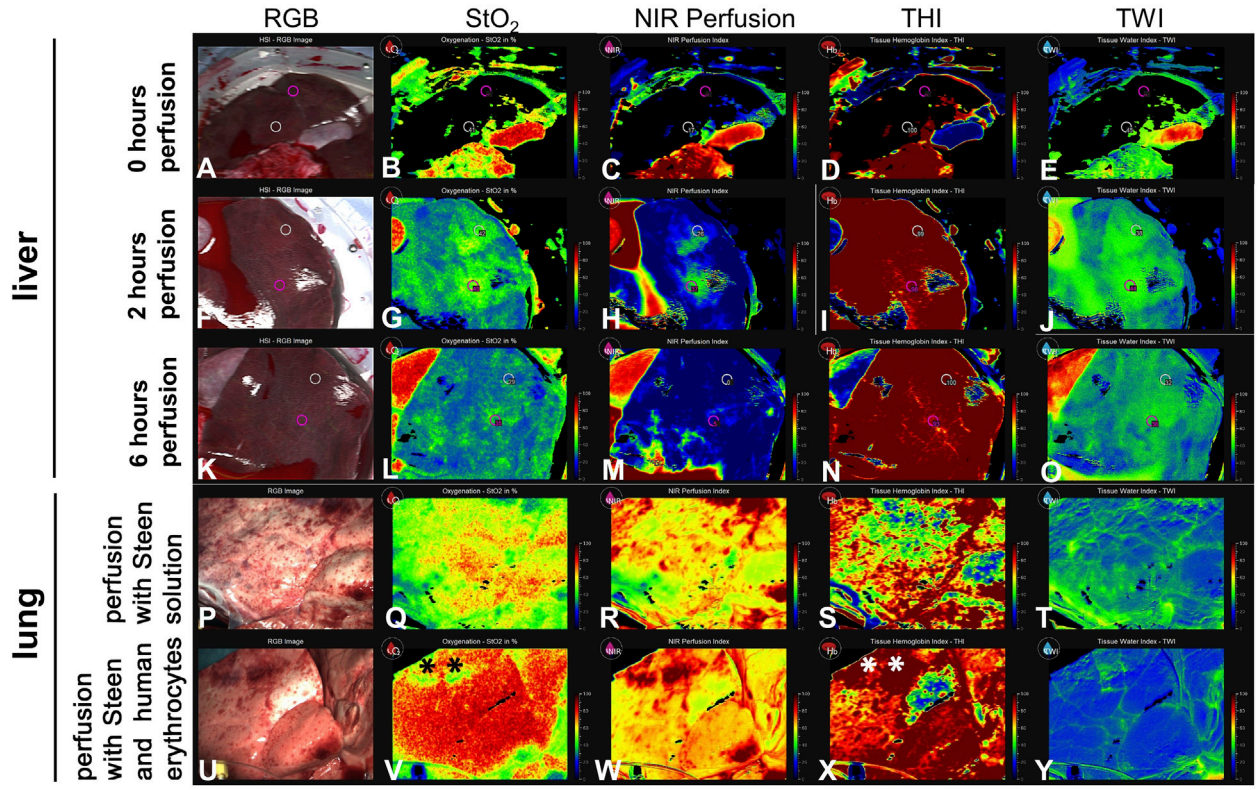


FIGURE 6 | Series of images showing hyperspectral imaging of liver and lung during NMP. **(A–O)** The images illustrate different parameters of liver tissue, including **(B, G, L)** tissue oxygen saturation (StO_2), **(C, H, M)** NIR Perfusion Index, **(D, I, N)** Tissue Hemoglobin Index (THI), **(E, J, O)** Tissue Water Index (TWI) at 0 h, 2 h, 6 h of NMP. Measurements in livers were taken in a central and a peripheral area (white and pink circles) **(P–T)** Lung perfused with cell-free STEEN Solution™. **(U–Y)** Lung perfused with STEEN Solution™ containing human erythrocytes, directly after recruitment. **(*)** Area of venous congestion. Values for StO_2, NIR perfusion index, THI, TWI are arbitrary units.

The question of how long a liver can be safely perfused prior to irreversible damage needs to be addressed in future experiments. Therefore, further developments should involve optimizing the perfusion parameters to ensure consistent oxygen delivery throughout the perfusion period. Additionally, exploring other genetically modified pig lines could yield better outcomes.

Another significant observation was the release of porcine immune cells into the perfusate, including NK cells (CD56), monocytes (CD14), T cells (CD4/CD8), and B cells (CD21). This phenomenon of passenger leukocytes is also observed in lung perfusion, leading to the implementation of leukocyte filters in clinically used perfusion circuits [19]. Understanding the nature and impact of these immune cells is crucial for developing strategies to mitigate any adverse effects [20, 21].

Ex Vivo Lung Perfusion

The lung is particularly challenging for xenotransplantation, because there is no preclinical or clinical model demonstrating long-term survival after xenotransplantation. Even human lung transplantation remains suboptimal compared to other organs, with a median survival of approximately 7 years according to ISHLT data [22].

Here, by applying Steen solution we have successfully demonstrated reproducible cell-free perfusion of genetically modified porcine donor lungs exhibiting excellent functionality. This method forms the basis for our future preclinical research to enable xenogeneic lung transplantation. In pig-to-non-human primate (NHP) lung transplantations most lungs failed to function and showed rapid loss of barrier function and extravasation of human erythrocytes [23]. Although, tested only as an example and without functional human immune cells, platelets, antibodies or complement, PAP, PVR and extravasation of human erythrocytes increased after addition of human erythrocyte concentrates. In our opinion, specific interactions between porcine lungs and human erythrocytes are responsible for the extravasation of human erythrocytes, even though some damage to the endothelium will occur in any organ perfusion. Some mechanisms have been reported, such as binding of human erythrocytes by porcine macrophages using sialoadhesin [24]. We speculate that there may be other, as of today unknown, mechanisms or molecular incompatibilities responsible for these observations.

In general, the use of human erythrocyte concentrates in the perfusate instead of human whole blood, is the major limitation of this study. The absence of functional human complement, antibodies and immune cells might explain the good perfusion results achieved with organ from pig #1396 that appeared to

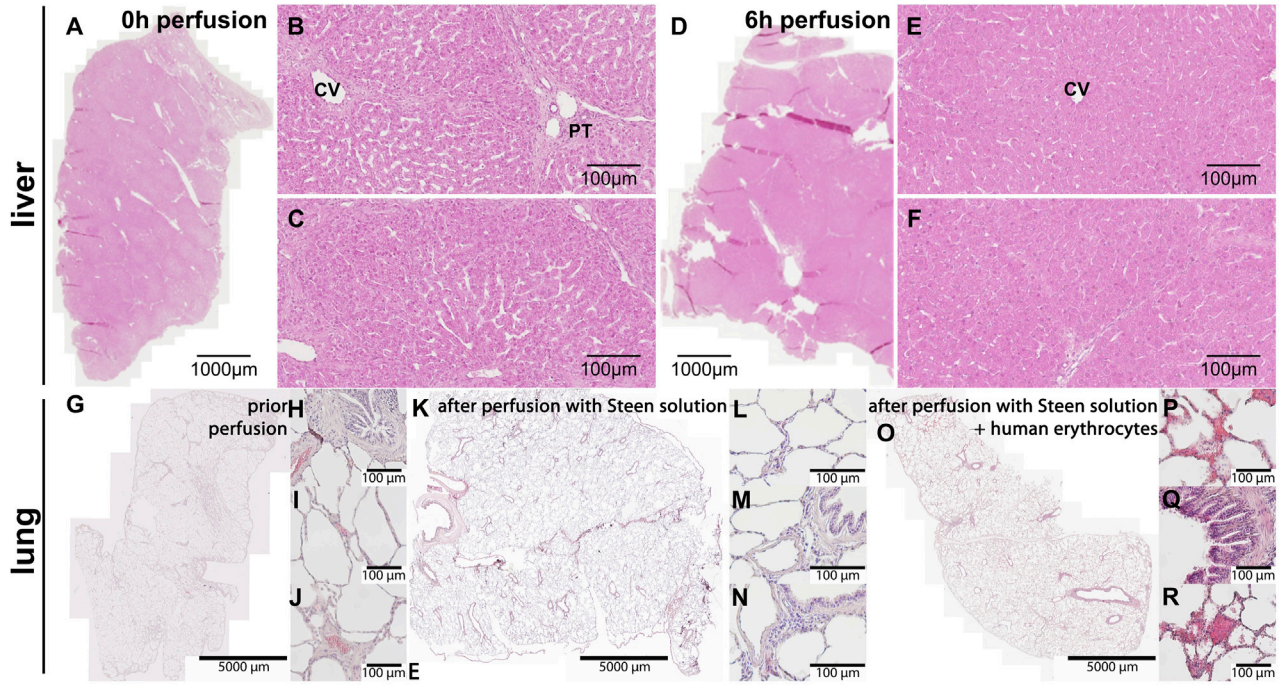


FIGURE 7 | Hematoxylin & Eosin staining of the liver and lung tissue samples. **(A–C)** Liver and **(G–J)** lung before the start of perfusion. **(D–F)** Liver and **(K-N)** lung at the end of perfusion. **(B, C)** At 0 h intact hepatic parenchyma with clearly visible sinusoids around central vein and a portal triad is visible. **(E, F)** At 6 h perfusion hepatocytes still look intact, but sinusoidal space has markedly collapsed. CV central vein, PT portal triad. **(G–J)** The images show intact lung histoarchitecture without edema formation in sections directly after retrieval and **(K–N)** after 2 h of cell-free perfusion with STEEN Solution™. **(H–J)** Porcine erythrocytes were visible within small vessels and capillaries directly after retrieval, **(L–N)** but were absent after 2 h of cell-free perfusion. **(O–R)** Following perfusion with human erythrocytes, erythrocytes were found within alveoli and bronchial epithelium. Scale bars represent **(A, D)** 1,000 µm, **(G, K, O)** 5,000 µm or 100 µm.

express αGal in certain amounts on PBMCs, possibly through chromosomal recombination.

Our results show that the use of porcine DCD organs without premedication of the animal is feasible in this context, especially for lungs confirming previous experiments using wildtype pigs [25, 26]. Tissue integrity and functional recovery of retrieved organs could improve by further reducing cold and warm ischemia times and donor animal anti-coagulation [27].

CONCLUSION AND OUTLOOK

Here, we demonstrate that normothermic machine perfusion of genetically modified porcine lungs and livers using human erythrocyte concentrates for up to six hours is feasible in an interdisciplinary setting. Despite facing challenges due to the use of DCD organs and the absence of certain human blood components in the perfusion medium, our results are encouraging, especially regarding the establishment of a xenotransplantation hub. Using clinically approved solutions and perfusion devices, physiological perfusion conditions, confirmed by hyperspectral imaging and blood gas analysis.

The results of this study, including the successful perfusion of genetically modified porcine organs, representing first steps in the development of the Hannover Xenotransplantation Hub. The development of this hub is essential for focusing the knowledge of genetic modeling as well as functional assessment of porcine organs in order to transfer this innovative concept into clinical practice. Taking this approach as well as the presented results into account, a variety of clinical applications from short-term to long-term organ replacement can be considered.

Our next step will be the development of a organ assist device as a treatment option for patients with acute organ failure. Especially for the liver, advanced NMP of porcine livers is becoming a treatment option for patients with acute liver failure. To achieve this aim, long-term studies using whole human blood as well as multi-knockout, multi-transgene animals will be performed. Furthermore, detailed studies on the immunological interactions between the porcine grafts and the human recipient's need have to be performed in preparation for a subsequent organ transplantation.

Considering the establishment of specialized organ perfusion centers around the world, donor organ evaluation and conditioning is becoming popular and is reaching the level of a commercial service.

These centers benefit from centrally available equipment and high level of expertise in handling technology and offer a path towards organ repair [28]. Based on the comprehensive analysis and findings from our research, it is our strong opinion that the establishment and advancement of a dedicated perfusion hub is essential for future clinical application of xenotransplantation.

The creation of such a hub would facilitate interdisciplinary collaboration, bringing together expertise from various fields including surgery, immunology, molecular genetics and bioengineering. This collaborative environment is crucial for addressing the complex challenges associated with xenogeneic organ perfusion and transplantation. The recently shown potential of NMP mediated knock-down of porcine MHC class-I (SLA-I) to avoid organ rejection [29] after allotransplantation might be promising also for porcine xenografts prior to clinical transplantation to terminally ill patient.

The concept of a perfusion hub not only allows for the pooling of resources and specialized equipment but also promotes the exchange of knowledge and best practices among experts. This set up would enable refinement of perfusion techniques, optimization of genetic modifications, and development of new protocols to improve the viability and functionality of donor organs. By centralizing these efforts, we could accelerate the translation of research into clinical practice, ultimately improving patient outcomes and addressing the critical shortage of donor organs.

In conclusion, the establishment of perfusion hubs, coupled with interdisciplinary collaboration, holds great promise for the future of xeno- and allotransplantation, providing new hope for patients in need.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The human erythrocyte concentrates used in the perfusion experiments were expired and provided to us by the department of transfusion medicine of the MHH. All animals employed in this study were kept in compliance with the German animal welfare law. The generation of the genetically modified pigs was approved by an external ethics committee of the supervisory authority, the Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES, Az. 33.19-42502-04-16/2343) in Oldenburg. According to German law (Tierschutzgesetz $4, Abs. 3), no ethical approval is required for the sole removal of organs after humane euthanasia. However, the local animal welfare officer of the Friedrich-Loeffler-Institute (Mariensee, Neustadt am Rübenberge) approved all euthanasia procedures, checked that all methods have been carried out in accordance with the relevant guidelines and regulations, and reports the number of euthanized animals to the Bundesamt für Risikoforschung (BfR, Berlin).

AUTHOR CONTRIBUTIONS

SS and SK contributed equally as first authors, performed experiments, analyzed data and wrote the manuscript. PW, HL, HZ, KH, JS, NR, VM, ST, AS, KC, and TG performed experiments. WK, KS, RS, HN, KS-O, and MS critically reviewed the data and draft of the manuscript. TG, PZ, FI, AR, and MS secured funding and designed the study. JM performed experiments, analyzed data and wrote the manuscript. PF and RR contributed equally as last authors, designed the study, performed experiments, analyzed data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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Economic Burden and Healthcare Trajectories of Patients Awaiting Heart Transplantation in a French Tertiary Center

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Heart transplantation (HT) is the gold standard treatment of end-stage heart failure, but organ shortage remains a challenge. This retrospective cohort study assesses the economic burden and healthcare pathways of patients awaiting HT in a French tertiary center. Direct healthcare resources were collected and valued, and a state sequence analysis was performed. Ninety-two adult patients were included, with 67 (73%) undergoing HT within a median waiting time of 2 months. The mean cost per patient was €21,324.05 with an average of 2.71 hospitalizations. Four clusters were identified. Type 1 patients (n = 43) underwent HT within 1 month, with a mean cost of €5,820.12 per patient. Only 4 (25%) Type 2 patients (n = 16) underwent HT within 30 months, as they were not prioritized for HT, with a mean cost of €22,285.32 per patient. Type 3 patients (n = 20) underwent HT within 10 months, but incurred higher costs (€27,541.11) compared to Type 2 patients over a shorter period. Despite high transplant priority, Type 4 patients (n = 13) died before HT within 3 months, with a mean cost of €61,858.45 and 3 hospitalizations. This work highlights the economic burden of organ shortage. The use of novel heart preservation devices (such as *ex-vivo* perfusion systems) could help to expand the donor pool and alleviate this burden, but these aspects need to be further investigated.

Keywords: health economics, heart transplantation, pathway, waiting list, donor pool

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INTRODUCTION

Heart transplantation is still the gold standard for carefully selected patients with end-stage heart failure refractory to guidelines-directed optimal medical treatment, with a reported median survival of 12.5 years [1–3]. Moreover, one-year survival on the heart transplantation waiting list has increased up to 67.8% in the 2011–2017 period due to improvements in the management of these severe patients [4]. Nevertheless, one of the key challenges worldwide is to overcome the large imbalance between organ supply and demand for heart transplantation [5]. In France in 2019 before the pandemic coronavirus disease, 573 patients were scheduled on the heart transplantation waiting list but only 425 underwent cardiac transplantation during the same year due to a shortage of available donors [6].

Data on the costs associated with the medical management (apart surgery) of patients with end-stage heart failure listed for heart transplantation are lacking. These data are important because they highlight the economic burden of organ shortage and the potential of strategies to expand the donor pool to help alleviate this burden, such as using *ex vivo* perfusion systems [7]. In a context of limited healthcare resources, our objective was to evaluate the economic burden of patients awaiting heart transplantation in a French tertiary center. A cost of illness (COI) study was conducted alongside a state sequence analysis to compare the economic outcomes with patients' healthcare trajectories.

MATERIALS AND METHODS

Study Design, Setting and Population

A retrospective cohort study was conducted in accordance with the provisions of the French Law and the European General Data Protection Regulation. The study was registered on the National Data Protection Commission register authorized for the Lyon University Hospital (n°22-5946) and has received a favorable opinion from our ethics and scientific committee on 21 December 2022 (n°22-946). All eligible patients were informed and could object to the use of their data.

We included adult patients (aged 18 or older) who were newly scheduled on our heart transplantation waiting list between 1 January 2018 and 31 December 2020. January 2018 was chosen because a new heart allocation system was introduced in France at that time [8]. Participants awaiting multi-organ transplantations were excluded. The main outcome was access to heart transplantation. The cohort entry date was the date of registration on the waiting list. The cohort exit date was the date of heart transplantation surgery, death or the end of the study period (30 June 2022), whichever came first. Patients lost to follow-up would be considered non-transplanted (worst-case scenario).

Data Collection

Data were collected through computerized medical records at individual level for all participants. Baseline patient clinical characteristics were collected at the time of registration on the heart transplantation waiting list: age, body mass index (BMI), New York Heart Association (NYHA) functional classification, indication for heart transplantation, Cardiac Risk Index (CRI) (i.e., a one-year waitlist mortality predictive score based on candidate characteristics, and part of the 2018 French heart allocation system [9]), mechanical circulatory support (temporary or durable), inotropic support, medical history, comorbidities and risk factors. Direct healthcare resource consumptions (i.e., hospitalizations, outpatient medical consultations and outpatient medical procedures) were also collected.

State Sequence Analysis

State Sequence Analysis is an epidemiological method derived from social sciences which can be used to describe and characterize typologies of longitudinal sequences such as healthcare trajectories [10–12]. Herein, six states were predefined: hospitalization, medical procedure, medical consultation, heart transplantation, death, waiting list. Once a patient experienced heart transplantation or death, he would remain in this state (irreversible states). The distance between each pair of patient sequences was then measured using Optimal Matching, a commonly used dissimilarity measure method with an insertion/deletion cost of 1 and a substitution cost matrix estimated based on observed transition rates between states [13]. Agglomerative hierarchical clustering using Ward's criterion on the dissimilarity matrix was then performed to create homogeneous clusters of patients and optimal number of clusters was chosen using the inertia curve [11].

Economic Evaluation

COI studies are designed not only to evaluate the costs attributable to the treatment of a particular illness but also to estimate actual illness-related costs [14]. The economic evaluation was conducted from the healthcare system perspective, which focuses solely on healthcare production and accounts for all monetary costs of healthcare, regardless of who bears the cost [15, 16]. Time horizon was set from the cohort entry to cohort exit dates. Given that our goal was to assess the economic burden of patients on the heart transplantation waiting list (not to compare any interventions at different points in time), we chose not to discount costs regardless of patients awaiting for more than 12 months. This methodological choice was consistent with our objective to estimate the actual expenses involved to manage these patients. All costs were expressed in euros (€) at 2023 price year and adjusted for inflation based on the French National Institute of Statistics and Economic Studies (INSEE) Consumer Price Indices of the healthcare products and services [17].

A top-down micro-costing approach was taken [18]. After identification, hospital stays were classified per Diagnosis Related Group (DRG) using the local Medicine-Surgery-Obstetrics Medical IT system (PMSI). Hospital stays were then valued using the French National Cost Study (NCS), a study based on the cost-accounting of a sample of public and private French institutions, which produces the closest valuation to the hospital production cost [15]. The average cost of stay excluded structural costs as well as cost of products (medicines and medical devices) funded on top of Healthcare Resource Group (HRG) based tariffs, which were additionally valued on the basis of their reference price stated in the French Official Gazette. Outpatient medical consultations and outpatient medical procedures were respectively valued on the basis of reimbursement tariffs of the French National Health Insurance and the French Joint Classification of Medical Procedures (CCAM).

In order to respect the cohort entry and exit dates and to properly exclude heart transplantation-related costs from the evaluation, we performed a specific valuation methodology on certain hospital stays (**Supplementary Figure S1**). When the date of enrolment on the waiting list occurred during a given hospital stay, the DRG provided by the Medical IT system was valued

TABLE 1 | Baseline patient characteristics.

	All patients (n = 92)	Type 1 (n = 43)	Type 2 (n = 16)	Type 3 (n = 20)	Type 4 (n = 13)	p-value[a]
Age (years), median (Q1-Q3)	52 (43–59)	53 (44–59)	47 (42–56)	52 (42–60)	58 (51–60)	
Sex, n (%)						0.045
Male	65 (71)	26 (60)	15 (94)	13 (65)	11 (85)	
Female	27 (29)	17 (40)	1 (6.3)	7 (35)	2 (15)	
BMI (kg/m^2), median (Q1-Q3)	26.0 (23.2–29.6)	24.7 (22.0–28.1)	26.1 (25.6–28.2)	28.9 (26.0–30.3)	25.2 (23.7–28.3)	
BMI ≥30 kg/m^2, n (%)	21 (23)	9 (21)	3 (19)	6 (30)	3 (23)	
Indication for heart transplantation, n (%)						
Ischemic cardiomyopathy	38 (41)	19 (44)	6 (38)	6 (30)	7 (54)	
Dilated cardiomyopathy	31 (34)	16 (37)	4 (25)	7 (35)	4 (31)	
Hyperthrophic cardiomyopathy	7 (7.6)	1 (2.3)	3 (19)	3 (15)	0 (0)	0.041
Valvular cardiomyopathy	1 (1.1)	0 (0)	1 (6.3)	0 (0)	0 (0)	
Adult congenital heart disease	3 (3.3)	2 (4.7)	1 (6.3)	0 (0)	0 (0)	
Graft failure	2 (2.2)	1 (2.3)	0 (0)	1 (5.0)	0 (0)	
Graft coronary heart disease	1 (1.1)	0 (0)	1 (6.3)	0 (0)	0 (0)	
Others	9 (9.8)	4 (9.3)	0 (0)	3 (15)	2 (15)	
NYHA Functional Classification, n (%)						<0.001
Class II	24 (26)	8 (19)	8 (50)	6 (30)	2 (15)	
Class III	46 (50)	22 (51)	7 (44)	14 (70)	3 (23)	
Class IV	22 (24)	13 (30)	1 (6.3)	0 (0)	8 (62)	
CRI, median (Q1-Q3)	21 (14–28)	23 (18–29)	11 (9–16)	19 (16–25)	33 (26–36)	<0.001
Temporary MCS (i.e., ECMO), n (%)	17 (18)	9 (21)	0 (0)	2 (10)	6 (46)	0.009
Durable MCS (i.e., LVAD), n (%)	10 (11)	2 (4.7)	1 (6.3)	6 (30)	1 (7.7)	0.028
Inotropic support, n (%)	24 (26)	13 (30)	0 (0)	1 (5.0)	10 (77)	<0.001
Cardiovascular risk factors, n (%)						
Hypertension	19 (21)	5 (12)	4 (25)	4 (20)	6 (46)	
Diabetes	20 (22)	9 (21)	4 (25)	3 (15)	4 (31)	
Smoking						
Active smoking	11 (12)	7 (16)	1 (6.3)	2 (10)	1 (7.7)	
Previous smoking	46 (50)	19 (44)	9 (56)	11 (55)	7 (54)	
Comorbidities, n (%)						
Chronic renal failure	13 (14)	6 (14)	0 (0)	4 (20)	3 (23)	
Arrhythmia	55 (60)	26 (60)	11 (69)	11 (55)	7 (54)	
ICD	63 (68)	28 (65)	15 (94)	13 (65)	7 (54)	
Cardiac resynchronisation therapy	24 (26)	11 (26)	4 (25)	6 (30)	3 (23)	
Familial cardiomyopathy	16 (17)	5 (12)	6 (38)	3 (15)	2 (15)	
Peripheral arterial disease	5 (5.4)	1 (2.3)	1 (6.3)	1 (5.0)	2 (15)	
Concomitant pulmonary disease	4 (4.3)	2 (4.7)	0 (0)	2 (10)	0 (0)	
Previous CVA	11 (12)	3 (7.0)	3 (19)	4 (20)	1 (7.7)	
History of cancer	9 (9.8)	4 (9.3)	0 (0)	4 (20)	1 (7.7)	
Previous cardiac surgery	18 (20)	11 (26)	2 (13)	3 (15)	2 (15)	
Previous thoracic surgery	1 (1.1)	0 (0)	0 (0)	0 (0)	1 (7.7)	
Venous thromboembolic disease	4 (4.3)	3 (7.0)	0 (0)	1 (5.0)	0 (0)	

BMI, Body Mass Index; CRI, Cardiac Risk Index; CVA, Cerebrovascular Accident; MCS, Mechanical Circulatory Support; ECMO, Extracorporal Membrane Oxygenation; LVAD, Long term Ventricular Assist Device; ICD, Implantable Cardioverter Defibrillator; NYHA, New York Heart Association.

[a]Chi2 test or Fisher's exact test for qualitative variables; Kruskall Wallis test for quantitative variables. A significance threshold of 5% was set, and all tests were two-tailed. For clarity, only statistically significant p-values are shown.

using the NCS and divided by its mean national length of stay (also provided by the NCS) to obtain a mean hospital cost per day. It was then multiplied by the actual patient's length of stay between the date of enrolment and the date of hospital discharge. When the date of enrolment on the waiting list and the date of heart transplantation surgery occurred on the same hospital stay, a standardized DRG of cardiac decompensation (05M093) was applied instead of the heart transplantation Medical IT system DRG and patient's length of stay between the date of enrolment and the date of heart transplantation was taken into account. The same standardized DRG was applied when a given hospital admission led to transplantation (i.e., heart transplantation was not the hospitalization reason) but patient's length of stay from admission to heart transplantation was taken into account.

Statistical Analysis

Descriptive quantitative data were presented using medians and first and third quartiles. Descriptive qualitative data were presented using integer numbers and percentage frequencies. Homogeneous clusters of patients obtained from the state sequence analysis were described according to patient baseline characteristics and to their healthcare resource consumption. The status at the cohort exit date (transplanted, dead, non-transplanted) and the time from heart transplantation list registration to heart

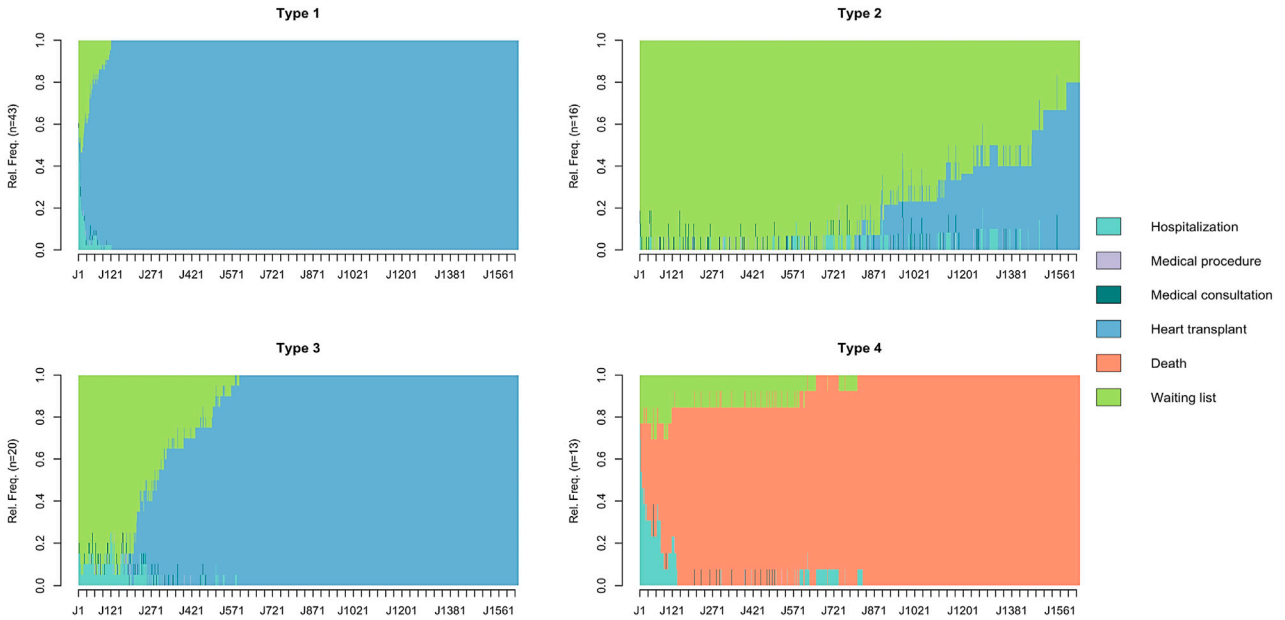

FIGURE 1 | Chronograms of patient healthcare trajectories by cluster type. The X-axis represents the time from registration on the waiting list (time step = day). The Y-axis represents the relative frequency (Rel.Freq) of patients in the different states. Chronograms were obtained from a State Sequence Analysis with six states predefined: hospitalization, medical procedure, medical consultation, heart transplantation, death, waiting list. Optimal Matching was as the chosen dissimilarity measure method with an insertion/deletion cost of 1 and a substitution cost matrix estimated based on observed transition rates between states. Agglomerative hierarchical clustering using Ward's criterion on the dissimilarity matrix was then performed to create homogeneous clusters of patients and optimal number of clusters was chosen using the inertia curve. Four homogeneous clusters of patients were identified (referred as "Types").

transplantation (or death) were also presented per cluster. Exploratory bivariate analyses were conducted to analyze patient baseline covariates according to cluster types. A bivariate association was sought using the Chi2 test for the qualitative variables (or the Fisher's exact test in case of insufficient conditions of performance) and using the Kruskall Wallis non parametric test for the quantitative variables. A significance threshold of 5% was set, and all tests were two-tailed.

Mean costs per patient and mean quantities per cost item were presented, assorted with their Bias-Corrected and accelerated bootstrapped (R = 10,000) 95% Confidence Intervals (CI) to assess uncertainty around our point estimates. Cost differences between groups were considered statistically significant if the bootstrapped 95% CIs did not overlap. All analyses were performed using R (version 4.2.2) within R Studio software. The R package "TraMineR" was used to perform the state sequence analysis [13].

RESULTS

Baseline Patient Characteristics

During the study period, 92 patients were included (median age of 52 years, male sex 71%). Medical history, comorbidities and risk factors are also summarized in **Table 1**. Ischemic cardiomyopathy and dilated cardiomyopathy were the most common indications for heart transplantation (41% and 34%, respectively). The median CRI, which assesses priority for heart transplantation based on candidate characteristics was 21. Inotropic support was required before heart transplantation in 24 (26%) patients. Twenty-seven (29%) patients were bridged to heart transplantation on temporary (i.e., extracorporeal membrane oxygenation [ECMO]; n = 17, 18%) or durable (i.e., long term ventricular assist device [LVAD]; n = 10, 11%) mechanical circulatory support.

Description of Clusters

After clustering, four homogeneous clusters of patients were identified based on the similarity of their healthcare trajectories (referred to as "Types" below). Chronograms are presented in **Figure 1**.

Type 1 patients (n = 43, 47%) were predominantly NYHA Class III (51%), Type 2 patients (n = 16, 17%) NYHA Class II (50%) and NYHA Class III (44%), Type 3 patients (n = 20, 22%) NYHA Class III (70%) and Type 4 patients (n = 13, 14%) NYHA Class IV (62%). Type 4 patients were characterized by the highest median age (58 years). Temporary mechanical circulatory support was the leading support (46%) in Type 4 patients while durable mechanical circulatory support was the leading support (30%) in Type 3 patients. One patient (6%) received durable mechanical circulatory support among Type 2 patients. The distribution of the CRI according to the type of cluster is shown in **Figure 2**.

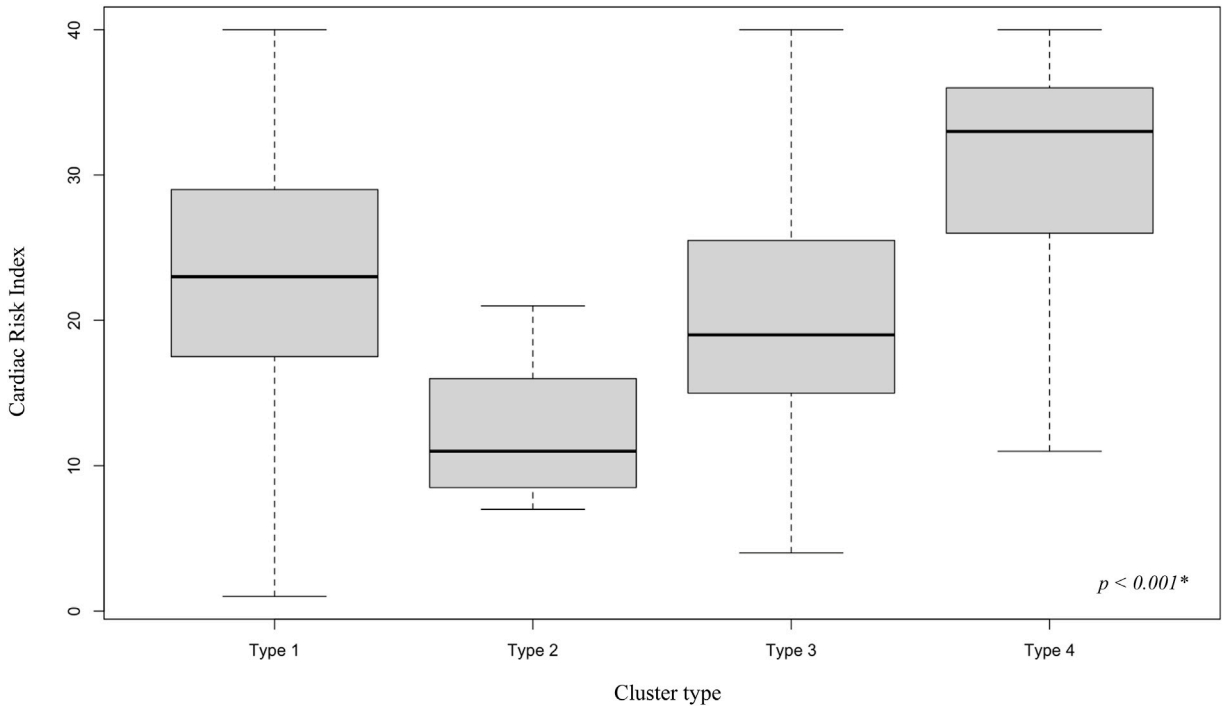

FIGURE 2 | Cardiac Risk Index by cluster type. Distribution of the Cardiac Risk Index, a one-year waitlist mortality predictive score based on candidate characteristics, and part of the 2018 French heart allocation system, by cluster type. * Kruskall Wallis two-tailed test. A significance threshold of 5% was set.

TABLE 2 | Direct healthcare resource consumptions and costs, in euro price year 2023 from the health system perspective.

	All patients (n = 92)	Type 1 (n = 43)	Type 2 (n = 16)	Type 3 (n = 20)	Type 4 (n = 13)
Overall patient trajectory, Mean cost (€)	21,324.05	5,820.12	22,285.32	27,541.11	61,858.45
[95% CI]	[14,661.89; 31,314.91]	[3,823.34; 9,448.58]	[11,254.33; 50,850.47]	[13,654.4; 55,149.85]	[32,130.42; 103,396.4]
All hospitalizations, mean	2.71	1	4.12	5	3.08
[95% CI]	[1.99; 4.76]	[0.74; 1.35]	[2.62; 6.69]	[2.35; 14.25]	[1.69; 5.85]
Mean cost (€)	21,004.68	5,572.9	21,683.02	27,285.41	61,550.7
[95% CI]	[14,392.35; 31,242.44]	[3,537.69; 9,209.7]	[10,588.7; 50,689.53]	[13,228.77; 53,641.06]	[32,392.09; 103,561]
Hospitalizations for heart failure, mean	0.75	0.49	0.62	1.1	1.23
[95% CI]	[0.54; 1]	[0.3; 0.77]	[0.19; 1.6]	[0.55; 1.75]	[0.69; 1.77]
Mean cost (€)	10,812.55	2,325.9	3,171.05	18,728.11	36,110.92
[95% CI]	[5,985.37; 18,915.73]	[1,260.87; 5,412.92]	[1,134.39; 9,308.26]	[5,558.03; 46,912.62]	[13,651.36; 74,642.67]
Hospital medical consultations, mean	3.46	2.4	6.75	2.7	4.08
[95% CI]	[2.58; 4.54]	[1.44; 3.77]	[4.25; 9.44]	[1.25; 5.1]	[1.92; 8.92]
Mean cost (€)	190.11	131.74	371.25	148.5	224.23
[95% CI]	[142.88; 250.49]	[79.3; 211.05]	[233.75; 522.5]	[66; 269.09]	[105.77; 477.12]
Hospital medical procedures, mean	1.36	1.21	2.38	1.15	0.92
[95% CI]	[0.96; 1.84]	[0.7; 2.05]	[1.38; 3.69]	[0.5; 2.25]	[0.23; 2]
Mean cost (€)	129.26	115.48	231.05	107.2	83.52
[95% CI]	[92.94; 176.3]	[65.31; 190.79]	[133.93; 374.01]	[49.31; 213.34]	[22.27; 181.88]

CI, confidence interval.

All costs were expressed in euros (€) at 2023 price year and adjusted for inflation based on the French National Institute of Statistics and Economic Studies (INSEE) Consumer Price Indices of the healthcare products and services.

Cost differences between groups were considered statistically significant if the bootstrapped 95% CIs did not overlap.

Results from the exploratory bivariate analyses identified sex, hypertrophic cardiomyopathy (as the indication for heart transplantation), NYHA Class, CRI, temporary mechanical support, durable mechanical support, and inotropic support as patient covariates associated with cluster membership (**Table 1**).

TABLE 3 | Description of hospitalizations motives.

Hospitalization motives (n = 249 hospitalizations)	n (%)
Cardiac decompensation	69 (27.7%)
Cardiac examinations/assessments	43 (17.3%)
Infection related to the cardiovascular disease	19 (7.6%)
Arrhythmia	16 (6.4%)
Implantation/Follow-up/Complication of ICD	16 (6.4%)
Acute Kidney Injury	10 (4.0%)
Other cardiac-related hospitalizations	76 (30.5%)

ICD, Implantable Cardioverter Defibrillator.

Follow-Up and Access to Heart Transplantation

The median follow-up was 4 months (Q1–Q3 = 1–14). Two (2%) patients were lost to follow-up and considered non-transplanted (worst-case scenario). During the follow-up period, 67 (73%) patients underwent heart transplantation, 12 (13%) remained non-transplanted and 13 (14%) died. All Type 1 and Type 3 patients underwent heart transplantation while only 4 (25%) patients of Type 2 were transplanted. Patients dead during the follow-up were exclusively Type 4 patients. Median wait time from listing to transplantation was 2 months (1–8) overall, 1 month (0–2) for Type 1, 30 months (28–32) for Type 2 and 10 months (7–15) for Type 3. Type 4 patients died at a median of 3 months (0–4) after listing.

Costs

The mean total cost for the entire patient trajectory was €21,324.05 [95% CI: €14,661.89–€31,314.91], mainly driven by hospitalization-related costs of €21,004.68 [95% CI: €14,392.35–€31,242.44]. The mean number of hospitalizations was 2.71 [95% CI: 1.99–4.76] (**Table 2**). Hospitalization for heart failure was the most common reason for admission, accounting for 27.7% (n = 69) of all admissions (**Table 3**). Costs varied significantly between Type 1 patients (€5,820.12 [95% CI: €3,823.34–€9,448.58]) and all patients, as well as between Types 2, 3 and 4 patients. Type 4 patients (€61,858.45 [95% CI: €32,130.42–€103,396.4]) had significantly different costs from all patients and from Type 1 patients. Type 3 patients had the highest mean number of hospitalizations with 5 admissions [95% CI: 2.35–14.25], whereas Type 1 patients had the lowest with 1 admission [95% CI: 0.74–1.35]. Type 4 patients had the highest mean cost for hospitalizations (€61,550.7 [95% CI: €32,392.09–€103,561]), 3 (23%) patients receiving a durable mechanical circulatory support during a hospitalization. Type 2 patients had the highest mean number of hospital medical consultations and procedures, 6.75 [95% CI: 4.25; 9.44] and 2.38 [95% CI: 1.38; 3.69] respectively. Average costs per year are presented in **Table 4**.

DISCUSSION

To the best of our knowledge, this is the first economic evaluation of illness-related costs of patients with end-stage heart failure eligible for heart transplantation, using waiting list enrolment as the entry point. It is also the first study to characterize clusters of patients awaiting for heart transplantation based on their healthcare trajectories after listing.

The mean cost associated with managing these patients was €21,324.05, hospitalization being the main component. These results are consistent with a systematic review of cost-of-illness studies on heart failure published between 2004 and 2016, which found prevalence-based annual cost estimates ranging from $868 to $25,532 [19]. The review also found that hospitalization costs contributed significantly to total direct costs, from 44% to 96% [19]. However, few studies have focused on end-stage heart failure. Russo et al. estimated the mean cost of medical management of patients with advanced

TABLE 4 | Average costs per year, in euro price year 2023 from the health system perspective.

Year of follow-up after waiting list inscription	All patients (n = 92)	Type 1 (n = 43)	Type 2 (n = 16)	Type 3 (n = 20)	Type 4 (n = 13)
First, n (%)	92 (100)	43 (100)	16 (100)	20 (100)	13 (100)
Mean cost (€) [95% CI]	16,616.3 [10,797.33; 25,770.64]	5,820.12 [3,823.34; 9,448.58]	3,665.76 [1,207; 12,045.37]	26,376.3 [12,323.29; 52,709.52]	53,941.49 [27,614.77; 90,905.34]
Second, n (%)	24 (26)	—	16 (100)	6 (30)	2 (15)
Mean cost (€) [95% CI]	6,818.52 [2,805.56; 14,706.02]	—	3,753.14 [839.23; 14,870.45]	4,408.46 [1,168.53; 12,021.89]	38,571.74 [27,029.34; 38,571.74]
Third, n (%)	15 (16)	—	14 (89)	—	1 (7)
Mean cost (€) [95% CI]	8,057.19 [3,741.51; 14,129.01]	—	6,924.94 [2,804.22; 12,868.08]	—	23,908.68[a]
Fourth, n (%)	9 (10)	—	9 (57)	—	—
Mean cost (€) [95% CI]	16,300.88 [1,076.51; 61,845.37]	—	16,300.88 [1,076.51; 61,845.37]	—	—
Fifth, n (%)	2 (2)	—	2 (14)	—	—
Mean cost (€) [95% CI]	951.18 [55; 951.18]	—	951.18 [55; 951.18]	—	—

CI, confidence interval.

[a]*Impossible to compute a confidence interval (n = 1).*

All costs were expressed in euros (€) at 2023 price year and adjusted for inflation based on the French National Institute of Statistics and Economic Studies (INSEE) Consumer Price Indices of the healthcare products and services.

Cost differences between groups were considered statistically significant if the bootstrapped 95% CIs did not overlap.

heart failure in the last 2 years of life in the United States, on the basis of the REMATCH trial (using date of death as reference point) to be $156,169, but this assessment was based on a health system significantly different from France, which may explain the higher costs, and included patients who were contraindicated to heart transplantation [20, 21]. Delgado et al. estimated costs for patients with symptomatic chronic heart failure in Spain, highlighting higher costs for patients with severe forms of heart failure including NYHA Class II (€3,789.30) and NYHA Class III-IV (€6,832.18) patients [22]. It was therefore of interest to use waiting list enrolment to define our end-stage heart failure population and assess its economic burden, as this population is usually difficult to characterize due to its inherent heterogeneity [23].

The state sequence analysis has also helped to understand patient pathways while waiting for transplantation, which is one of the objectives of the Ministerial Plan for Organ and Tissue Donation and Transplantation 2022–2026 in France [24]. Four clusters were identified. Type 1 patients had a low economic burden, as they survived until transplantation and were transplanted quickly (median 1 month). Despite high transplantation priority, Type 4 patients died before transplantation (median 3 months). The outcome of these patients, characterized by their critical condition, reflects the challenge of limited access to heart transplantation. Indeed, they are older (58 years), with 46% requiring ECMO and 77% dependent on inotropes, indicating greater severity. Their human leukocyte antigen (HLA) sensitization status would have been interesting but was not available. They also represented a major economic burden on the healthcare system with an average of 3 hospitalizations per patient. Additionally, 3 patients (23%) were bridged to heart transplantation on durable mechanical circulatory support after listing. These devices, funded separately from HRG-based fees and reimbursed in France at a price of €87,565, further contributed to the overall costs. Type 2 patients were not prioritized for heart transplantation. Consequently, they remained on the waiting list for an extended period, and only 4 (25%) patients underwent transplantation. Despite their initial milder condition, they still incurred significant healthcare costs due to deteriorating health, averaging 4.12 hospitalizations after listing. With 6 patients (30%) bridged to heart transplantation on durable support at enrolment, Type 3 patients underwent transplantation within a median of 10 months. However, they were heavy consumers of healthcare resources, averaging 5 hospitalizations and incurring higher costs compared to Type 2 patients over a significantly shorter period.

Heart transplantation remains the standard of care in selected, eligible patients, and is cost-effective [23]. This analysis further highlights the current issues related to its access, the economic consequences of organ shortage for healthcare systems, and the need to support strategies that can expand the donor's pool [25–28]. Results from our COI study could therefore help inform decisions about health system resource allocation for this specific population and along the pathways identified [29, 30]. These results provide information on the economic burden of the disease, which could be reduced by health technologies designed to improve access to heart transplantation by expanding the donor pool, such as *ex vivo* perfusion systems [31, 32]. Indeed, our study showed that despite a priority status for transplantation, the average cost of patients who died before receiving a heart (i.e., Type 4 patients) was €61,550.7 [95% CI: €32,392.09–€103,561]. In comparison, the unitary purchase price of the consumables for one of these *ex vivo* perfusion systems (i.e., the TransMedics Organ Care System (OCS™) Heart (TransMedics; Andover, MA) is €54,000 including taxes (one consumable per procedure).

Therefore, we could hypothesize that the additional costs associated with the use of these expensive devices in routine in heart transplant centers, could be compensated by the reduction in the economic burden associated with the management of end-stage heart failure patients on the list, especially the most severe (i.e., Type 4). In addition, expanding the donor pool could lead to better health outcomes and health-related quality of life for these patients, which are of primary considerations within a cost-effectiveness analysis framework. These hypotheses need to be further investigated in a complete model-based cost effectiveness analysis. Here, we have provided real-world illness-related cost estimates in a French setting which could be further used for this economic evaluation and, more broadly for economic evaluations comparing treatment strategies for end-stage heart failure. Special emphasis should be placed on developing economic models based on real-world patient pathways [33].

Our study does have limitations. Data on changes in CRI during the time spent on the waiting list would have been interesting to capture changes in patient priority status, but the score was only reported at listing in the computerized medical records. Patients' post-transplant prognosis and economic data according to their pre-transplant healthcare trajectory would also have been interesting. However, the primary objective of this study focused on the pre-transplant pathway, as economic data on these aspects are particularly scarce in the literature. In addition, a long follow-up period would have been required to collect this data. This retrospective cohort study was conducted in a single tertiary center and included a small number of patients. This limited the possibility to properly investigate associations between baseline patient characteristics (at the time of waiting list registration) and cluster membership using multivariate statistical modelling. This model could be of interest for predicting future healthcare trajectories and resource use based on patient characteristics at registration on the waiting list. These health economic estimates could be considered as complementary indicators for ranking candidates for heart allocation. Here, only exploratory bivariate analyses were conducted to identify which patient covariates may influence cluster type belonging (i.e., sex, hypertrophic cardiomyopathy as the indication for heart transplantation, NYHA Functional Classification, Cardiac Risk Index, temporary mechanical support, durable mechanical support and inotropic support). However, although these findings are exploratory and based on a small dataset, they may be of interest to clinicians managing these patients and involved in their care pathway. Furthermore, despite being single-centered, this study is a fairly good reflection of the French national situation in terms of access to heart transplantation over the same period, with one-year

access at 76.7% [34]. The potential impact of the COVID-19 pandemic cannot be overlooked, as the number of heart transplants per year in France, according to data from the French Agency of Biomedicine, was 450 in 2018 and 425 in 2019, before decreasing to 370 in 2020, followed by 409 in 2021 and 411 in 2022 [35]. Of notice and in contrast with other solid organ transplant programs, heart transplant programs kept running during the COVID era and its access did not seem deeply affected by the outbreak. Finally, this economic evaluation was conducted from the healthcare system perspective and only focused on hospital care. A broader perspective may be of interest, especially when considering informal care, which may be an important cost component in end-stage heart failure [19]. However, this was not feasible here.

In conclusion, this study assessed the economic burden of patients waiting for heart transplantation and helped characterizing patients with higher healthcare resource utilization. It may provide insights for better informed decisions on the medical management of these patients, and help inform resource allocation along this pathway, particularly regarding strategies designed to expand the donor pool.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

This study was approved by the Ethics and Scientific committee of Hospices Civils de Lyon on December 21, 2022 (n°22-946), and was registered on the National Data Protection Commission register (n°22-5946). The study was conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

Conceptualization: JA, PG, MP, and LH. Methodology: JA, PG, and LH. Data curation: JA. Formal analysis: JA. Supervision: LH. Validation: PG, LS, MP, and LH. Writing–original draft: JA. Writing–review and editing: JA, PG, LS, MP, and LH. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.13703/full#supplementary-material

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ORIGINAL RESEARCH
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Donor and Recipient Polygenic Risk Scores Influence Kidney Transplant Function

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Kidney transplant outcomes are influenced by donor and recipient age, sex, HLA mismatch, donor type, anti-rejection medication adherence and disease recurrence, but variability in transplant outcomes remains unexplained. We hypothesise that donor and recipient polygenic burden for traits related to kidney function may also influence graft function. We assembled a cohort of 6,060 living and deceased kidney donor-recipient pairs. We calculated polygenic risk scores (PRSs) for kidney function-related traits in both donors and recipients. We investigated the association between these PRSs and recipient eGFR at 1- and 5-year post-transplant as well as graft failure. Donor: hypertension PRS ($P < 0.001$), eGFR PRS ($P < 0.001$), and intracranial aneurysm PRS ($P = 0.01$), along with *recipient* eGFR PRS ($P = 0.001$) were associated with eGFR at 1-year post-transplantation. Clinical factors explained 25% of the variation in eGFR at 1-year and 13% at 5-year, with PRSs cumulatively adding 1% in both cases. PRSs were not

Abbreviations: DeKAF, Deterioration of kidney allograft function; eGFR, estimated glomerular filtration rate; FRCBS, Finnish Red Cross Blood Service; GEN03, Genomics of kidney transplantation; GWAS, genome-wide association study; HLA, human leukocyte antigen; KDPI, kidney donor profile index; KiT-GENIE, Kidney transplantation - genomic investigation of essential clinical concerns; PRS, Polygenic risk score; SNP, single nucleotide polymorphism; TKV, total kidney volume; TL, Transplant lines; UKIRTC, United Kingdom and Ireland renal transplant consortium; QUB, Queen's University Belfast.

associated with long-term graft survival. We demonstrate a small, but statistically significant association between donor and recipient PRSs and recipient graft function at 1- and 5-year post-transplant. This effect is, at present, unlikely to have clinical application and further research is required to improve PRS performance.

Keywords: polygenic risk scores, eGFR, graft survival, graft function, multivariable models

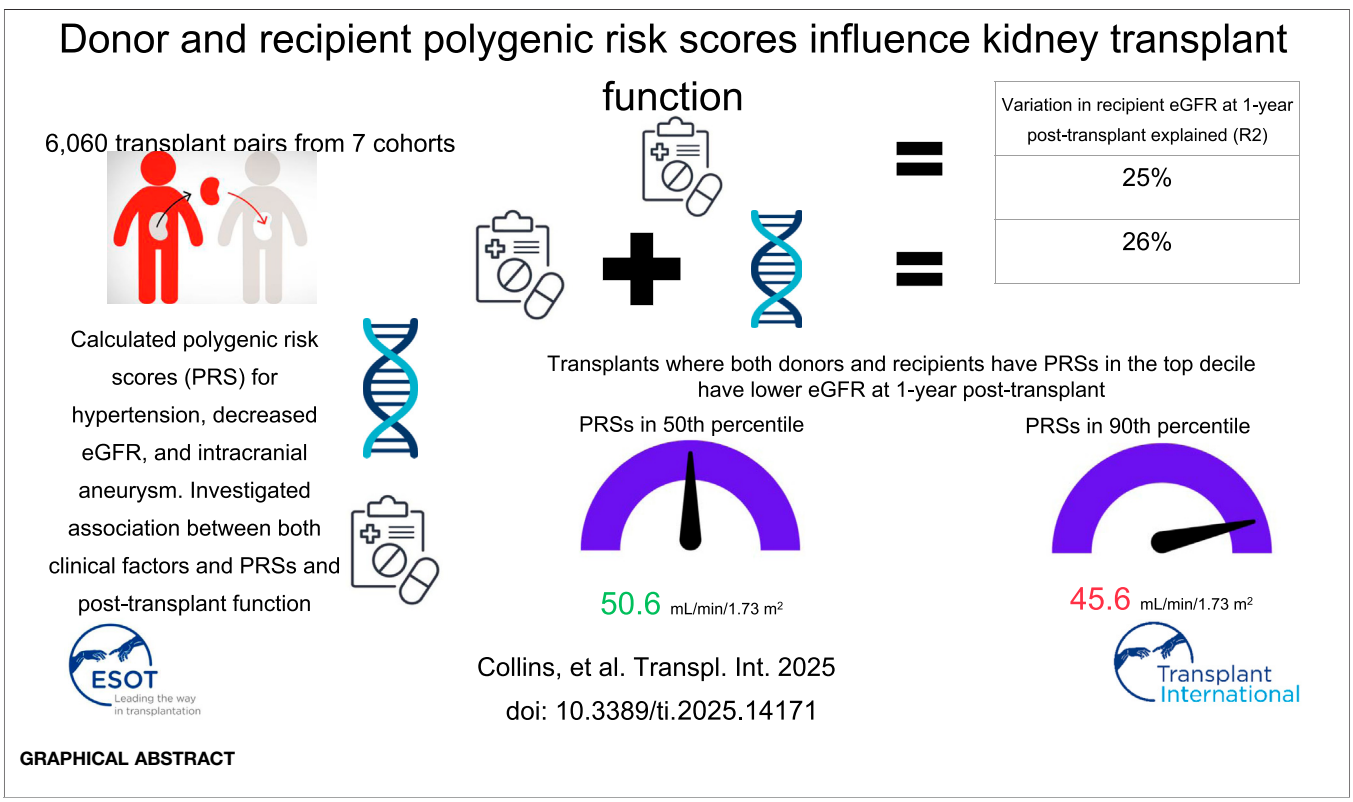
INTRODUCTION

Kidney transplant outcomes are influenced by a wide array of factors including donor age and sex, whether the donor is living or deceased, clinical era of transplant, donor cause of death, and HLA mismatch [1, 2]. While significant progress has been made in improving short-term graft survival, enhancing medium- and long-term graft survival and function still remains a challenge [3].

HLA mismatch and blood group are the only genetic factors currently used in transplant allocation decisions. It is well established that graft survival is inversely related to the number of mismatched HLA alleles [4]. However, in many centres, less than 5% of transplants are fully matched across the 6 HLA antigens tested [5]. Thanks to modern immunosuppression, it is possible to have good outcomes even with poorly HLA matched kidneys [6]. It has also been reported that mismatches between donor and recipient in non-synonymous single nucleotide polymorphisms (SNPs) in genes for transmembrane and secreted proteins and outside the HLA were significantly associated with graft survival [7]. However, a subsequent replication attempt, involving nearly 8,000 pairs, found no significant associations between these variants and graft outcome [8]. A more recent study reported an association between donor and recipient genetic mismatch and graft survival [9]. Genetic mismatch in this context was defined as the sum of variant mismatches in transmembrane, secretory, and kidney-related proteins.

Polygenic risk scores (PRSs) quantify individual genetic burden for a trait using summary statistics from genome-wide association studies (GWAS). Specifically, they estimate the cumulative effect of common genetic variation on an individual's disease status weighted by estimated effect size [10].

PRSs for various traits of the donor kidney ("donor PRS") have been reported to be associated with transplant outcome. Donor burden for estimated glomerular filtration rate (eGFR) has been correlated with eGFR post-transplant [11]. Other studies have shown an association between donor genetic risk scores in interleukin-6 and biopsy proven rejection [12, 13]. A recent study from our group has shown that donor kidneys in the top decile of PRS for traits related to stroke have eGFR at 1-year post-transplant approximately 5 mL/min/1.73 m^2 lower than those in the bottom decile of risk [14]. The effect of recipient



GRAPHICAL ABSTRACT

polygenic burden on transplant outcome has also been established for several outcomes of interest. Recipient polygenic burden of eGFR has been shown to be associated with post-transplant eGFR [11] and recipient burden for skin cancer has been associated with skin cancer post-transplant [11, 15]. Recipient PRS for type 2 diabetes was shown to be associated with the development of post-transplant diabetes, and the same study found that the same PRS in donors was a significant predictor of post-transplant diabetes, but only in liver transplants [16]. Shaked et al also found that combining both donor and recipient PRS for type 2 diabetes significantly improved type 2 diabetes prediction [16].

We assembled 6,060 genotyped donor-recipient transplant pairs across seven cohorts. We calculated kidney function related PRS for seven traits in both donors and recipients. We test the correlation between polygenic burden and transplant outcomes, particularly eGFR at 1 and 5 years post-transplant, as well as long-term graft survival.

MATERIALS AND METHODS

Inclusion Criteria

The inclusion criteria were as follows: (1) availability of SNP array genotyping data for both the donor and recipient in a transplant pair; (2) availability of data on donor age, sex, and kidney donation type (living, deceased of stroke, deceased of other cause), recipient age, sex, year of transplant, and whether it was the recipient's first transplant; (3) at least one of the following outcome variables was also required: death-censored graft survival, eGFR at 1-year post-transplant, eGFR at 5-year post-transplant (plus or minus 3 months for each). If a graft had failed by 1- or 5-year, then individuals were assigned values of eGFR at 1- or 5-year respectively of 0 mL/min/1.73 m^2.

Patient Cohort Descriptions

We included seven predominantly European ancestry cohorts from the following regions: USA: Deterioration of Kidney Allograft Function (DeKAF), Genomics of kidney transplantation (GEN03). Finland: Finnish Red Cross Blood Service (FRCBS). Netherlands: Transplant Lines (TL). France: Kidney Transplantation - Genomic Investigation of Essential Clinical concerns (KiT-GENIE). UK and Ireland: United Kingdom and Ireland Renal Transplant consortium (UKIRTC), Queen's University Belfast (QUB). See **Supplementary Materials** for more detailed information on the recruitment and characteristics of each of these cohorts involved. There were 924 missing values of HLA mismatch, so we performed multiple imputation using the R package mice based on the variables for donor type, donor age, recipient age, donor sex, recipient sex, and whether it was the recipient's first transplant.

Calculation of PRS

SNP array genotype data was subject to quality control for minor allele frequency, missingness per marker, and missingness per individual (see **Supplementary Materials**). We calculated PRSs in each individual for hypertension [17], eGFR [18], rapid decline in eGFR [19], albuminuria [20], total kidney volume (TKV) [21], stroke [22], and intracranial aneurysm [23] using published GWAS for each trait. These traits were selected as they were directly related to kidney function and risk factors for progression of kidney disease. We have previously demonstrated the impact of donor intracranial aneurysm and stroke as a cause of death to be associated with recipient graft function [14]. Further details of these GWAS can be found in **Supplementary Table S1**. PRSs were calculated using PRSice2 [24], selecting alleles with a p-value threshold greater than 0.5 (see **Supplementary Materials** for further details). All analysis was conducted in R, using version 4.2.1 (2022-06-23) [25].

For two of these PRSs (eGFR and total kidney volume), we hypothesised that higher values would be associated with better kidney function [26], while for the others (hypertension, albuminuria, stroke, intracranial aneurysm, and rapid kidney function decline), one might expect that higher values would be associated with worse kidney function. To simplify interpretation, we standardised the directionality of all PRSs, such that one might expect higher scores to be associated with negative outcomes. We did this by inverting the sign of the eGFR and total kidney volume PRSs to create "new" PRSs, which we will refer to as "decreased eGFR," and "decreased total kidney volume."

Univariable Analysis

A series of univariable linear models for recipient eGFR at 1- and 5-year post-transplant were created for all the clinical factors (donor age, donor sex, recipient age, recipient sex, HLA mismatch, year of transplant, donor type, and whether it was the recipient's first transplant), as well as the donor PRSs, and recipient PRSs. The variance in the outcome explained (R^2) was also calculated for each factor. In a similar manner, a series of univariable Cox proportional hazards models for death-censored graft survival were created for each of the clinical factors, donor PRSs, and recipient PRSs.

Multivariable Analysis

For each of the three outcomes of interest in the univariable analysis (eGFR at 1-year, eGFR at 5-year, and graft survival), multivariable models were created with just the factors that had a p-value less than 0.2in the univariable analysis. Assumptions of a linear model (residuals vs. fitted, normal Q-Q, scale-location, and residuals vs. leverage) and Cox model (proportional hazards, nonlinearity and influential observations respectively) were also checked. The adjusted R^2 for each model was also calculated. The adjusted R^2 for a model without the PRSs (with just the clinical factors), was then calculated. Using the R function *anova*, an ANOVA test was then carried out to investigate if there was a statistically significant difference between these models.

Comparison of Outcomes Between Individuals With High and Low Polygenic Burden

We used these multivariable linear models to predict eGFR at 1- and 5-year for two transplant recipients: one with high PRSs (in the 90th percentile), and the other with average PRSs (in the 50th percentile), but are otherwise completely identical. We did this for

TABLE 1 | Demographic characteristics of study participants. For further details regarding the recruitment and characteristics of each cohort, see the **Supplementary Materials**.

Variable	Overall	DeKAF	FRCBS	GEN03	KiT-GENIE	QUB	TL	UKIRTC
Number of transplants	6,060	684	888	472	1,463	68	608	1877
Donor age, median (range)	50 (18–90)	44 (18–70)	58 (18–77)	45 (18–71)	56 (18–90)	44 (18–66)	46 (18–72)	47 (18–81)
Female donor, n (%)	2,839 (47)	405 (59)	417 (47)	271 (57)	616 (42)	32 (47)	292 (48)	806 (43)
Donor type								
Living, n (%)	1,470 (24)	684 (100)	0 (0)	472 (100)	265 (18)	0 (0)	49 (8)	0 (0)
Died of stroke, n (%)	2,826 (47)	0 (0)	585 (66)	0 (0)	699 (48)	42 (62)	319 (52)	1,181 (63)
Died of other causes, (n %)	1764 (29)	0 (0)	303 (34)	0 (0)	499 (34)	26 (38)	240 (39)	696 (37)
Recipient age, median (range)	51 (0–84)	51 (0–83)	57 (18–79)	51 (1–81)	55 (18–84)	44 (10–72)	50 (16–74)	47 (18–79)
Female recipient, n (%)	2,148 (35)	231 (34)	275 (31)	177 (38)	497 (34)	30 (44)	246 (40)	692 (37)
First transplant, n (%)	5,337 (88)	603 (88)	888 (100)	418 (89)	1,140 (78)	68 (100)	555 (91)	1,665 (89)
HLA mismatch, median (range)	3 (0–6)	3 (0–6)	3 (0–6)	3 (0–6)	4 (0–6)	NA	NA	2 (0–6)
Unknown	924 (15)	1 (0.1)	0 (0)	13 (3)	0 (100)	68 (100)	608 (100)	234 (12)
Year of transplant, median	2007	2008	2014	2014	2011	2002	2000	2001
Follow up, median (range)	5 (0–25)	2 (0–5)	3 (0–10)	2 (0–3)	6 (0–21)	7 (0–24)	7 (0–17)	8 (0–25)
Graft status, n (%)								
Censored	5,098 (84)	671 (98)	831 (94)	470 (100)	1,138 (78)	46 (68)	509 (84)	1,433 (76)
Rejected	962 (16)	13 (2)	57 (6)	2 (0.4)	325 (22)	22 (32)	99 (16)	444 (24)
eGFR at 1-year, median (range)	52 (0–185)	60 (0–178)	54 (0–135)	62 (16–185)	50 (0–129)	0 (0–0)	45 (0–124)	49 (0–124)
Unknown, n (%)	726 (12)	0 (0)	247 (28)	0 (0)	59 (4)	53 (78)	24 (4)	343 (18)
eGFR at 5-year, median (range)	44 (0–124)	0 (0–0)	38 (0–106)	0 (0–0)	45 (0–122)	0 (0–0)	47 (0–124)	44 (0–121)
Unknown, n (%)	3037 (50)	671 (98)	747 (84)	470 (99.5)	491 (34)	49 (72)	139 (23)	470 (25)

DeKAF, deterioration of kidney allograft function; FRCBS, finnish red cross blood service; GEN03, genomics of kidney transplantation; KiT-GENIE, kidney transplantation - genomic investigation of essential clinical concerns; QUB, Queen's University Belfast; TL, TransplantLines; UKIRTC, united kingdom and ireland renal transplant consortium; eGFR, estimated glomerular filtration rate.

the median transplant recipient, which in our cohort, took place in 2007, with a 51 year old male recipient, on his first transplant, with a 50 year old male donor who died of stroke, with whom he has three HLA mismatches.

RESULTS

Table 1 shows the characteristics for the 6,060 kidney transplants ascertained from seven sites that passed genotyping quality control. The median donor age was 50 years and there were more males (3,221, 53%) than females. 1,470 (24%) of the donors were living, 2,826 (47%) died of stroke, while 1,764 (29%) died of other causes. The median recipient age was 51 years, with more male recipients than female (3,912, 65%). First transplants comprised 88% of the cohort, and HLA mismatch data was available for 85% of the cohort. The median number of HLA mismatches in a donor: recipient pair was 3. One-year graft survival was 97%, 5-year graft survival was 89%, and 10-year graft survival was 76%. Recipient eGFR at 1-year post-transplant was available for 88% of the cohort, with a median of 52 mL/min/1.73 m^2 while eGFR at 5-year post-transplant was only available for 50% of the cohort, with a median of 44 mL/min/1.73 m^2. Power calculations indicated that the smallest sample size required to detect an effect that explains at least 1% of the variation in outcome was 272 individuals (see **Supplementary Materials**).

Univariable Models to Identify Factors Associated With Transplant Outcome

In order to investigate the impact of donor and recipient PRSs on eGFR at 1- and 5-year, we created univariable linear models for each PRS. Similarly, we also created univariable Cox models for each PRS to predict graft failure (see *Materials and Methods*). The association between each of the clinical factors, seven donor PRSs, and seven recipient PRSs and recipient eGFR at 1-year, 5-year, and graft failure are detailed in **Table 2**. We observed a significant univariable association between the following ***donor*** characteristics and recipient eGFR at 1-year: age (Estimate = −0.63; P < 2e-16), male sex (Estimate = 3.4; P = 6.1e-8), stroke cause of death (Estimate = −17; P < 2e-16), other cause of death (Estimate = −8.6; P < 2e-16), and year of transplant (Estimate = 0.45; P < 2e-16). Standard deviation increases in donor hypertension, decreased eGFR, and intracranial aneurysm PRSs correspond to decreases in eGFR at 1-year of 1.6 (P = 6.7e-7), 1.5 (P = 5.4e-6), and 1.0 (P = 0.001) mL/min/1.73 m^2 respectively. We also observed significant associations between ***recipient*** age (Estimate = −0.46; P = 8.2e-12), recipient decreased eGFR PRS (Estimate = −1.5; P = 4.4e-4) and recipient eGFR at 1-year. None of the other PRSs were significantly associated with eGFR at 1-year. The factors with the highest R^2 were donor age, recipient age, and donor type (0.16, 0.08, 0.10 respectively).

Univariable ***donor*** factors associated with eGFR at 5-year post-transplant included: age (Estimate = −0.64; P < 2e-16), male sex (Estimate = 3.3; P = 0.001), stroke cause of death (Estimate = −13; P = 1.7e-10), other cause of death (Estimate = −4.2; P = 0.04), and HLA mismatch (Estimate = −1.1; P = 0.002). Standard deviation increases in donor hypertension PRS, donor decreased eGFR PRS, and recipient decreased eGFR PRS correspond to decreases in eGFR at 5-year of 1.2 (P = 0.02), 1.8 (P = 4.4e-4), and 1.1 (P = 0.02) mL/min/1.73 m^2 respectively. ***Recipient*** factors included whether it was the recipient's first transplant (HR =

TABLE 2 | Univariable linear models for recipient eGFR at 1- and 5-year post-transplant, and Cox model for death-censored graft failure.

	eGFR at 1-year			eGFR at 5-year			Graft failure	
	Estimate (SE)	*P* value	R^2	Estimate (SE)	*P* value	R^2	HR (95% CI)	*P* value
Clinical factors								
Donor age	***−0.63 (0.02)***	***<2e-16***	***0.16***	***−0.64 (0.03)***	***<2e-16***	***0.12***	***1.02 (1.02–1.03)***	***<2e-16***
Male donor sex	***3.4 (0.62)***	***6.1e-8***	***0.005***	***3.3 (0.98)***	***0.001***	***0.004***	0.98 (0.86–1.1)	0.72
Male recipient sex	1.2 (0.65)	0.06	0.001	***2.5 (1.01)***	***0.01***	***0.002***	1.1 (0.93–1.2)	0.34
Recipient age	***−0.46 (0.02)***	***<2e-16***	***0.08***	***−0.24 (0.04)***	***8.2e-12***	***0.02***	1.00 (0.99–1.01)	0.29
HLA mismatch	−0.16 (0.20)	0.43	0	***−0.77 (0.33)***	***0.02***	***0.001***	***1.1 (1.02–1.11)***	*0.006*
First transplant	−0.2 (0.94)	0.83	0	***3.0 (1.38)***	***0.03***	***0.002***	***0.66 (0.56–0.78)***	***7.9e-7***
Year of transplant	***0.45 (0.047)***	***<2e-16***	***0.02***	−0.15 (0.08)	0.06	0.001	***0.98 (0.98–0.99)***	***0.003***
Donor type			***0.1***			***0.03***		
Living	-	-		-	-		-	-
Stroke cause of death	***−17 (0.72)***	***<2e-16***		***−13 (1.95)***	***1.7e-10***		***3.5 (2.5–4.7)***	***1.6e-14***
Other cause of death	***−8.6 (0.79)***	***<2e-16***		***−4.2 (2.01)***	***0.04***		***2.7 (2.0–3.8)***	***1.4e-9***
Donor PRSs								
Donor hypertension PRS	***−1.6 (0.32)***	***6.7e-7***	***0.005***	***−1.2 (0.49)***	***0.02***	***0.002***	***1.07 (1.00–1.14)***	***0.049***
Donor decreased eGFR PRS	***−1.5 (0.32)***	***5.4e-6***	***0.004***	***−1.8 (0.5)***	***4.4e-4***	***0.004***	1.05 (0.99–1.13)	0.11
Donor albuminuria PRS	0.52 (0.32)	0.1	0.001	−0.34 (0.5)	0.5	0	1.00 (0.94–1.06)	0.92
Donor rapid eGFR decline PRS	−0.37 (0.38)	0.34	0	−0.15 (0.51)	0.77	0	0.99 (0.92–1.06)	0.73
Donor intracranial aneurysm PRS	***−1.03 (0.31)***	***0.001***	***0.002***	−0.6 (0.48)	0.21	0.001	1.04 (0.98–1.11)	0.18
Donor stroke PRS	−0.12 (0.31)	0.7	0	0.58 (0.5)	0.23	0	0.95 (0.89–1.01)	0.09
Donor decreased TKV PRS	0.25 (0.31)	0.43	0	0.08 (0.5)	0.87	0	0.98 (0.92–1.05)	0.58
Recipient PRSs								
Recipient hypertension PRS	0.53 (0.30)	0.08	0	0.63 (0.49)	0.2	0.001	0.99 (0.92–1.1)	0.64
Recipient decreased eGFR PRS	***−1.5 (0.31)***	***1.0e-6***	***0.004***	***−1.1 (0.47)***	***0.02***	***0.002***	1.05 (0.99–1.1)	0.09
Recipient albuminuria PRS	0.56 (0.32)	0.08	0.001	0.14 (0.48)	0.77	0	1.02 (0.96–1.1)	0.5
Recipient rapid eGFR decline PRS	0.34 (0.36)	0.35	0	0.65 (0.48)	0.17	0.001	0.97 (0.91–1.0)	0.4
Recipient intracranial aneurysm PRS	0.12 (0.32)	0.7	0	−0.43 (0.5)	0.38	0	1.00 (0.94–1.1)	0.97
Recipient stroke PRS	−0.29 (0.32)	0.36	0	−0.83 (0.49)	0.09	0.001	1.10 (0.99–1.1)	0.12
Recipient decreased TKV PRS	0.1 (0.31)	0.74	0	0.1 (0.49)	0.83	0	0.94 (0.88–1.0)	0.06

eGFR, estimated glomerular filtration rate; HR, hazard ratio; TKV, total kidney volume; SE, standard error.
Statistically significant (P < 0.05) predictors are bolded and italicised.

0.66; $P = 7.9e\text{-}7$), age (Estimate = −0.24; $P = 8.2e\text{-}12$), and male sex (Estimate = 2.5; $P = 0.01$).

Univariable ***donor*** factors associated with graft failure included age (HR = 1.02; $P < 2e\text{-}16$), HLA mismatch (HR = 1.1; $P = 8.1e\text{-}5$), stroke cause of death (HR = 3.5; $P = 1.6e\text{-}14$), other cause of death (HR = 2.7; $P = 1.4e\text{-}9$), year of transplant (HR = 0.98; $P = 0.003$), and hypertension PRS (HR = 1.07; $P = 0.049$). No recipient factors were associated with graft failure. A standard deviation increase in donor hypertension PRS corresponds to a 7% greater risk of graft failure.

Multivariable Models to Identify Factors Associated With Transplant Outcome

For each of the three outcomes of interest (eGFR at 1-year, 5-year and graft failure), multivariable models were created using only the statistically significant factors from the univariable analysis (**Table 3**).

In a multivariable model the following ***donor*** factors were independently associated with eGFR at 1-year: age, sex, year of transplant, donor type, hypertension PRS, decreased eGFR PRS, and intracranial aneurysm PRS. ***Recipient*** factors associated with eGFR at 1-year in the multivariable model included age, and decreased eGFR PRS. This model had an adjusted R^2 of 0.26, compared to the adjusted R^2 of a model with just the clinical factors of 0.25. There was a significant difference between the two models, according to the ANOVA test (F = 14.4, $P = 9.9e\text{-}12$), indicating that the addition of PRSs increases the predictive power of a model with just clinical factors.

In the multivariable model for eGFR at 5-year, ***donor*** factors associated included age, donor type, and decreased eGFR PRS. ***Recipient*** factors included sex, age, and decreased eGFR PRS. The adjusted R^2 of the model with the PRSs was higher (0.14) than that of the model with just the clinical predictors (0.13). There was a significant difference between the two models, according to the ANOVA test ($P = 0.003$), again indicating that the addition of PRSs to a model of clinical factors significantly increases predictive ability.

The following factors were associated with graft failure in the multivariable model: donor age, HLA mismatch, whether it was the recipient's first transplant, year of transplant, and donor cause of death. None of the PRSs were significantly associated with graft failure.

Comparison of Outcomes Between Individuals With High and Low Polygenic Burden

To demonstrate the utility of these models, we used the models created in the previous section to predict recipient eGFR at 1- and

TABLE 3 | Multivariable models for recipient eGFR at 1- and 5-year post-transplant, and graft failure, keeping statistically significant factors from univariate models. Effect of polygenic risk scores is highlighted in grey.

eGFR at 1-year (adjusted R^2 = 0.26)		
	Estimate (95% CI)	***P* Value**
Intercept	−1,267 (−1,460, −1,098)	<2e-16
Donor age	−0.54 (−0.59, −0.50)	<2e-16
Male donor sex	2.7 (1.5, 3.7)	21.6e-6
Male recipient sex	1.68 (0.52, 2.72)	0.004
Recipient age	−0.25 (−0.29, −0.21)	<2e-16
Year of transplant	0.68 (0.59, 0.78)	<2e-16
Donor type		
Living	-	-
Stroke cause of death	−7.6 (−9.1, −6.2)	<2e-16
Other cause of death	−6.3 (−7.8, −4.7)	<2e-16
Donor albuminuria PRS	0.24 (−0.32, 0.80)	0.40
Donor hypertension PRS	−1.3 (−1.7, −0.6)	9.2e-6
Donor decreased eGFR PRS	−1.2 (−1.8, −0.7)	4.33e-5
Donor intracranial aneurysm PRS	−0.66 (−1.2, −0.14)	0.01
Recipient hypertension PRS	0.47 (−0.07, 1.02)	0.09
Recipient albuminuria PRS	−0.05 (−0.62, 0.50)	0.85
Recipient decreased eGFR PRS	−1.0 (−1.5, −0.49)	0.001
eGFR at 5-year (Adjusted R^2 = 0.14)		
	Estimate (95% CI)	***P* value**
Intercept	−621 (−952, −290)	0.0002
Donor age	−0.68 (−0.7, −0.6)	<2e-16
Male donor sex	1.01 (−0.8, 2.9)	0.28
Recipient age	0.04 (−0.0, 0.1)	0.26
Male recipient sex	2.8 (0.9, 4.6)	0.004
Year of transplant	0.35 (0.2, 0.5)	3.7e-5
HLA mismatch	−0.61 (−1.3, 0.1)	0.09
First transplant	3.24 (0.7, 5.8)	0.01
Donor type		
Living	-	-
Stroke cause of death	−8.0 (−11.8, −2.8)	3.0e-5
Other cause of death	−6.7 (−10.5, −2.8)	0.0007
Donor hypertension PRS	−0.7 (−1.9, −0.1)	0.16
Donor decreased eGFR PRS	−1.6 (−2.6, −0.8)	0.0003
Recipient decreased eGFR PRS	−0.9 (−1.8, −0.0)	0.04
Recipient stroke PRS	−0.95 (−1.9, −0.1)	0.04
Graft failure(R^2 = 0.23)		
	HR (95% CI)	***P* value**
Donor age	1.02 (1.02, 1.03)	1.8e-13
HLA mismatch	1.13 (1.07, 1.19)	3.3e-6
First transplant	0.61 (0.52, 0.72)	6.1e-9
Year of transplant	0.97 (0.96, 0.98)	2.3e-8
Donor type		
Living	-	-
Stroke cause of death	2.6 (1.9, 3.6)	8.7e-9
Other cause of death	2.5 (1.8, 3.5)	5.6e-8
Donor Hypertension PRS	1.06 (0.99, 1.1)	0.09
Donor decreased eGFR PRS	1.04 (0.98, 1.1)	0.18
Donor intracranial aneurysm PRS	1.03 (0.96, 1.1)	0.39
Donor stroke PRS	0.95 (0.90, 1.01)	0.11
Recipient decreased eGFR PRS	1.05 (0.98, 1.11)	0.15
Recipient stroke PRS	1.06 (0.98, 1.12)	0.08
Recipient decreased TKV PRS	0.96 (0.90, 1.02)	0.17

eGFR, estimated glomerular filtration rate; CI, confidence interval; HR, hazard ratio.

5-year post-transplant in the median transplant recipient (see *Materials and Methods*), one with high PRSs (in the 90th percentile) and the other with average PRSs (in the 50th percentile). Transplants where both the donor and recipient had high PRSs were predicted to have an eGFR at 1-year of 45.6 mL/min/1.73 m^2, whereas transplants where both the donor and recipient had average PRSs were predicted to have an eGFR at 1-year of 50.6 mL/min/1.73 m^2. Transplants where both the donor and recipient had high PRSs were predicted to have an eGFR at 5-year of 40.0 mL/min/1.73 m^2, whereas transplants where both the donor and recipient had average PRSs were predicted to have an eGFR at 5-year of 42.8 mL/min/1.73 m^2.

DISCUSSION

We have explored the influence of donor and recipient PRSs for traits related to kidney function on post-transplant outcome. We have confirmed the previously reported clinical factors associated with graft function, and have additionally demonstrated, across seven cohorts comprising 6,060 donor-recipient transplant pairs, that higher donor and recipient decreased eGFR PRS was associated with lower eGFR at 1-year post-transplant, with similar effects observed at 5-year post-transplant. We further demonstrated that donor hypertension and intracranial aneurysm PRSs are also associated with reduced eGFR at 1-year post-transplant. Transplants where both the donor and recipient had high polygenic burden were predicted to have recipient eGFR at 1-year post-transplant that was over 5 mL/min/1.73 m^2 lower than those with average polygenic burden.

To our knowledge, this is the first study to combine donor and recipient PRS into a single predictive model in the transplant setting. Previous studies have investigated the effect of either donor PRS [11] or recipient PRS [13, 15], but none have combined the two in a single predictive model. While the impact of PRS on transplant outcome is relatively modest (accounting for 1% of the variation in recipient post-transplant eGFR), these results align with a growing body of literature demonstrating the utility of PRS in predicting kidney disease [27] and kidney transplant outcome [11]. They are also consistent with recent results demonstrating the association of combined donor and recipient genetic factors with transplant outcome [7, 9, 28]. As GWAS continue to grow in size and predictive power, PRS could potentially explain a more substantial proportion of graft function. It is likely that a GWAS focused on kidney failure would result in significantly better PRS for kidney failure rather than just a GWAS for eGFR, as it is quite possible that the variants involved in low eGFR may be quite different from those involved in kidney failure.

This study has replicated the well described significant impact of clinical factors on long-term graft function and survival including donor age, donor cause of death, HLA mismatch, era of transplantation, and donor type. These clinical factors explain approximately 25% of variation in eGFR at 1-year and

significantly outweighs the impact of PRSs on transplant function.

This study has several limitations. The participants included were of predominantly European ancestry. The performance of PRSs in non-European ancestry populations is generally lower, though much work is currently being done to address this issue [28]. Data on HLA mismatch and/or eGFR at 5-year was unavailable on 15% and 50% of participants respectively. We were unable to detect a significant effect of PRSs on graft failure, which may be on account of the effect potentially being stronger in immediate graft function rather than long-term survival. Additionally, 16% of these transplants date from before the year 2000. This means that we have a long follow-up time for many of our transplants, but treatment regimens have improved significantly since some of the earlier transplants in the 1980s and 1990s. We accounted for this by controlling for the year of transplant in our analysis. We lack data on several factors which may influence graft function including history of hypertension, history of diabetes, hepatitis C virus status, terminal serum creatinine, and donor height and weight and thus were unable to calculate the kidney donor profile index (KDPI). However, this is not likely to significantly impact our results, as it has been previously shown that while KDPI was predictive of post-transplant eGFR, it does not significantly add to donor age as a predictor of graft failure [29].

Additionally, the focus in this study is on common variation. Large scale donor-recipient exome studies are currently underway which will address the question of the impact of rare variation on graft function. It is possible, and even likely [30], that incorporating such information on rare variation may yield results of larger effect.

In summary, this study demonstrates that the combined effect of donor and recipient PRSs for decreased eGFR has an impact on post-transplant eGFR. Donors and recipients who both have high PRSs result in an average recipient eGFR at 1-year post-transplant that is over 5 mL/min/1.73 m^2 lower than the average from transplants where both donor and recipient have average polygenic burden. At this point in their development, these PRSs have minimal added benefit over existing clinical risk factors, but we anticipate that as PRSs become increasingly powerful, that they will become an important tool in clinical decision-making. These results may have potential implications for transplant allocation decisions. Any incorporation of PRSs into such decisions would likely first take place in living donor transplants, where potential donors could be genotyped and analysed without the time pressures that exist around deceased donor transplantation. Before this can take place, further studies are required to validate these results and construct a transplant risk prediction tool based on clinical factors and PRSs.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Privacy concerns prevent these datasets being made publicly available. Requests to access these datasets should be directed to Graham Lord, graham.lord@manchester.ac.uk.

ETHICS STATEMENT

The studies involving humans were approved by Hammersmith and Queen Charlotte's and Chelsea ResearchEthics Committee REC No 08/H0707/1. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

The authors confirm contribution to the paper as follows: study conception and design: KC, EG, PC, GC, and ML; data collection: KC, EG, VM, KB, EE, CO'S, CH, AmM, AlM, PM, MB, WG, PJ, AI, BK, GL, SM, IH, KH, JP, and SL; analysis and interpretation of results: KC, EG, GC, PC, SM, and ML; draft manuscript preparation: KC, EG, GC, and PC. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

IH reports receiving research funding from MSD and Hansa Biopharma, and has ongoing consultancy agreements with Novartis, Hansa Biopharma, and Takeda. ML has received advisory and consulting fees from Bayer, Sanofi, Reata, GlaxoSmithKline, and Otsuka. GC and PC report receiving research funding relating to kidney disease genetics from Biomarin.

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.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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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.2025.14171/full#supplementary-material

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ORIGINAL RESEARCH
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Anti-TNFα as an Adjunctive Therapy in Pancreas and Kidney Transplantation

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The rate of early pancreas allograft failure remains high due to thrombosis but also to severity of rejection episodes. We investigated if adjunct anti-TNFα therapy was safe and could improve outcomes after pancreas transplantation. We investigated all pancreas transplants performed in our institution between 2010 and 2022. Etanercept, an anti TNFα therapy, was added to our standard immunosuppressive regimen since 2017 after approval from our institutional human ethics committee. Pancreas survival, rejection episodes, as well as infectious complications were analyzed. A total of 236 pancreas transplants were included, among whom 87 received Etanercept for induction. In multivariable analysis, after adjustment on confounding variables, pancreas survival did not differ between groups (HR = 0.92, CI 95% = 0.48; 1.73, p = 0.79). However, patients receiving Etanercept presented a significantly lower occurrence of pancreas rejection in multivariate analysis (HR = 0.36, CI 95% = 0.14; 0.95, p = 0.04). Patients receiving Etanercept did not experienced a higher risk of bacterial, fungal, CMV nor BK virus infections compared to the non-treated group. The use of anti-TNFα after pancreas transplantation was safe and did not increase infectious complications. Despite a similar rate of thrombosis, anti-TNFα significantly reduced pancreatic rejection, thus supporting its use among pancreas transplant recipients.

Keywords: anti-TNFα, pancreas transplantation, allograft thrombosis, allograft rejection, ischemia/reperfusion, inflammation

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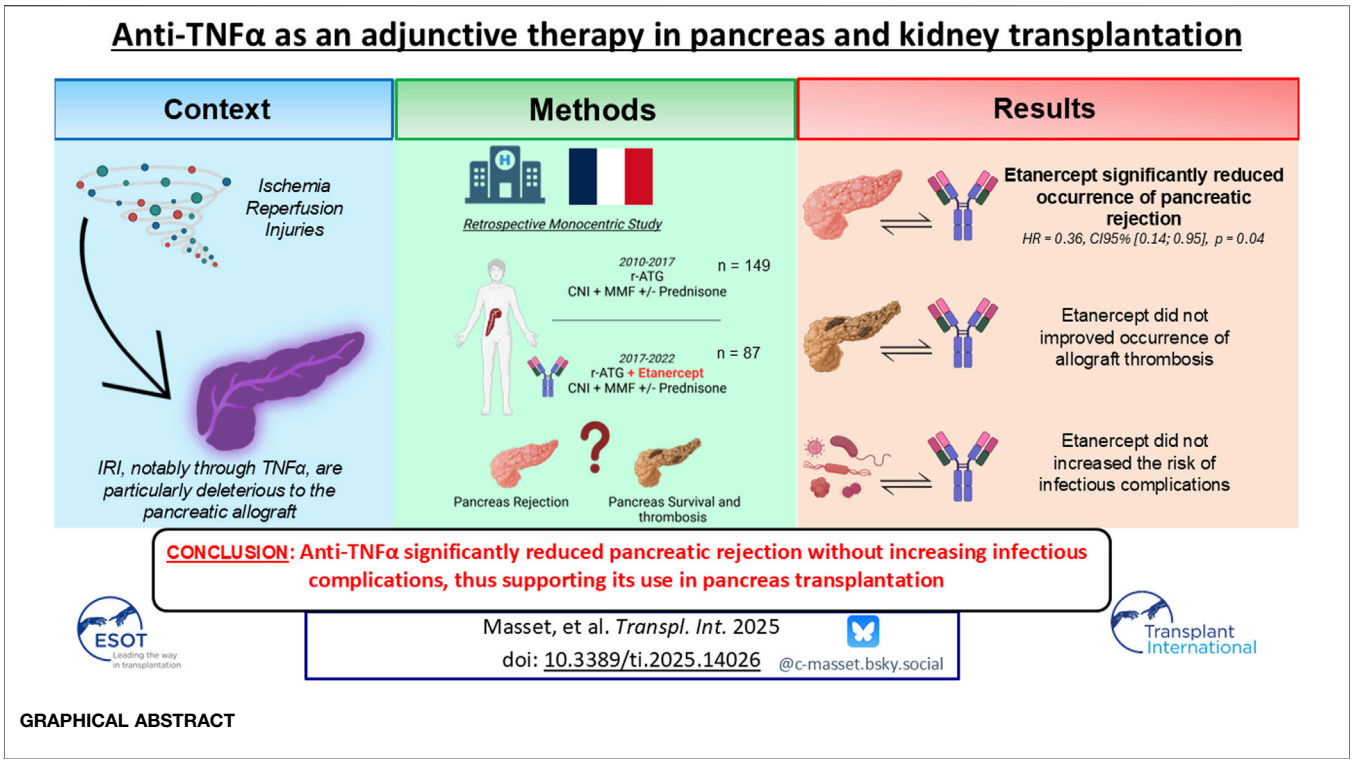
INTRODUCTION

Despite improvement in recent decades, pancreas allografts still face early failure, with approximately 7%–10% experiencing complete thrombosis, leading to significant morbidity and mortality [1–3]. While traditionally categorized as a "technical failure," its association with prolonged cold ischemia time, along with established risk factors such as donor age and BMI, suggests a connection with an immune response related to ischemia/reperfusion [4–6]. Our group recently described the mechanisms of sterile inflammation further conducing to pancreatic thrombosis and/or rejection [7]. This includes activation of endothelial cells, innate immune cells (neutrophils, monocytes), and

Abbreviations: SPK, Simultaneous Pancreas-Kidney; PAK, Pancreas After Kidney; PTA, Pancreas Transplantation Alone; DSA, Donor Specific Antibodies; CIT, Cold Ischemia Time; eGFR, estimated Glomerular Filtration Rate; SOC, Standard of Care; HR, Hazard Ratio.



GRAPHICAL ABSTRACT

platelets [8, 9]. Inflammatory cytokines play a pivotal role in driving the pathophysiological pathways leading to immunothrombosis. Specifically, TNFα acts as a potent activator of endothelial cells and neutrophils, promoting the expression of adhesion molecules, secretion of cytotoxic molecules, and activation of coagulation [10, 11]. In addition, TNFα is well known to promote infiltration of immune cells into allografts and thus promote further rejection [12]. In particular, pancreas allografts are recognized as being very sensitive to alloimmune responses with a high rate of pancreatic loss following a rejection episode [13–15].

Etanercept is a recombinant fusion protein with anti-TNFα activity. It has been used widely as an anti-inflammatory drug for numerous arthritic conditions and used since several years following islet transplantation due to the *in-vitro* toxicity of TNFα on β-cells [16]. Initial reports demonstrated promising results, including high rates of insulin independence at 1 year [17]. Consistent with these findings, Etanercept is currently extensively used among islet transplant centers, as it may facilitate islet engraftment by mitigating the innate inflammatory response observed during ischemia/reperfusion but also reduce occurrence of rejection [18].

Drawing from the experience of islet transplant recipients, we opted several years ago to modify the immunosuppressive strategy in pancreas transplant recipients by incorporating Etanercept during the early post-operative period. Indeed, blocking TNFα in the early post-transplantation period appears to be a very promising strategy, as it helps reduce the cytokine storm associated with ischemia-reperfusion injury and the subsequent risk of allograft rejection. This approach is particularly relevant in the context of pancreatic transplantation, given the highly inflammatory nature of the digestive segment transplanted alongside the pancreas to ensure exocrine drainage. We thus hypothesized that an anti-TNFα therapy may be beneficial by reducing activation of immune system following ischemia/reperfusion, and thus reduce occurrence of pancreas rejection and immunological thrombosis.

Here, we present an evaluation of the outcomes of anti-TNFα therapy as an adjunctive treatment to prevent rejection in a large single-center cohort of pancreas transplant recipients.

MATERIALS AND METHODS

Studied Population

All patients who underwent pancreas transplantation (simultaneous pancreas-kidney (SPK), pancreas after kidney (PAK), and pancreas transplant alone (PTA) between 1st January 2010, and 30th April 2022, at our institution were included in the study. Data were extracted from the French prospective DIVAT cohort of transplanted patients.[1]

Available Data

Complete available data are presented in **Table 1**. Donor and recipient characteristics, as well as peri-transplant parameters, were prospectively collected. Pancreas failure was defined by

[1]http://www.divat.fr

TABLE 1 | Description of the studied cohort depending on the administration of Anti-TNFα in the early post-operative time (p-values are obtained using Chi-square test or Fisher exact test for categorical variables and using Student's t-test or Mann-Whitney U for continuous variables).

	Whole cohort (n = 236)			Anti-TNFα (n = 87)			Standard of care (n = 149)			p-value
	NA	N	%	NA	n	%	NA	N	%	
Type of graft	0			0			0			
SPK		182	77.1		72	82.7		110	73.8	0.1481
PAK		22	9.3		4	4.6		18	12.1	0.0651
PTA		32	13.6		11	12.6		21	14.1	0.8451
Male recipient	0	133	56.3	0	46	52.9	0	87	58.4	0.4181
Retransplantation	0	29	12.3	0	8	9.2	0	21	14.1	0.3096
Pancreas preservation fluid	13			3			10			
Celsior		65	29.2		8	9.5		57	41.0	<0.0001
IGL		89	39.9		53	63.1		36	25.9	<0.0001
Other		69	30.9		23	27.4		46	33.1	0.4560
Male donor	0	157	66.5	0	56	64.4	0	101	67.8	0.2350
Vascular cause of donor death	0	92	38.9	0	34	39.1	0	58	38.9	>0.9999
Donor hypertension history	0	16	7.2	9	5	6.4	5	11	7.3	0.7572
History of donor cardiac arrest sampling	0	61	25.1	1	25	29.1	1	36	24.3	0.4431
Use of vasopressive drug	0	203	89.4	8	74	93.7	1	129	87.2	0.1741
Depleting induction	0	218	92.4	0	87	100	0	131	87.9	0.0002
Initial maintenance therapy	0			0						
Cyclosporine		2	0.8		0	0	0	2	1.3	0.5325
Tacrolimus		234	99.1		87	100	0	147	98.6	0.5325
Antiproliferative drugs		235	99.6		87	100	0	148	99.3	>0.9999
mTOR inhibitors		0	0		0	0	0	0	0	>0.9999
Oral steroids		231	97.9		87	100	0	144	96.6	0.2963
Pre-formed DSA	0	25	10.6	0	10	11.5	0	15	10.4	0.6587
	NA	Mean	SD	NA	Mean	SD	NA	Mean	SD	
Recipient age (years)	0	40.6	7.9	0	39.6	7.3	0	41.3	8.3	0.1104
Recipient BMI (kg/m^2)	0	23.7	3.7	0	23.9	3.8	0	23.6	3.6	0.3313
Duration of diabetes (years)	8	26.4	8.7	8	24.6	8.8	0	27.4	8.5	0.0276
Pancreas CIT (min)	0	608	140	0	563	136	1	635	136	<0.0001
Kidney CIT (min)	0	753	155	0	688	133	0	794	154	<0.0001
Duration in ICU at post-op (days)	6	1.7	1.7	6	1.4	0.9	0	1.9	1.9	0.0194
Donor age (years)	0	32.9	10.9	0	33.1	11.2	0	32.7	10.8	0.7978
Donor BMI (kg/m^2)	0	23.1	3.0	0	22.8	2.9	0	23.2	3.1	0.4103
Donor creatininemia (μmol/L)	0	77	33	0	80	40	0	76	28	0.8970

BMI, body mass index; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; NA, not available (missing); PAK, pancreas after kidney; PTA, pancreas transplant alone; SD, standard deviation; SPK, simultaneous pancreas-kidney; CIT, Cold Ischemia Time.

either the persistence of insulin requirement, allograft removal, or retransplantation. Kidney failure was defined by either a return to dialysis or retransplantation. Rejection episodes were diagnosed based on pancreatic biopsy findings or if no biopsy was available, pancreas rejection was considered in cases of dysfunction (hyperglycemia + increase in lipase levels) with a biopsy-proven diagnosis of kidney rejection [19]. This strategy aimed to minimize unnecessary invasive biopsies, especially for the pancreatic allograft. Rejection episodes were categorized according to the Banff classification. Cellular rejection was usually treated with steroid pulses or r-ATG (Thymoglobulin), while humoral rejection was managed with plasma exchanges, intravenous immunoglobulins, and sometimes associated with CD20 depleting therapy. Donor specific antibodies (DSA), assessed pre-transplant, in case of rejection, and at 1 year post-transplantation were determined by Luminex® assay and considered positive when mean fluorescence index values were superior to 1000. Infectious complications, including CMV viremia (either asymptomatic or associated with CMV disease), BK virus (BKV) viremia (either asymptomatic or associated with BKV nephropathy), fungal infections, and severe bacterial infections, were recorded. Prospective follow-up of pancreatic and kidney allograft functions included fasting glycemia, fasting C-peptide, HbA1c levels, estimated glomerular filtration rate (eGFR, using the CKD-EPI formula), collected every 3 months during the first year and then annually. Follow-up and data collection ceased upon transplant failure or death.

Immunosuppressive Protocol

The management of pancreas transplantation was consistent across all categories (SPK, PAK, and PTA) and remained globally unchanged during the study period, except for the addition of Etanercept. The surgical technique remains globally unchanged during the study period, with digestive anastomosis performed in all cases for exocrine diversion. Induction therapy consisted mostly in rabbit antithymocyte globulin (rATG) for five alternate days, or either basiliximab in some rare cases, along with two pulses of 500 mg methylprednisolone. From April 2017, pancreas transplant recipients received an additional course of Etanercept at a similar dosage than for islet recipients: 50 mg on day 0

TABLE 2 | Univariate and multivariate cause-specific Cox model associated with the risk of pancreas graft failure at 3 years post-transplantation. The following variables were forced into the multivariate model due to their known association with pancreas failure: pancreas cold ischemia time, donor age, donor BMI, donor vascular cause of death, donor history of hypertension (47 events were observed during follow-up, 1 observation was excluded because of missing data).

	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Anti-TNFα	0.80	0.43–1.48	0.480	0.92	0.49–1.73	0.7880
Pancreas Cold Ischemia Time	1.00	1.00–1.00	0.016	1.002	1.001–1.004	0.0335
Donor's age	1.00	0.98–1.03	0.771	1.01	0.98–1.04	0.4479
Donor's BMI	1.00	0.91–1.10	0.991	1.00	0.91–1.11	0.8978
Donor's vascular cause of death	0.88	0.48–1.59	0.663	0.54	0.25–1.17	0.1190
Donor's history of hypertension	1.27	0.45–3.53	0.652	1.43	0.47–4.35	0.5251
Donor's gender (Female)	1.81	1.02–3.21	0.043	1.90	1.02–3.53	0.0424
Type of transplant: SPK	0.56	0.31–1.02	0.058			
T cell depleting induction	1.91	0.46–7.88	0.370			
Recipient's age	1.00	0.97–1.04	0.870			
Recipient's gender (Female)	1.64	0.92–2.91	0.092			
Recipient's BMI	1.06	0.98–1.14	0.128			
Preemptive SPK	1.12	0.77–1.63	0.541			
Retransplantation	1.59	0.74–3.39	0.235			
Duration of diabetes	1.01	0.97–1.04	0.742			
Pretransplant C peptide	0.95	0.73–1.23	0.678			
Pretransplant HbA1c	0.98	0.80–1.19	0.822			
Donor's cardiac arrest	0.63	0.31–1.31	0.218			
Donor's eGFR	1.01	1.00–1.02	0.217			
Use of vasopressive drugs	0.88	0.35–2.23	0.782			
Number of HLA mismatches	1.16	0.86–1.57	0.325			
Use of Cyclosporine (Ref: Tacro)	2.31	0.72–7.42	0.162			
Use of non CNI treatment	0.56	0.08–4.06	0.566			
Anti HLA class I at baseline	1.34	0.70–2.56	0.375			
Anti HLA class II at baseline	0.76	0.35–1.64	0.479			
DSA at baseline	1.17	0.49–2.78	0.718			

(intravenous), followed by 25 mg (subcutaneous) on days 3, 7, and 10. All patients underwent screening for latent tuberculosis and hepatitis viruses before Etanercept administration. Maintenance immunosuppressive therapy included a calcineurin inhibitor (mainly tacrolimus) and mycophenolate mofetil or mycophenolic acid, with oral prednisone tapered and withdrawn from postoperative day 7. Our anticoagulation protocol involved per-operative administration of intravenous aspirin (250 mg) and heparin (25 UI/kg) at the time of clamping, followed by preventive anticoagulation using low molecular weight heparin within the first days post-surgery, typically for 10 days. In the absence of allograft thrombosis, detected on purpose or by systematic CT-scan on day 10, preventive heparin was replaced by long-term administration of antiplatelet therapy. Finally, our strategy for treating pancreatic rejection episodes remained largely consistent throughout the study period (i.e., steroid pulses for cellular rejection, with rATG used in cases of grade II or grade III cellular rejection or steroid resistance, and plasma exchange, IV Ig and Rituximab for treatment of humoral rejection).

Statistical Analyses

The characteristics at transplantation were described using frequency and proportion for categorical variables and mean and standard deviation for continuous variables. To assess the impact of anti-TNFα treatment on a specific phenotype over time, survival curves were generated using the Kaplan-Meier estimator. Statistical comparisons were conducted using the log-rank test. For univariate analysis, the Student's t-test or Mann-Whitney test was employed, while multivariate analysis used the Cox model. The anti-TNFα variable was consistently included in the statistical models to evaluate its effect on the different studied outcomes. Initial variable selection was performed retaining only those with a p-value of less than 0.2 according to the Wald test for inclusion in the final Cox model [20]. In addition, five variables were forced selectively into the Cox model for pancreas survival due to their known association with complete thrombosis (pancreas cold ischemia time, and donor-related variables: age, BMI, vascular cause of death, and history of hypertension). Similarly, induction therapy (r-ATG or Basiliximab) was forced into the Cox model for pancreatic rejection. Subsequently, a stepwise forward selection process was conducted, whereby variables were added to the model if their inclusion improved the Bayesian information criterion. The final model comprised the forced variables along with any additional selected variables. Of note, patients with missing data on the variables of interest were excluded from the final analysis. The hazard proportionality assumption was tested from the Schoenfeld residuals [21]. The absence of multicollinearity of the model was verified using the Variance Inflation Factor. To visualize the results, adjusted survival curves were generated to observe the impact of anti-TNFα use over time while holding other variables constant. While one-year endpoints were assessed to accurately determine the impact of anti-TNFα, we also conducted a three-year analysis to gain insights into its long-term effects. Even if some confounding factors may arise

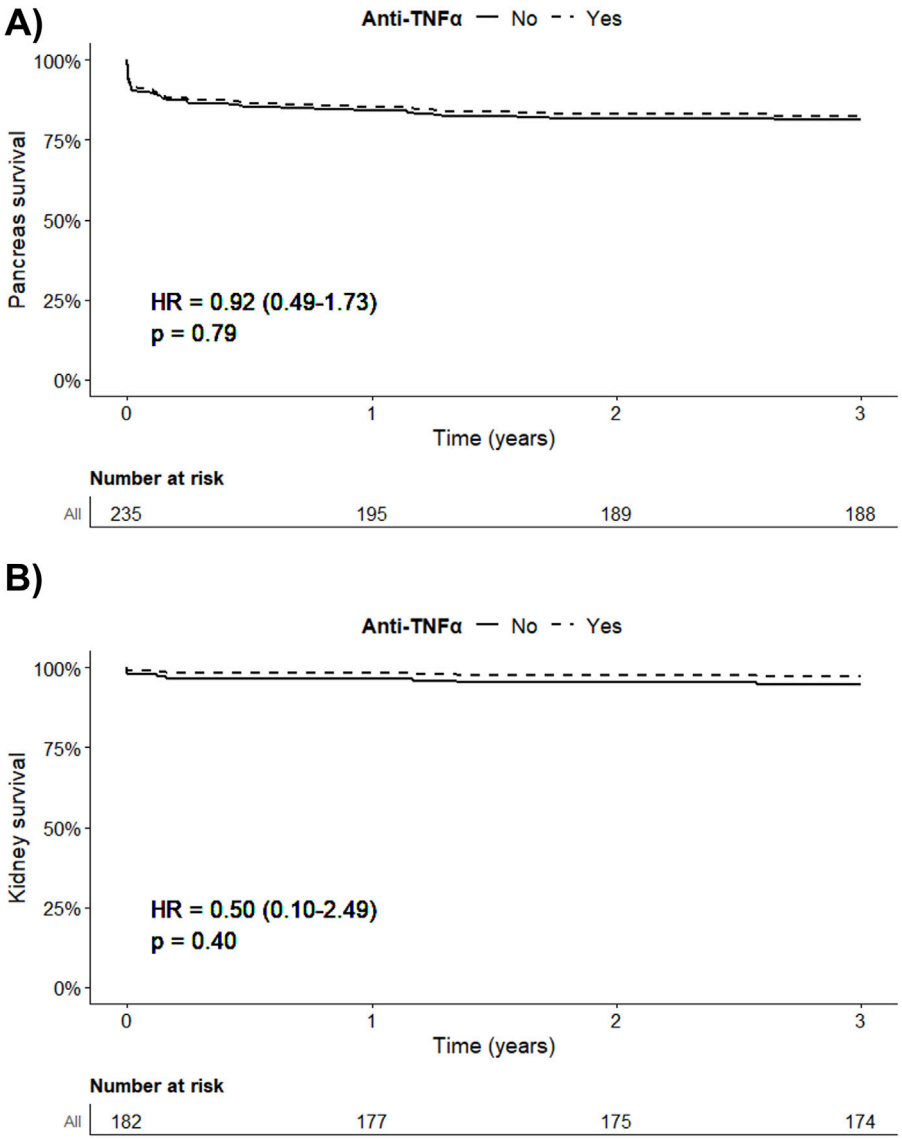


FIGURE 1 | (A) Confounder-adjusted death-censored pancreas allograft survival according to the administration of anti-TNFα. **(B)** Confounder-adjusted death-censored kidney allograft survival according to the administration of anti-TNFα among the SPK recipients.

TABLE 3 | Description of pancreatic rejection episodes occurring in the studied period and their long-term evolution, depending on the administration or not of anti-TNFα.

	Anti-TNFα (n = 5)			No anti-TNFα (n = 26)		
	NA	**N**	**%**	**NA**	**n**	**%**
TCMR	0	1	20	0	10	38.5
Allograft loss post-TCMR	0	0	0	0	5	50
ABMR	0	2	40	0	7	27
Allograft loss post-ABMR	0	1	50	0	4	57
Mixed rejection	0	2	40	0	9	34.5
Allograft loss post Mixed rejection	0	2	100	0	5	55
All pancreatic loss post-rejection	0	3	60	0	14	54

TABLE 4 | Univariate and multivariate cause-specific Cox model associated with the risk of pancreas graft rejection in the first year post-transplantation. The type of induction therapy variable was forced into the multivariate model due to its known association with pancreas rejection (27 events were observed during follow-up, 0 observations were excluded because of missing data).

	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Anti-TNFα	0.20	0.06–0.66	0.008	0.23	0.07–0.75	0.0161
Type of transplant: SPK	0.24	0.11–0.52	0.001	0.29	0.13–0.62	0.0015
T cell depleting induction	1.02	0.24–4.29	0.983	0.96	0.22–4.21	0.9569
Donor's gender (Female)	2.28	1.07–4.86	0.032	2.31	1.08–4.95	0.0305
Recipient's gender (Female)	1.20	0.57–2.56	0.631			
Recipient's age	1.00	0.96–1.05	0.930			
Recipient's BMI	1.05	0.95–1.15	0.335			
Preemptive SPK	0.54	0.29–0.99	0.047			
Pancreas Cold Ischemia Time	1.00	1.00–1.01	0.030			
Retransplantation	2.19	0.88–5.42	0.091			
Duration of diabetes	1.00	0.96–1.05	0.846			
Pretransplant C peptide	0.58	0.24–1.39	0.222			
Pretransplant HbA1c	1.28	1.06–1.55	0.010			
Donor's age	1.04	1.00–1.07	0.043			
Donor's BMI	1.12	0.99–1.27	0.077			
Donor's vascular cause of death	1.29	0.60–2.75	0.516			
Donor's history of hypertension	0.99	0.23–4.18	0.989			
Donor's cardiac arrest	0.22	0.05–0.91	0.037			
Donor's eGFR	1.00	0.98–1.01	0.551			
Use of vasopressive drugs	1.46	0.35–6.19	0.606			
Number of HLA mismatches	1.20	0.80–1.78	0.376			
Use of Cyclosporine (Ref: Tacro)	7.37	2.54–21.35	0.001			
Use of non CNI treatment	4.34	1.30–14.41	0.017			
Anti HLA class I at baseline	1.72	0.77–3.85	0.190			
Anti HLA class II at baseline	0.86	0.32–2.30	0.768			
DSA at baseline	0.97	0.29–3.23	0.954			

well after the induction treatment; these are part of the causal pathway of the initial treatment (i.e., they result from it) and should be considered as part of the evaluation process.

The analysis was conducted using R version 4.1.3, with statistical significance defined as a p-value of less than 0.05.

Ethical Consent

All data were extracted from the Nantes DIVAT database. This study received data privacy approval from CNIL (09-17-2004, number n°891735, Réseau DIVAT:10.16.618). The patient's non-opposition regarding access to their medical records, collection and data processing is mandatory under French law. All data were anonymized before analysis. The use of Etanercept in pancreas transplant recipients was approved by the local human ethics committee (n°23-115-09-211). The quality of the DIVAT data bank is validated by an annual audit. The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the Declaration of Istanbul on Organ Trafficking and Transplant Tourism.

RESULTS

Description of the Population

During the study period, 236 pancreas transplant recipients were included, among whom 87 received anti-TNFα and 149 received standard of care (SOC). The complete characteristics of the population are described in **Table 1**. Briefly, 77.1% received simultaneous pancreas-kidney (SPK) transplants, 13.6% received pancreas transplant alone (PTA), and 9.3% received pancreas after kidney (PAK) transplants, with a mean age of 40 years. The mean donor's age was 33 years, with a mean BMI of 23, and 39% of them died from cardiovascular events, without any significant differences observed among groups. Of note, patients receiving anti-TNFα were more often transplanted with shorter pancreatic and kidney cold ischemia times (563 vs. 635 min, $p < 0.0001$ and 688 vs. 794 min, $p < 0.0001$ respectively). 10.6% of patients presented with preformed donor-specific antibodies (DSA) at the time of transplantation. Induction therapy consisted of a T-cell depleting agent in 92.4% of the cohort, followed by maintenance therapy comprising a calcineurin inhibitor (mainly tacrolimus: 99.1%) and an antiproliferative agent, either mycophenolate mofetil or mycophenolic acid (99.6%). Oral steroids were administered to 97.9% of patients, followed by rapid tapering during the first weeks post-transplantation.

Impact of Anti-TNFα on Allograft Survival and Function

At 3 years post-transplantation, the overall pancreatic allograft survival rate was 80.1%. The main causes of failure were

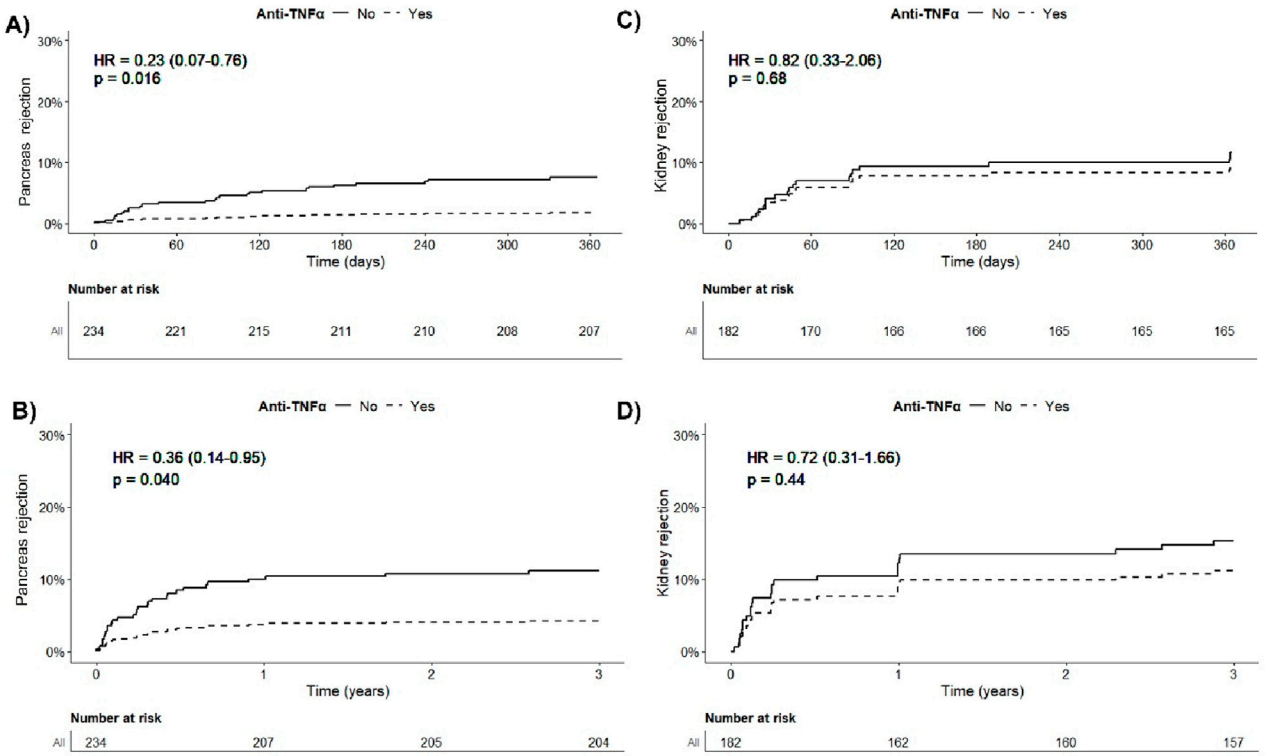


FIGURE 2 | (A) Confounder-adjusted death-censored occurrence of pancreas rejection according to the administration of anti-TNFα during the first year post-transplantation. **(B)** Confounder-adjusted death-censored occurrence of pancreas rejection according to the administration of anti-TNFα at 3 years post-transplantation. **(C)** Confounder-adjusted death-censored occurrence of kidney rejection according to the administration of anti-TNFα among SPK recipients during the first year post-transplantation. **(D)** Confounder-adjusted death-censored occurrence of kidney rejection according to the administration of anti-TNFα among SPK recipients at 3 years post-transplantation.

allograft thrombosis (68.1%), allograft rejection (17.0%), and surgical complications (10.6%). Numerically, there were 15 allograft failures in the anti-TNFα group (17.2%, of whom 13/15 were complete thrombosis) and 32 in the SOC group (21.5%, of whom 19/32 were complete thrombosis) at 3 years. After adjusting for confounding variables and factors associated with allograft failure due to thrombosis, the adjusted hazard ratio (HR) for pancreas survival was 0.92 (95% CI = 0.49; 1.73, p = 0.79) for patients receiving anti-TNFα therapy, **Table 2**. The cumulative adjusted probability of pancreatic allograft survival is presented in **Figure 1A**. Among SPK recipients, the adjusted HR for kidney allograft survival was 0.50 (95% CI = 0.10; 2.49, p = 0.40) for patients receiving anti-TNFα therapy compared to the SOC group, **Supplementary Table S1**. The cumulative adjusted probability of kidney allograft survival is presented in **Figure 1B**.

We further investigated pancreatic and kidney allograft function censored for allograft loss (**Supplementary Figure S1**). Regarding the pancreas, no differences were found in fasting glycemia, fasting C-peptide levels, and HbA1c levels during the first 3 years post-transplantation in the anti-TNFα group vs. SOC. Similarly, in the subgroup of SPK recipients, eGFR were globally comparable even if we observed a higher eGFR slope between 3 months and 3 years among patients from the SOC group vs. anti-TNFα (respectively −12.1% and −2.3%).

Impact of Anti-TNFα on Occurrence of Rejection and *De Novo* DSA

At 3 year post-transplantation, there were 5 pancreatic rejection episodes (5.7%) diagnosed in the anti-TNFα group (3 proven by pancreatic biopsy) and 26 (17.4%) in the SOC group (17 proven by pancreatic biopsy). The complete description of these rejection episodes is provided in **Table 3**. The occurrence of a pancreatic rejection episode led to further allograft loss in around 60% of cases. In the multivariate analysis, after adjusting for confounding factors—particularly induction therapy—adjunctive treatment with anti-TNFα was significantly protective against the occurrence of pancreatic rejection during the first year post-transplantation (HR = 0.23, 95% CI = 0.07–0.76, p = 0.01; **Table 4**; **Figure 2A**). Importantly, this protective effect persisted over time and remained significant up to 3 years post-transplantation (HR = 0.36, 95% CI = 0.14–0.95, p = 0.04; **Table 5**; **Figure 2B**). Notably, among the 18 patients who received non-depleting induction therapy and no anti-TNFα, the incidence of pancreatic rejection at 3 years was

TABLE 5 | Univariate and multivariate cause-specific Cox model associated with the risk of pancreas graft rejection in the first 3 years post-transplantation. The type of induction therapy variable was forced into the multivariate model due to its known association with pancreas rejection (30 events were observed during follow-up, 2 observations were excluded because of missing data).

	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Anti-TNFα	0.32	0.12–0.83	0.019	0.36	0.14–0.95	0.0396
Type of transplant: SPK	0.26	0.13–0.53	0.001	0.29	0.14–0.59	0.0008
T cell depleting induction	1.14	0.27–4.79	0.856	1.15	0.27–4.99	0.8484
Recipient's age	1.00	0.95–1.04	0.9			
Recipient's gender (Female)	1.3	0.64–2.66	0.474			
Recipient's BMI	1.02	0.93–1.12	0.612			
Preemptive SPK	0.67	0.39–1.14	0.14			
Pancreas Cold Ischemia Time	1.00	1.00–1.01	0.025			
Retransplantation	2.35	1.01–5.48	0.048			
Duration of diabetes	1.01	0.96–1.05	0.785			
Pretransplant C peptide	0.59	0.27–1.31	0.197			
Pretransplant HbA1c	1.25	1.03–1.50	0.022			
Donor's age	1.03	1.00–1.07	0.045			
Donor's gender (Female)	2.13	1.04–4.35	0.039			
Donor's BMI	1.09	0.97–1.23	0.155			
Donor's vascular cause of death	1.07	0.52–2.22	0.854			
Donor's history of hypertension	0.89	0.21–3.72	0.870			
Donor's cardiac arrest	0.54	0.21–1.40	0.204			
Donor's eGFR	1.00	0.98–1.01	0.691			
Use of vasopressive drugs	1.65	0.39–6.93	0.495			
Number of HLA mismatches	1.18	0.81–1.72	0.392			
Use of Cyclosporine (Ref: Tacro)	6.78	2.36–19.49	0.001			
Use of non CNI treatment	3.91	1.18–12.89	0.025			
Anti HLA class I at baseline	1.46	0.67–3.22	0.342			
Anti HLA class II at baseline	0.94	0.38–2.32	0.892			
DSA at baseline	0.85	0.26–2.80	0.784			

11.1%, which aligns with the rejection incidence in patients who received a T-cell depleting induction without anti-TNFα. This may be linked to a higher level of maintenance immunosuppressive burden administered during the first year in these patients (**Supplementary Figure S2**). Finally, occurrence of DSA at 1 year was comparable between groups (16.4% vs. 10.4%, p = 0.55). The protective effect of anti-TNFα on pancreatic rejection was particularly notable as maintenance therapy was significantly reduced in the anti-TNFα group compared to the SOC group, especially regarding tacrolimus trough levels and steroid use during the first months, **Figure 3**.

Conversely, anti-TNFα did not significantly impact the risk of kidney rejection (HR = 0.72, 95% CI = 0.31; 1.66, p = 0.44), as shown in **Figures 2C, D** and **Supplementary Tables S2, S3**. Nevertheless, we observed a shift in the kidney Banff classification, with a trend toward fewer TCMR and ABMR and more Borderline lesions among SPK patients treated with anti TNFα, **Supplementary Figure S3**.

Impact of Anti-TNFα on Occurrence of Infectious Complications

During the first year post-transplantation, we did not observe an increased risk of infectious complications following the administration of anti-TNFα. Regarding the occurrence of severe bacterial infections, the adjusted HR was 0.69, 95% CI = 0.50; 0.95, p = 0.02 for patients receiving anti-TNFα, as shown in **Figure 4A**, and **Supplementary Tables S4, S5**. Concerning the occurrence of fungal infections, the adjusted HR was 0.53, 95% CI = 0.26; 1.07, p = 0.08 for patients receiving anti-TNFα, as depicted in **Figure 4B** and **Supplementary Tables S6, S7**. The risk of CMV viremia was similar among patients receiving anti-TNFα compared to others (adjusted HR = 0.89, 95% CI = 0.37; 1.24, p = 0.21), **Figure 4C** and **Supplementary Tables S8, S9**. Finally, the risk of BKV viremia was also similar following the administration of anti-TNFα (HR = 0.58, 95% CI = 0.31; 1.07, p = 0.08), **Figure 4D**, **Supplementary Tables S10, S11**. No cases of tuberculosis or viral hepatitis replication were observed among patients having received anti-TNFα therapy. Finally, anti-TNFα therapy did not impact patient survival (**Supplementary Figure S4**).

DISCUSSION

Our study highlights for the first time the significant reduction in the incidence of pancreatic rejection among patients who received anti-TNFα during the first week following pancreas transplantation. This result is all the more notable given that the maintenance therapy in the anti-TNFα group was significantly less intense, particularly with regard to tacrolimus trough levels and the use of oral steroids. Other published *in-vitro* data have reported the benefit of early treatment using anti-TNFα for reducing cytokine storm and leukocyte infiltration in the allograft [11, 12, 22, 23].

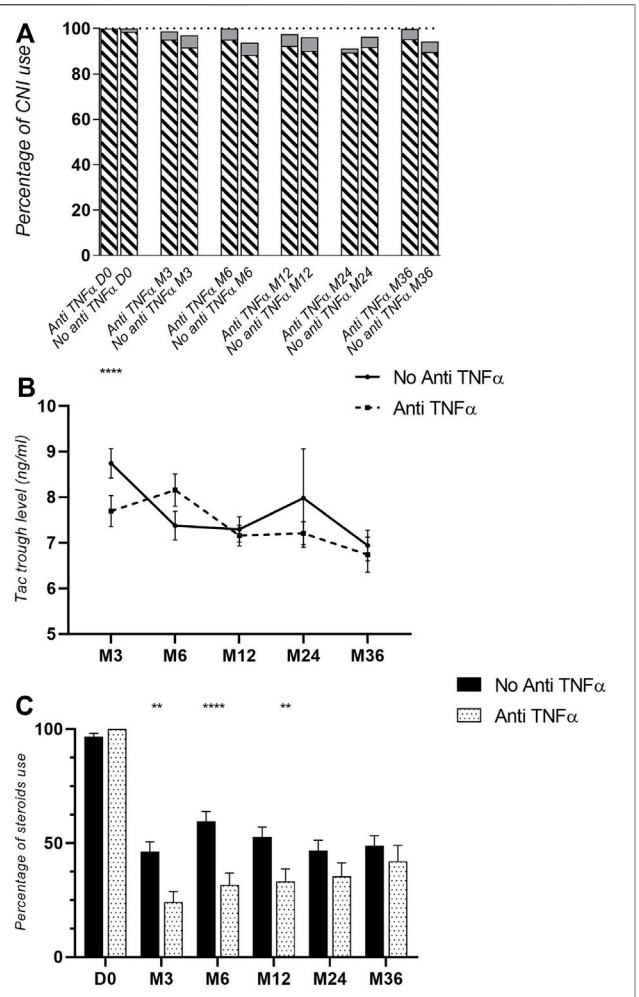


FIGURE 3 | Representation of maintenance therapy during the first year post-transplantation. **(A)** Percentage of calcineurin inhibitors use (tacrolimus: crosshatched bars and cyclosporine: Gy bars). **(B)** Tacrolimus trough levels. **(C)** Use of steroids. *: $p < 0.05$; **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

However, to the best of our knowledge, no clinical data in humans support its use for the prevention of rejection. This result is all the more important as the occurrence of pancreas rejection exacerbates further allograft loss [24–26], which was not attenuated by anti-TNFα therapy in our series. The effect of anti-TNFα therapy on pancreas rejection might be linked to the duodenal part of the pancreatic allograft which might trigger important inflammatory reactions and further alloimmune responses [27]. The benefit of TNFα blockade for digestive inflammatory diseases has been well known for several years [28, 29]. Anti-TNFα therapy has also been used in some cases of refractory intestinal rejection episodes to allow resolution of the alloimmune response [30]. In recipients of a pancreas transplant, a correlation between duodenal rejection and pancreatic rejection has been observed in some cases, suggesting possible interconnected mechanisms [31–33]. This hypothesis is moreover supported by the absence of a significant effect of anti-TNFα on the incidence of kidney allograft rejection. Finally, the observed trend toward a higher incidence of humoral/mixed rejection in patients who received anti-TNFα warrants further investigation and close monitoring to assess the potential for more severe rejection episodes in these patients. In the context of pancreatic transplantation, basic science data regarding the specific effects of anti-TNFα blockade will be of great interest.

Nevertheless, despite the addition of anti-TNFα, we did not observe an improvement in pancreatic allograft survival nor thrombosis. This is certainly due to the complex pathophysiology of pancreatic allograft thrombosis, which involve both immune and non-immune mechanisms [6, 34, 35], as well as implication of multiple inflammatory cytokins such as IL1β. In islet transplantation, the combination of anti-TNFα and anti-IL-1β has proven to be effective in improving grafted islets and long-term survival [36, 37], whereas the use of Etanercept alone did not benefit islet survival [38]. This is consistent with murine models, which report a synergy in the blockade of anti-TNFα and IL-1β regarding islet survival, whereas their respective effects were low independently [39]. Further research on the pathophysiology of pancreas thrombosis will undoubtedly allow a better understanding of this complication and an improvement in strategies to prevent its occurrence.

Importantly, we observed an overall safety profile of anti-TNFα in pancreas transplant recipients. Notably, we did not observe any increase in the risk of severe bacterial or fungal infections, CMV viremia, nor BKV viremia. We even observed a trend towards fewer infectious complications, which can be explained by a reduced maintenance immunosuppressive treatment in patients receiving anti-TNFα. This contrasts with previously reported data in kidney transplant recipients [40, 41] but aligns with findings in liver transplantation [42]. Differences in maintenance therapy, particularly the use of steroids, might explain these discrepancies. Furthermore, although anti-TNFα has been reported to induce rare cases of renal injuries [43], our patients did not exhibit worsened kidney allograft function.

Our study has several limitations, the most significant being its retrospective, single-center design, which may introduce unforeseen confounding factors due to variations across different time periods. However, it is important to note that during the study period, there were no major changes in our surgical techniques or perioperative management of pancreas transplant recipients, except for the use of anti-TNFα and the administration of basiliximab as induction therapy in a small proportion of non-immunized patients. The differences in the initial use of a T-cell-depleting agent, stemming from a local protocol implemented in our center in 2014 to reserve Thymoglobulin for the treatment of pancreatic acute rejection episodes, may have introduced a potential confounding bias regarding rejection occurrence. However, we observed a similar incidence of rejection among patients who did not receive a T-cell-depleting agent compared to those who did. Furthermore, the use of T-cell-depleting agents was accounted for and adjusted in our multivariate analysis, ensuring that the observed difference in rejection rates is attributable to anti-TNFα rather than variations in the use of T-cell-depleting agents.

Additionally, the lack of systematic pancreatic biopsies, either for cause or protocolar, may introduce bias in the definition of rejection

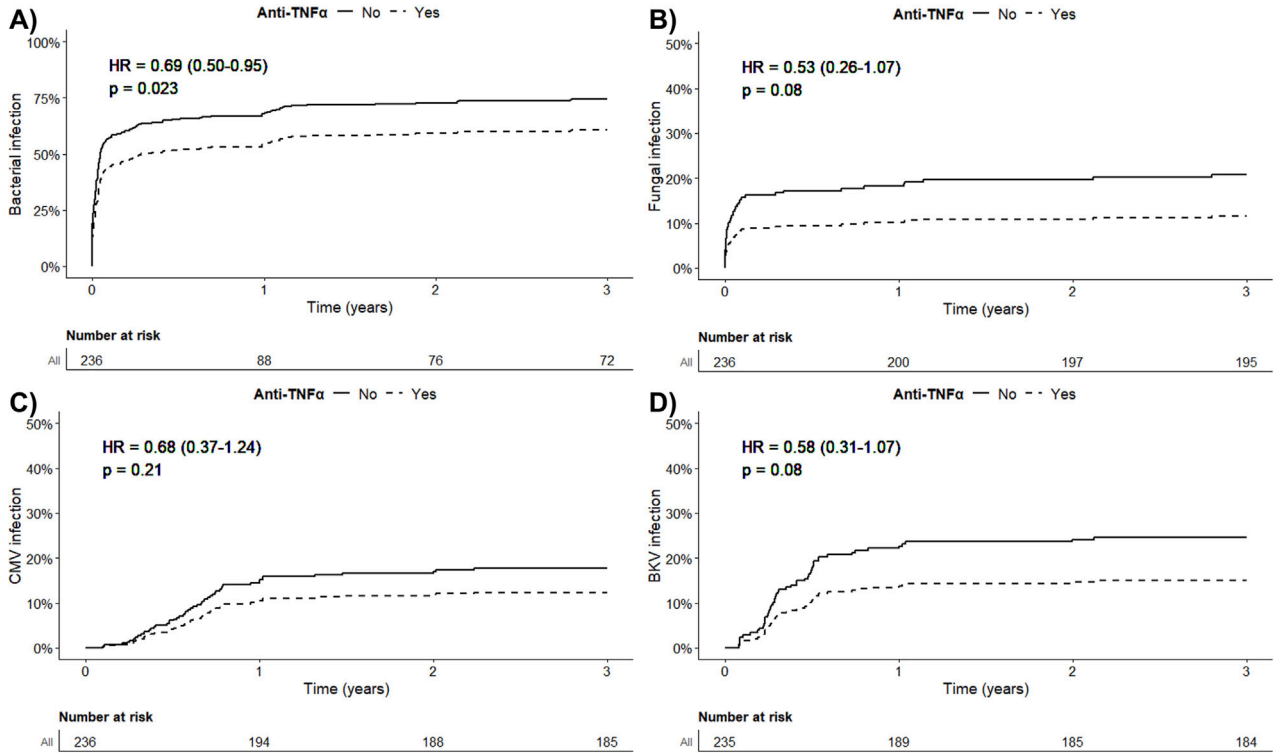


FIGURE 4 | (A) Confounder-adjusted death-censored occurrence of a severe bacterial infection during the first year post-transplantation according to the administration of anti-TNFα. **(B)** Confounder-adjusted death-censored occurrence of a fungal infection during the first year post-transplantation according to the administration of anti-TNFα. **(C)** Confounder-adjusted death-censored occurrence of a CMV viremia during the first year post-transplantation according to the administration of anti-TNFα. **(D)** Confounder-adjusted death-censored occurrence of a BK virus viremia during the first year post-transplantation according to the administration of anti-TNFα.

episodes. Nevertheless, in our cohort, the rate of biopsy-proven pancreatic rejection compared to the global rate of diagnosed rejection was similar among patients receiving anti-TNFα compared to others, suggesting a relatively low impact on our final results.

Finally, it will be of great interest to confirm the benefit of anti-TNFα therapy in pancreas transplant recipients in a multicenter prospective study.

In conclusion, we report the first use of anti-TNFα adjunctive therapy in pancreas transplantation. Although it did not improve neither the rate of early failure due to thrombosis nor overall allograft survival, anti-TNFα significantly reduced the occurrence of pancreatic rejection without increasing infectious complications. Given the retrospective monocentric of our cohort, further evaluation of anti-TNFα would be of interest to properly define its role in pancreas transplantation.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Data are available upon reasonable request to the corresponding author. Requests to access these datasets should be directed to christophe.masset@univ-nantes.fr.

ETHICS STATEMENT

All data were extracted from the Nantes DIVAT database. This study received data privacy approval from CNIL (09-17-2004, number n°891735, Réseau DIVAT:10.16.618). The patient's non-opposition regarding access to their medical records, collection and data processing is mandatory under French law. All data were anonymized before analysis. The use of Etanercept in pancreas transplant recipients was approved by the 180 local human ethics committee (n°23- 115-09-211). The quality of the DIVAT data bank is validated by an annual audit. The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the Declaration of Istanbul on Organ Trafficking and Transplant Tourism.

AUTHOR CONTRIBUTIONS

DC elaborated design and research project, supervised analysis, helped in writing the manuscript and critically revising it. CM and OR analyzed the data. CM collected the data, participated in the study analysis, and wrote the manuscript. All authors participated in writing and revising 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.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.14026/full#supplementary-material

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LETTER TO THE EDITOR
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Successful Organ Donation After Yew Intoxication

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Keywords: ECLS, yew intoxication, organ donation, organ protection, intensive care medicine

Dear Editors,
We would like to report on a case of a successful organ donation under ongoing extracorporeal life support following a yew intoxication.

The European yew (*Taxus baccata*) is as an ornamental conifer widespread throughout Europe. The poisonousness of yews has been known since ancient times and can lead to life threatening intoxications. All parts of the plant, with exception of the red aril, are poisonous. Measured by its cardiotoxic effect, Taxin B is the most important of the alkaloids contained in yews called taxanes. An ingestion of about 3–6.5 mg/kg bodyweight Taxine B is described as potentially lethal for humans [1]. In central Germany there is approximately one severe intoxication per year; most intoxications occur with suicidal intent in young adults [2, 3].

In Germany, brain death (BD) is a necessary condition for organ donation. The second frequent cause of BD is the hypoxic-ischemic encephalopathy (HIE) following cardiac arrest [4]. Life threatening yew intoxications are rare [2], but can lead to cardiac arrest and so to HIE.

A few years ago, we treated a middle-aged patient with a lethal yew intoxication. For ethical reasons, age and gender can not be specified. The medical history included paranoid schizophrenia, recurrent depressive episodes, and several suicide attempts. The patient ingested around 50 crushed yew needles with suicidal intent. Later, regretting the ingestion, the patient sought medical assistance.

Emergency services were called to the location and found a hemodynamically stable patient, already showing a broad complex tachycardia on the ECG. Arriving at a primary care hospital, the patient showed a hemodynamic relevant ventricular tachycardia, which deteriorated into an asystole following electrical cardioversion. After a brief, successful cardiopulmonary resuscitation (CPR), an esophagogastroduodenoscopy (EGD) with application of activated charcoal was performed, and the patient was transferred to a higher-level care facility. As the broad complex tachycardia persisted and a cardiogenic shock developed, a multi-faceted therapy with administration of high dose catecholamines, application of lidocaine, sodium bicarbonate, and digitalis antidote was established. Additionally, a continuous hemodialysis with hemadsorption was applied. Under this therapy the ECG rhythm stabilized temporally before suddenly another cardiac arrest with an asystole occurred. A pacemaker could not be implanted. This time, CPR was prolonged and, as a return of spontaneous circulation (ROSC) did not occur, an extracorporeal life support system (ECLS) was established successfully 1 hour after the start of CPR. The treatment, after ROSC, addressed a post-cardiac arrest syndrome, with persistent hemodynamic instability, renal failure, and a prolonged metabolic acidosis. During therapy, a lack of wake-up reaction and a loss of brainstem reflexes were observed. As a CT scan of the head showed a pronounced cerebral edema indicating severe HIE, an isoelectric EEG was derived. All criteria of BD were fulfilled. In accordance with the patient's wishes, as expressed by the relatives, the patient was evaluated as an organ donor.

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TABLE 1 | Results of the organ donation.

Organ	Time of explanation		1-year after transplantation			
	Visual organ quality	Transplantation	Creatinine [mmol/L]	Urea [mmol/L]	GFR [mL/min/ 1,73 m^2]	Summarized organ quality[a]
Left Kidney	Good	Yes	166	5,6	38	Good
Right Kidney	Good	Yes	142	6,3	43	Good
Liver	Good	No Histology: microvesicular steatosis	-	-	-	-

[a]*Assessment through the ministering transplantation centers.*

We continued intensive care therapy for 3 days, fully aware of the yew intoxication, to ensure that no toxins remained in the blood. Blood levels of 3,5-dimethoxyphenole and other taxanes, including Taxin B, measured by liquid chromatography–mass spectrometry (Triple Quad 5500+, Sciex, Framingham, USA) were taken on days 1, 2, and 3. It was only on day 3 that no toxins were detected, and therefore BD diagnostics were initiated. Organ-protective intensive care, including ECLS, was maintained until the irreversible BD was confirmed. Subsequently, the liver and both kidneys were successfully donated. The organ quality was assessed as good. Histologic examination of the liver showed a microvesicular fibrosis and this organ was rejected for transplantation. The kidneys were successfully transplanted and showed a good one-year-organ function (**Table 1**).

The ingestion of 50 yew needles represents as a potential lethal dose [1]. By inhibition of myocardial sodium and calcium channels, Taxin B induces a transient myocardial channelopathy [5, 6]. Clinically, patients present with malignant cardiac arrhythmias and cardiogenic shock. Patients with a potentially fatal poisoning should immediately be admitted to a tertiary care center with access to a full range of treatments. Otherwise, sufficient therapy may be delayed. Many therapy options are described in the literature, very few of them with evidence. Gastrointestinal decontamination seems to be an useful therapy option, because Taxin B induces a prolonged gastric passage. Therefore, the repetitive administration of activated charcoal is recommended [7]. An EGD is an individual measurement and not generally recommended. For potential lethal yew intoxications both well-established therapies should be applied.

There are no options for secondary toxin elimination: Due to the large size of Taxin B molecules (534 kDa) neither hemodialysis nor hemadsorption filters are able to eliminate yew alkaloids. Symptomatic therapy options for yew intoxication are limited as well. With a half-life of 11–13 h [8], the effect of yew toxins can be expected to last for 2 days. Symptomatic therapy should bridge this time. This is consistent with the toxicological measurements in the presented case. There is some evidence of ineffectiveness of sodium bicarbonate, Lidocaine should be avoided due to its similar effect to Taxine B. The use of pacemakers or an electrical cardioversion is uncertain, because of the underlying myocardial channelopathy. A reported cross-reactivity of yew alkaloids with digitalis antidote was the rationale of its use [9]. Best data exists for ECLS [9], despite the fact that it is a procedure prone to complications.

The rationale for the use of digoxin immune fab is the potential cross-reactivity of the taxane constituents in the yew plant with the digoxin-specific antibody fragments.

Taxin B has a cardioselective effect [6], so that organ damage in severe yew intoxications does not primary result from the toxin but secondarily by the induced cardiogenic shock and cardiac arrest. The observed microvesicular steatosis of the liver is caused by an impaired mitochondrial beta-oxidation. There is no described effect of taxanes on mitochondrial functions and thus considered as the impact of hepatic hypoxia due to a prolonged cardiogenic shock and CPR time. At the time of the declaration of BD, taxanes could not be detected. Postmortem histological analysis of the myocardium showed no signs of inflammation or other pathologies. No long-term effects of taxanes are known, and none were observed in the transplanted organs.

In conclusion, an organ donation after a lethal yew poisoning is possible and leads to good transplantation outcomes. Therefore, ECLS is not only the option with most evidence in therapy of yew intoxications, it also provides an option to optimize organ protective intensive care therapy towards organ donation.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article because the study protocol was approved by the Ethics Committee of the Medical Faculty of the University of Leipzig, Germany (reference number 308/24-ek) and was conducted in accordance with the local legislation

and institutional requirements. The need for a signed informed consent was waived by the Ethics Committee based on study design.

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PA and AS wrote the manuscript. All authors contributed to the article and approved the submitted version.

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LETTER TO THE EDITOR
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Liver Transplant for Acute Cholestatic Crisis in Sickle Cell Disease

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Keywords: liver transplant, acute liver failure, exchange transfusion, sickle cell disease, sickle cell-associated intrahepatic cholestasis

Dear Editors,

We here report on the management of a 54-year-old female patient who developed severe liver and multi-organ failure due to a severe veno-occlusive crisis in the context of sickle cell disease (SCD) and eventually underwent high urgency transplantation.

After presentation to the emergency department with clinical signs of severe acute liver failure (ALF), a heterozygous sickle cell disease with recurrent symptomatic hemolytic crisis without any preexisting liver disease was diagnosed. The laboratory markers of liver function on admission are outlined in **Table 1**. In the past, a splenectomy and femoral head replacement after aseptic bone necrosis had been performed due to severe hemolytic crisis.

A liver biopsy showed sinusoidal obstruction and congestion due to sickle cell aggregates (**Figure 1**) with no evidence of drug-toxic damage or advanced fibrosis. Sickle cell hepatopathy in sickle cell-associated intrahepatic cholestasis (SCIC) with ALF was diagnosed based on the histological picture, the clinical presentation, and the high sickle cell hemoglobin (HbS content (83%).

Supportive drug therapy was initiated for ALF by means of continuous intravenous administration of ornithine aspartate, lactulose enemas, and continuous acetylcysteine infusion. In addition, the sickle cell crisis was treated with continuous glucose infusion and exchange transfusions, which lowered the HbS content to <20%. Due to the pre-existing immunization as result of the extensive pre-transfusions with detection of irregular erythrocyte antibodies, erythrocyte concentrates without anti Cw and anti E were administered.

Despite these measures, the patient developed progressive liver failure (**Table 1**), which ultimately met the requirements for "high urgency" listing for liver transplantation, which was arranged after excluding contraindications 2 days after admission. In the course, the patient developed progressive multi-organ failure with severe encephalopathy, coagulopathy, lactic acidosis, and circulatory insufficiency. In terms of a bridge-to-transplant strategy, therapeutic plasma exchange and human albumin dialysis were initiated to treat the ALF and the hepatic encephalopathy, respectively, resulting in sufficient stabilization. A suitable donor organ was available 3 days after admission. The transplantation of the whole organ was performed in one stage without complications. The arterial anastomosis was performed as a branch patch of the recipient and donor gastroduodenal artery due to the existing anatomical conditions. Anastomization of the bile duct was performed as an end-to-end anastomosis. The cold ischemia time was 498 min.

In the postoperative course, the HbS percentage rose to 41% necessitating further exchange transfusions. The patient suffered from prolonged postoperative delirium with inconspicuous neuro imaging results. In order to prevent further sickle cell crises and organ complications, a concept of a close HbS surveillance was initiated in close cooperation with the local treating hematologist to assure a sufficient lowering of HbS percentages below 20%, for which a kimal catheter was inserted prior to

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TABLE 1 | Laboratory results on the day of admission (d0), on the day of high urgency listing (d2), immediately before transplantation (d3, after plasmapheresis), 24 h after transplantation (d4), and 6 days after transplantation (d10).

	Admission (d0)	Transplant listing (d2)	Pre-transplant (d3)	Post-transplant (d4/d1 post Tx)	Post-transplant (d10/d6 post Tx)
Creatinine (45–84 µmol/L)	97	58	48	75	49
ALT (<31 U/L)	1,445	1,100	603	3,254	36
AST (<34 U/L)	1,111	999	611	2,448	318
GLDH (<5 U/L)	83	51	35	5,199	17
Bilirubin (2–21 µmol/L)	136	220	177	58	47
AP (35–104 U/L)	165	89	95	96	201
INR (0.9–1.25)	4.21	4.26	1.34	1.35	1.12
Ammonia (11–51 µmol/L)	150	92	56	Nm	Nm
FV (70%–180%)	11.9	10.2	59.1	40.8	Nm
MELD	31	32	19	-	-
Lactate (<2.4 mmol/L)	8.4	1.6	2.0	0.7	0.8
LDH (<247 U/L)	874	539	341	Nm	nm
HbS (%)	83	10.7	Nm	Nm	13.2

ALT, Alanine aminotransferase; AST, Aspartat aminotransferase; AP, Alkaline phosphatase; FV, Factor V; LDH, Lactate hydrogenase; GLDH, Glutamate dehydrogenase; MELD, Model of Endstage Liver Disease; nm, not measured; HbS, Sickle cell hemoglobin.

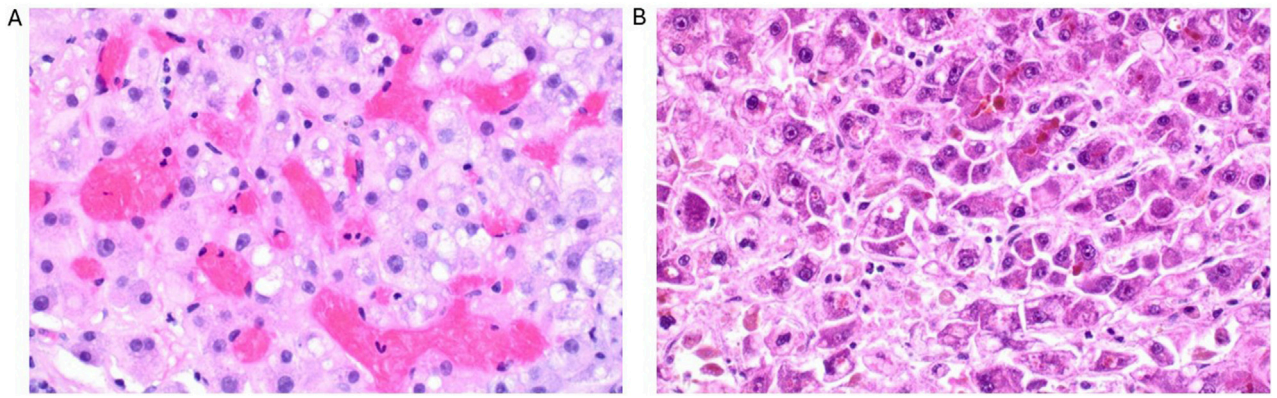

FIGURE 1 | Histological findings of the liver biopsy (1 day after admission) with sinusoidal obstruction and congestion due to sickle cell aggregates with hepatocyte swelling and numerous single cell deaths **(A)** as well as the liver explant (3 days after admission) with significantly more pronounced necrosis compared to the prior biopsy **(B)**.

discharge. Prophylactic anticoagulation was administered during the inpatient stay, which was discontinued on discharge.

No complications, particularly no further sickle cell crises, occurred over the further course under close hematological care with regular exchange transfusions, hydroxycarbamide treatment, and specialized follow-up at our liver transplant outpatient department.

At the last presentation, 18 months after liver transplantation, the graft function was unrestricted with no evidence of advanced hepatic fibrosis (acoustic radiation force impulse imaging elastography 0.96 m/s). The patient was on combined immunosuppression with tacrolimus (trough level aim 5–8 ng/mL) and mycophenolate (500 mg b.i.d.), had no history of acute rejection episodes, and will now be included in our surveillance biopsy guided personalized immunosuppression program.

Vaso-occlusive crises with secondary organ failure including hepatobiliary damage can occur in the context of SCD [1]. SCIC is a rare and severe form of sickle cell hepatopathy leading to local hypoxia with infarction and ballooning of the hepatocytes via sickle cell aggregates in the liver sinusoids. This can cause severe cholestasis and, in very few instances, result in acute liver failure (ALF) [2]. The number of liver transplantations due to acute hepatic sickle cell crisis with irreversible organ failure is very low, so there is only limited experience.

Levesque et al. summarized 21 published cases of liver transplantations in SCD patients and formulated recommendations for the pre- and postoperative management [1]. The 5-year overall survival rate after transplantation was 65% in this cohort, comparable to that of recipients who undergo liver transplantation due to other diseases, while the rate of significant cerebral, micro- and macroangiopathic as well as septic complications was higher. The authors emphasized the need for close pre- and postoperative monitoring of hemoglobin S (HbS) levels for the early detection of imminent vascular occlusions. In addition, given the increased incidence of cerebral bleeding events and vascular occlusions in this patient population, it was recommended to clarify occuring neurological symptoms via neuro imaging with low threshold. Additionally, prophylactic anti-infective therapy was suggested in the light of increased sepsis rates.

The patient cohort reported was very heterogeneous: only 7 patients underwent liver transplantation in the context of SCIC-associated liver failure, while the majority of patients was electively listed for transplantation and/or required transplantation due to another end-stage liver disease, so that the transplantation did not take place in an acute sickle cell crisis.

In the few cases described in the literature, vascular complications such as thrombosis and bleeding occurred significantly more frequently after liver transplantation in SCIC [1, 2]. The patients with ALF and SCIC described by Levesque et al. predominantly had a homozygous mutation in the HbS gene and suffered from early and fatal vascular complications after transplantation despite HbS <20%. Patients with SCIC without early vascular complications, like the patient described in our case, had a HbS-β°-thalassaemia with consistent control of the HbS level to <20% using hydroxycarbamide and exchange transfusions. We support the statement of Levesque et al. that this secondary prophylaxis of new sickle cell crises plays a key role in the post-transplant course in order to avoid vascular complications.

Another important factor for beneficial post-transplant courses is the regular re-evaluation of immunosuppression, particularly due to pre-existing alloimmunization from multiple transfusions prior to transplantation, which can contribute to rejections. A low-threshold transplant biopsy in the event of elevated transaminases can help to detect and treat rejections at an early stage.

Taken together, after transplantation of acute liver failure in SCIC, low-threshold and consistent management of postoperative sepsis episodes and neurological complications is crucial. Exchange transfusions are essential if the HbS level is >20% to avoid vascular complications, as is a low-threshold transplant biopsy in the event of an increase in transaminases.

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.

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Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

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SW and RA wrote the manuscript. JK and RT reviewed the manuscript and the clinical case. BH contributed pathological examinations and images. All authors contributed to the article and approved the submitted version.

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