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DCD class 2 kidneys: NRP to the rescue!

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# DCD class 2 kidneys: NRP to the rescue!



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IN MEMORIAM
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# Jean-François Borel (1933–2025)

*Pierre-Alain Clavien* *

*Department of Surgery and Transplantation, University of Zurich and Swiss Medical Network, Zurich, Switzerland*

**Keywords: organ transplantation, cyclosporin A, organ transplant, rejection, immunity**

Transplant International mourns the profound loss of Jean-François Borel, who significantly advanced the field of solid organ transplantation through his breakthrough work on Cyclosporin.

Jean-François Borel, a Swiss microbiologist and immunologist, was born on 4 July 1933, in Belgium. After moving to his home country Switzerland during the second world war he studied at the Swiss Federal Institute of Technology in Zurich, where he earned his Ph.D. in immunological genetics in 1964. JF Borel's curiosity and passion for science and art drove him to pursue a career that would change the face of medicine.

JF Borel's most notable contribution was in the discovery of cyclosporin, a drug that has transformed the field of organ transplantation. His research demonstrated the drug's ability to selectively suppress T-cells, paving the way for its use in humans. Cyclosporin's impact on transplantation medicine has been profound, enabling hundreds of thousands of people to receive life-saving organ transplants.

Throughout his illustrious career, JF Borel received numerous honors for his work, including the Gairdner Foundation International Award, the Paul Ehrlich and Ludwig Darmstaedter Prize, as well as the Cloëtta Prize. He was also awarded an honorary doctorate from the University of Basel.

Beyond his scientific achievements, JF Borel was a man of diverse passions and talents. One of his greatest loves was painting (**Figure 1**). JF Borel's artwork reflects his keen eye for detail and his ability to capture the beauty in the world around him. His paintings often incorporated elements of nature, showcasing his deep appreciation for the natural world. Through his art, JF Borel expressed himself in a different way, exploring new ideas and perspectives that complemented his scientific pursuits. His love of painting brought him joy and fulfillment, and his artwork remains a testament to his creativity and talent.

Since 2000 we hold a yearly special lecture in honor of JF Borel at the University Hospital in Zurich, where leaders and innovators in transplantation medicine give a special lecture. While Jean François Borel withdrew from the scientific public life, he regularly attended his namesake lecture in Zurich, and spent his time enjoying art, literature and of course painting.

JF Borel's life was a testament to the idea that we are all multifaceted individuals, with many passions and interests that shape who we are. His love of science and art reminds us that these two seemingly disparate fields are, in fact, interconnected, and that together, they can lead to a richer, more fulfilling life.

As the scientific community and many patients around the globe mourn his passing, we take comfort in the knowledge that JF Borel's legacy will live on. His work will continue to inspire future generations of researchers, and his artwork will bring joy and inspiration to those who see it. His



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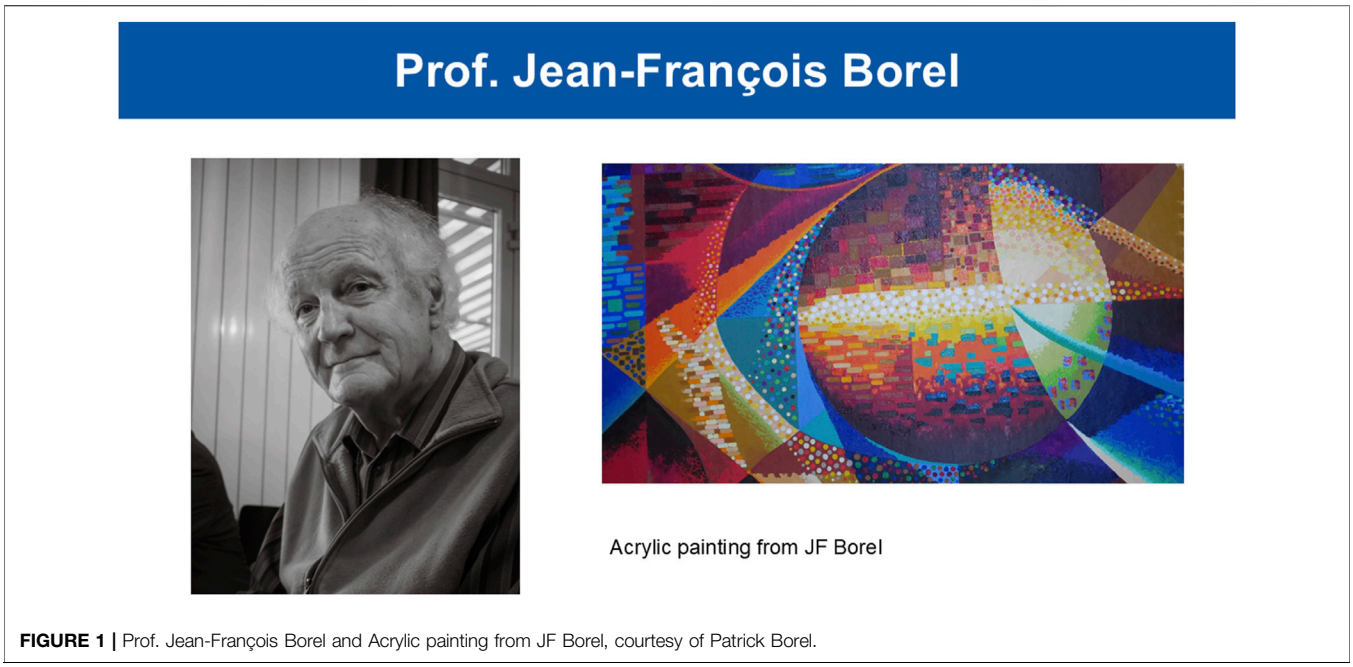


**FIGURE 1 |** Prof. Jean-François Borel and Acrylic painting from JF Borel, courtesy of Patrick Borel.

passing leaves a void, but his impact will endure, a reminder of the difference one person can make in the world.

Pierre-A. Clavien.

## DATA AVAILABILITY STATEMENT

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

## AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication. The accuracy of the information has been adjusted by Patrick Borel, the son of Jean-François Borel.

## CONFLICT OF INTEREST

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

## 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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# Kidney Transplantation From Uncontrolled Donation After Circulatory Death Maintained by Normothermic Regional Perfusion: An 8-Year Portuguese Single-Centre Experience

*Ana Pinho[1]*, Susana Sampaio[1,2], Inês Alencastre[2], Maria João Polidoro[3,4], Margarida Rios[5], Roberto Roncon-Albuquerque Jr.[2,6], João Silva[1,2], Carlos Silva[1,2] and Manuel Pestana[1,2]*

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In January 2016, our hospital started a program of uncontrolled donation after circulatory death (uDCD) to increase organ availability for kidney transplantation. We analysed the results of 523 consecutive kidney transplants (KT) performed from January 2016 to December 2023 in our center and compared the outcomes of 142 KT from uDCD maintained by abdominal normothermic regional perfusion (A-NRP) with those from 194 KT from standard-criteria brain-death donors (SCD) and 187 KT from expanded-criteria brain-death donors (ECD). Primary non-function (PNF) was similar in uDCD (16.9%) and ECD (13.4%, $p = 0.460$) and more common than in SCD (4.6%; $p < 0.001$). In addition, delayed graft function (DGF) differed among the groups, being higher in the uDCD (69.7%), followed by ECD (43.9%) and SCD (37.6%; $p \leq 0.05$). However, the estimated glomerular filtration rate (eGFR) at 7 years was similar in uDCD and SCD (62.27 ± 18.38 mL/min/1.73 $m^2$ vs. 65.48 ± 19.24 mL/min/1.73 $m^2$, $p = 1$) and higher than in ECD (47.67 ± 23.05 mL/min/1.73 $m^2$, $p < 0.001$). When excluding PNF, the 7-year death-censored graft survival was similar among the three groups (SCD, 91.4%; uDCD, 96.2%; ECD, 82.7%). Despite the increased risk of PNF and DGF, functional and survival outcomes of uDCD KT at 7 years were comparable to those of SCD, thus supporting the use of uDCD kidneys maintained under A-NRP as a successful resource to address organ scarcity.

**Keywords: kidney transplantation, uncontrolled donation after circulatory death, abdominal normothermic regional perfusion, delayed graft function, primary non-function, brain-death donors, expanded-criteria donors, extracorporeal membrane oxygenation**

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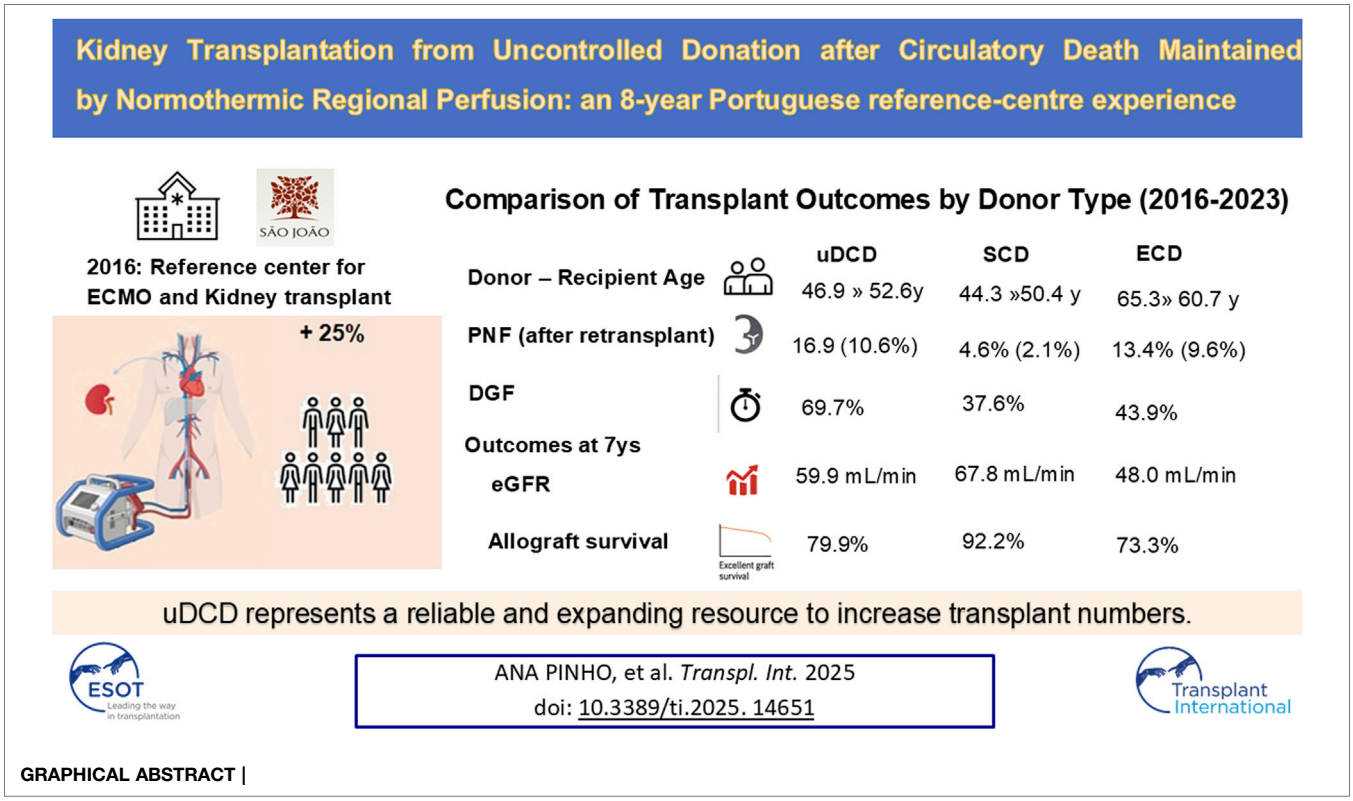



**GRAPHICAL ABSTRACT |**

## INTRODUCTION

Portugal has one of the highest prevalence rates of chronic kidney disease (CKD) requiring renal replacement therapy in the world (>2000 per million) [1]. Transplantation is the best and most cost-effective therapeutic option for eligible patients with end-stage kidney disease. Most kidney transplants (KTs) are carried out with donors declared dead based on neurologic criteria, that is, donation with brain death donors (DBD). Even considering the upward trend in kidney transplants with DBD observed in the last years, peaking at 35.8 per million in 2023, the time on the active waiting list for transplantation in Portugal is still very long [2].

The necessity to expand the donor pool led to an alteration in the Portuguese law in 2013, which opened up the possibility of organ recovery from uncontrolled donation after circulatory death (uDCD) donors, when the efforts after cardiopulmonary resuscitation are unsuccessful (Maastricht II category) [3].

The growing use of extracorporeal membrane oxygenation (ECMO) in critical care has reinvigorated the interest in uDCD donors. Adapting the ECMO technology to post-mortem A-NRP has been shown to reduce the detrimental effects of warm ischemia leading to better allograft-related outcomes with uDCD donors, compared with the previous technique of *in situ* perfusion [4, 5].

In January 2016 we started a project in Unidade Local de Saúde São João, EPE (ULS São João), the largest tertiary teaching hospital in the North of Portugal serving an urban area of ~1.5 million inhabitants, to optimize organ donation from uDCD donors after unsuccessful cardiopulmonary resuscitation. For this purpose, an area of integration of pre-hospital emergency with an in-hospital emergency was created, for assistance in refractory out-of-hospital cardiopulmonary arrest, in compliance with the recommendations of the European Council on matters of resuscitation and organ procurement from uDCD donors [6].

In the present study, we report the results of the first 8 years of KTs carried out with uDCD donors in our institution and compare the outcomes with those from KTs from DBD donors, including both standard criteria (SCD) and expanded criteria (ECD).

## PATIENTS AND METHODS

### Study Design

This retrospective study entails a cohort of all consecutive KTs performed at ULS São João from January 2016 to December 2023 (n = 523). During this period, 142 KTs were carried out with uDCD donors, 194 from SCD and 187 from ECD donors, defined according to the United Network for Organ Sharing (UNOS) [7].

Clinical data were collected using a standardized case report form.

The KTs were followed up for a minimum of 6 months and a maximum of 96 months. The last follow-up date was

**TABLE 1 |** Donor characteristics according to donation group.

| | uDCD | SCD | ECD | p value | | |
|---|---|---|---|---|---|---|
| | (n = 142) | (n = 194) | (n = 187) | uDCD vs. SCD | uDCD vs. ECD | SCD vs. ECD |
| Age (years), mean (SD) | 46.9 (11.2) | 44.3 (11.6) | 65.3 (6.4) | 0.114 | **<0.001** | **<0.001** |
| Gender, n (%) | | | | 0.091 | **0.032** | 0.571 |
| female | 40 (28.2) | 74 (38.2) | 67 (35.8) | 0.071 | 0.171 | 0.690 |
| male | 102 (71.8) | 120 (61.8) | 120 (64.2) | | | |
| Cause of death, n (%) | | | | | | |
| Cardiac arrest | 138 (97.2) | 54 (27.8) | 19 (10.2) | **<0.001** | **<0.001** | 0.006 |
| Stroke | 4 (2.80) | 73 (37.6) | 111 (59.4) | **<0.001** | **<0.001** | **<0.001** |
| Polytraumatism | — | 67 (34.6) | 57 (30.4) | **<0.001** | **<0.001** | 0.44 |
| Preoperative serum creatinine (mg/dL), mean (SD) | 1.2 (0.33) | 0.88 (0.38) | 0.75 (0.22) | **<0.001** | **<0.001** | <0.001 |
| Warm ischemia time (min),mean (SD) | 93.8 (32.1) | — | — | — | — | — |
| A-NRP (min), mean (SD) | 177.7 (31.9) | — | — | — | — | — |
| Cold ischemia time (hours), mean (SD) | 14.5 (3.5) | 13.1 (5.8) | 14.8 (5.8) | 0.031 | 0.860 | **0.0150** |

*Bold p values indicate significant variables.*

*A-NRP, abdominal normothermic regional perfusion; ECD, expanded-criteria brain-dead donors; SCD, standard-criteria brain-dead donors; SD, standard deviation; uDCD, uncontrolled donation after circulatory death.*

**TABLE 2 |** Recipient characteristics according to donation group.

| | uDCD | SCD | ECD | p value | | |
|---|---|---|---|---|---|---|
| | (n = 142) | (n = 194) | (n = 187) | uDCD vs. SCD | uDCD vs. ECD | SCD vs. ECD |
| Age (years), mean (SD) | 52.6 (11) | 50.4 (10.4) | 60.7 (7.9) | 0.170 | **<0.001** | **<0.001** |
| Gender, n (%) | | | | | | |
| female | 44 (31.0) | 79 (40.7) | 64 (34.2) | 0.090 | 0.610 | 0.230 |
| male | 98 (69.0) | 115 (59.3) | 123 (65.8) | | | |
| Etiology of Chronic Kidney Diseased, n (%) | | | | | | |
| Glomerulonephritis | 29 (20.4) | 54 (27.8) | 34 (18.2) | 0.161 | 0.661 | 0.030 |
| Polycystic kidney disease | 27 (19.0) | 22 (11.3) | 31 (16.6) | 0.061 | 0.630 | 0.194 |
| Diabetes | 15 (10.6) | 9 (4.6) | 38 (20.3) | 0.063 | 0.029 | <0.001 |
| HTA | 10 (7.0) | 14 (7.3) | 3 (1.6) | 1.000 | 0.024 | 0.021 |
| Unknown | 37 (26.1) | 50 (25.8) | 47 (25.1) | 1.000 | 0.890 | 0.961 |
| Others | 24 (16.9) | 45 (23.2) | 34 (18.2) | 0.141 | 0.663 | 0.321 |
| Dialysis modalilty, n (%) | | | | | | |
| Hemodialysis | 115 (81.0) | 164 (84.5) | 162 (86.7) | 0.191 | 0.461 | 0.651 |
| Peritoneal Dialysis | 26 (18.3) | 28 (14.5) | 24 (12.8) | 0.211 | 0.401 | 0.743 |
| Pre-emptive | 1 (0.7) | 2 (1.0) | 1 (0.5) | — | — | — |
| Dialysis vintage (months), mean (SD) | 50.5 (20.5) | 64.9 (38.1) | 61.2 (32.8) | **<0.001** | **0.001** | 0.67 |
| Previous Kidney transplant, n (%) | — | 22 (11.3) | 14 (7.5) | — | — | 0.15 |
| HLA-ABDR mismatches, n (%) | | | | | | |
| 0–3 | 69 (48.6) | 116 (59.8) | 83 (44.4) | 0.541 | 0.930 | 0.372 |
| 4–6 | 73 (51.4) | 78 (40.2) | 104 (55.6) | | | |
| PRA, n (%)[a] | | | | | | |
| 0% | 122 (85.9) | 146 (75.3) | 145 (77.5) | **0.010** | **0.030** | 0.791 |
| 1%–20% | 13 (9.2) | 31 (15.9) | 34 (18.2) | 0.056 | **0.010** | 0.591 |
| >20% | 7 (4.9) | 17 (8.8) | 8 (4.3) | 0.230 | 1.000 | 0.140 |
| Induction therapy, n (%) | | | | | | |
| ATG-based protocols | 139 (97.8) | 86 (44.3) | 63 (33.7) | **<0.001** | **<0.001** | **0.041** |
| Basiliximab | 3 (2.2) | 108 (55.7) | 124 (66.3) | **<0.001** | **<0.001** | **0.032** |

*Bold p values indicate significant variables.*

[a]*Pre-formed donor-specific antibodies were a criterion to rule out transplantation.*

*ADPKD, autosomal dominant polycystic kidney disease; ATG, Anti-thymocyte globulin; CMV, Cytomegalovirus; CKD, chronic kidney disease; RRT, Renal replacement therapy; HLA, Human leukocyte antigen; PRA, Panel reactive antibodies; SD, standard deviation.*

31 December 2023, unless graft loss or death occurred first. The follow-up period analysed was 7 years for at least 1 year of follow-up for each patient.

The main outcomes studied were 7-year graft survival (both uncensored and censored by death), recipient survival and longitudinal analysis of graft function over 8 years.

In addition, donor and recipient pre-transplant factors and post-transplant variables were assessed and analysed concerning their relevance in the above-described outcomes. Donor characteristics were (**Table 1**): age, gender, cause of death (cardiac arrest, stroke, trauma, and other), preoperative serum creatinine (SCreat, mg/dL), warm ischemia time (WIT, min),

**TABLE 3 |** Post-transplant characteristics according to donation group.

| | uDCD | SCD | ECD | p value | | |
|---|---|---|---|---|---|---|
| | (n = 142) | (n = 194) | (n = 187) | uDCD vs. SCD | uDCD vs. ECD | SCD vs. ECD |
| PNF, n (%) | 24 (16.9) | 9 (4.6) | 25 (13.4) | **<0.001** | 0.462 | **0.005** |
| Surgical complication | 13 (9.2) | 5 (2.6) | 19 (10.2) | | | |
| Rejection | 4 (2.82) | 1 (0.5) | 1 (0.5) | | | |
| Others | 7 (4.9) | 3 (1.6) | 5 (2.7) | | | |
| PNF after retransplantation, n (%) | 15 (10.6%) | 4 (2.1%) | 18 (9.62%) | **<0.001** | 0.371 | **0.003** |
| DGF, n (%) | 99 (69.7) | 73 (37.6) | 82 (43.9) | **<0.001** | **<0.001** | 0.050 |
| Sessions of HD until decrease of Cr, median (IQR) | 13 (7.5–19) | 7 (3–11) | 7.5 (3–12) | **<0.001** | **<0.001** | 0.813 |
| Biopsy-proven acute rejection, n (%) | 18 (12.7) | 14 (7.2) | 18 (9.6) | 0.130 | 0.481 | 0.510 |
| Acute cellular rejection | 4 (2.8) | 5 (2.6) | 4 (2.1) | | | |
| Acute humoral rejection | 2 (1.4) | 3 (1.6) | 3 (1.6) | | | |
| Bordline | 12 (8.5) | 7 (3.6) | 10 (5.35) | | | |
| Hospital stay (days), mean (SD) | 25.2 (15.2) | 15.5 (12.6) | 19.8 (16.4) | **<0.001** | **0.008** | **0.013** |
| *De novo* donor-specific antibodies, n (%) | 8 (4.1%) | 10 (5.3%) | 5 (3.5%) | 0.313 | 0.194 | 0.740 |
| Follow-up (months), median (IQR) | 31.5 (8–64) | 38.5 (22–67) | 24 (9.5–55) | 0.551 | 0.470 | **0.003** |

*Bold p values indicate significant variables. Cr, Creatinine; DGF, delayed graft function; HD, hemodialysis; IQR, 25%–75% quartil; PNF, primary nonfunction; SD, standard deviation.*

time on A-NRP (min) and cold ischemia time (CIT, hours). Recipient characteristics were (**Table 2**): age, gender, etiology of CKD (polycystic kidney disease, glomerulonephritis, diabetes, and others including undetermined), dialysis modality (haemodialysis or peritoneal dialysis), dialysis vintage, previous kidney transplant, immunosuppressive induction therapy and immunological risk based on panel reactive antibody (PRA, %) and human leukocyte antigen (HLA) mismatch levels.

Post-transplant characteristics included in the analysis were (**Table 3**): primary non-function (PNF), delayed graft function (DGF), biopsy-proven acute rejection (BPAR), surgical complications, hospitalization days, and months of follow-up.

The study was performed according to the ethical standards in the Helsinki and Istanbul declarations and was approved by the Local Institutional Review Board and Ethics Committee of ULS São João. Due to the retrospective and non-interventional nature of the investigation, the need for specific informed consent was waived.

Notwithstanding the Portuguese law concerning organ retrieval being an opting-out rule system, the local Organ Procurement Office obtained the agreement from each deceased donor's next of kin. All recipients were informed at the pre-transplant outpatient clinic about the different types of donors and signed an Informed Consent, according to the donor definition, upon admission for the transplant.

## Variables Definitions

Expanded-criteria brain-death donor was defined as a donor >60 years old or >50 years and with 2 of the 3 comorbidities: hypertension (HTA), death due cerebrovascular event, and SCreat >1.5 mg/dL.

Cold ischemia time was defined as the time from the donor´s aortic clamping during the recovery of the organs until the unclamping of the renal artery during the transplant surgery.

Warm ischemia time was defined for uDCD donors as the time elapsed after cardiac arrest until A-NRP was established.

The A-NRP time encompassed the period from the start of A-NRP until organ retrieval.

The immunological risk was assessed based on three PRA groups (0%; 1%–20%; >20%) and the number of mismatches between donor and recipient of HLA-A, HLA-B, and HLA-DR combined; this variable was dichotomized into two groups (0–3; 4–6).

Primary non-function was defined as permanent graft non-functioning leading to the immediate continuation of dialysis therapy, re-transplant or death. Many PNF cases were associated with early technical complications. Due to frequent overlap between events—such as simultaneous arterial and venous thrombosis, or thrombosis with bleeding or hematoma—a precise attribution of a single causal factor was often not possible.

To address this, we grouped all such intraoperative or early postoperative events under a single category: "surgical complications." This classification reflects the multifactorial nature of early graft failure and minimizes misclassification bias inherent in retrospective analyses.

Delayed graft function was defined as the need for at least one haemodialysis session during the first week post-transplant, with subsequent recovery of kidney function.

All the acute rejection episodes were biopsy-proven and classified as BPARs. In cases of non-satisfactory function, a percutaneous renal graft biopsy was routinely performed post-transplant during the first 10–14 days and repeated 7–10 days after in case of prolonged DGF.

The permanent return to dialysis or re-transplant defined the death-censored graft loss. Graft survival encompassed graft loss to dialysis or re-transplant as well as recipient death. Patient survival was determined until death, censored to return to dialysis (considering the deaths during the first 3 months post-transplantation), or until the last date of maximum follow-up, 8 years post-transplantation.

The graft function was assessed by the estimated glomerular filtration rate (eGFR, in mL/min/1.73m2) using the CKD-EPI equation [8]. The eGFR value following transplant was measured

at 1 month, 3 months, every 6 months until the end of the third year, and annually thereafter.

## Kidney Transplantation Program: Donation After Circulatory Death

The potential uDCD donors in our center must be ≥18 and ≤60 years old, although this is not an exclusion criterion. In addition, standard criteria for selecting uDCD donors were followed regarding neoplastic, infectious, or potentially transmissible diseases [9].

The timeline and order of events for unsuccessful resuscitation before identifying potential uDCD donors were carried out according to the ULS São João protocol [6]. Patients with out-of-hospital cardiac arrest are transported to the emergency department of our hospital on ventilation support and continuous mechanical chest compressions (LUCAS, Physio-Control Inc., Sweden). In case of unsuccessfully advanced life support without inclusion criteria for E-CPR, the intensivist of the emergency department declares death after 10 min (min) of no-touch when no signs of circulation or electrical activity are found. After this, mechanical chest compressions are restarted, and percutaneous cannulation of a femoral artery and vein is performed. An occlusion balloon is placed on the thoracic aorta through the contralateral femoral artery to exclude brain circulation from the extracorporeal circuit. After the establishment of the extracorporeal circuit, chest compressions is terminated.

The nRP is performed using a Maquet CardioHelp system (Maquet, Germany). This technique restores blood flow and oxygenation to the abdominal organs and allows the recovery of kidneys after a prolonged period of WIT. The target pump flow for nRP is 1.75–2.5 L/min, and a constant temperature of 37°C is maintained. Blood samples are obtained every 30 min for blood gases, and biochemistry analysis including serum lactate levels and hematocrit. The maximum preservation time of nRP is 240 min, but it can be extended to 360 min if blood gases, biochemistry, and hematocrit parameters are adequate for organ recovery.

The extraction of organs is performed through a median laparotomy, maintaining nRP support during surgery. Thereafter the kidneys are included in a preservation fluid (Custodiol HTK, Franz Kohler, Germany) and placed in "static" cold storage at 4°C. Implantation surgery is performed within the next 18 h.

In our center, the characteristics of the recipients considered for receiving a kidney from uDCD or DBD donors are similar. However, we avoid performing transplants with uDCD donors in candidates i) waiting for a retransplant, ii) with high immunological risk, iii) with heart failure with depressed ejection fraction and iv) in those undergoing anticoagulation therapy.

All uDCD transplanted patients and DBD recipients with high immunologic risk received rabbit anti-thymocyte globulin (ATG-Fresenius, Fresenius Biotech GmbH, Germany) as induction immunosuppression, at a maximum dose of 2.5 mg/kg/day, with a cumulative target dose of 12.5 mg/kg. High immunologic risk was defined in cases of cPRA >20%, as well as in recipients with prior transplants, the presence of donor-specific antibodies (even if weakly positive), and those with high mismatch levels (HLA 4–6 mismatches). This broader immunologic risk stratification justified the use of ATG in 44.3% of SCD and 33.7% of ECD recipients. In the remaining DBD recipients, we used anti-CD25 monoclonal antibodies (basiliximab) as induction therapy.

All uDCD and DBD recipients received maintenance immunosuppression with steroids, tacrolimus and mycophenolate mofetil. We start with a 500 mg dose of iv methylprednisolone before and immediately after surgery and transition to 1 mg/kg/day of oral prednisolone on the first-day post-transplantation, with subsequent tapering. Mycophenolate mofetil is started orally on the first day post-transplant at a 500 mg dose twice a day. After the end of the induction therapy, this dose is increased to 750 mg twice a day. Tacrolimus is started at a 0.2 mg/kg/day dose, 2 days before the predicted last dose of anti-thymocyte globulin, to ensure adequate levels by the end of the induction therapy. All patients receive chemoprophylaxis with cotrimoxazole and nistatin. Valganciclovir is administered during the first 6 months after transplant in recipients that receive induction therapy with ATG or in cases of CMV donor (+)/receptor (−) serostatus.

## Statistical Analysis

Categorical variables were expressed as absolute (n) and relative (%) frequencies. Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR: first and third quartiles) if the hypothesis of normality of the distribution was not verified. The hypothesis of normal distribution was tested using the Shapiro-Wilk test.

Categorical variables were compared between groups using Pearson's chi-square test or Fisher's exact test when the first cannot be used. Continuous variables were compared using Student´s t-test when data were normally distributed or using the Wilcoxon-Mann-Whitney´s non-parametric test otherwise. The Bonferroni correction was the method used to counteract the multiple comparison problems.

Survival curves for graft and patient survival were obtained using the Kaplan-Meier method, and differences between the groups were compared using the Log-rank test.

PNF and DGF risk factors were analysed using a binary logistic regression model. Relative risks are reported as odds ratios with 95% confidence intervals. Multivariable models included all significant factors in the univariable models and were determined with a forward stepwise procedure.

A p-value <0.05 was considered significant in all tests. The statistical analysis was undertaken using IBM SPSS Statics software (version 26.0) and R software (version 4.2.2).

# RESULTS

From January 2016 to December 2023, 523 KT have been carried out in our center: 142 KT from uDCD donors (27.2%), 194 KT from SCD donors (37.1%) and 187 KT from ECD donors (35.7%)

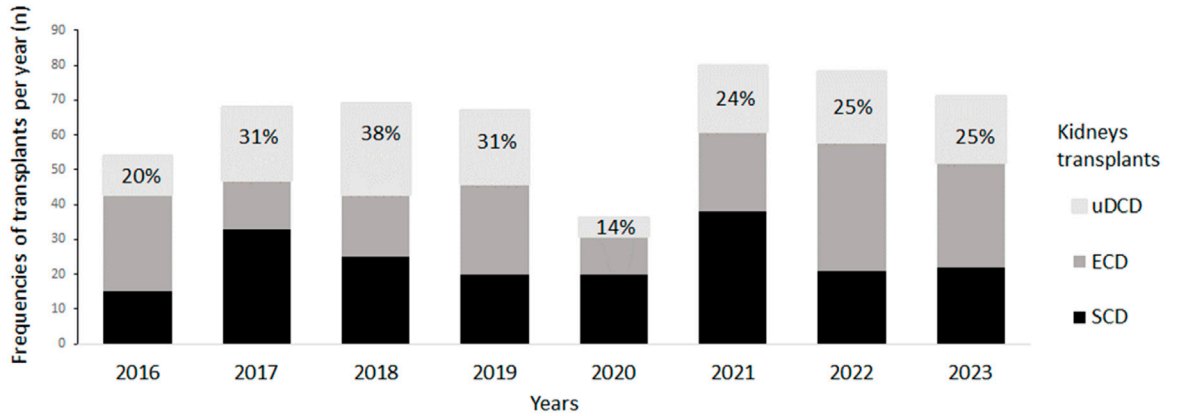



**FIGURE 1 |** uDCD Kidney transplant impact on the Transplantation Program. ECD, expanded-criteria brain-dead donors; SCD, standard-criteria brain-dead donors; uDCD, uncontrolled donation after circulatory death.

(**Table 1**). The impact of renal transplants from uDCD donors on our program is shown in **Figure 1**. As can be observed, KTs carried out with uDCD donors represented 20%–38% of the total number of transplants performed each year throughout this period except in 2020 (14%), which corresponded to the first year of the SARS-cov-2 pandemic when the retrieval of organs from uDCD donors was temporarily suspended according to a directive from the Portuguese National Coordination for Transplant.

## Baseline Evaluation

**Tables 1** and **2** show an overview of the donor and recipient characteristics according to donor group. The mean age of both donors and recipients in the ECD group was significantly higher (65.3 ± 6.4 and 60.7 ± 7.9 years, respectively) than in the other two groups. However, no significant difference was observed between uDCD (46.9 ± 11.2 and 52.6 ± 11.0 years) and SCD (44.3 ± 11.6 and 50.4 ± 10.4 years).groups regarding the median age of both donors and recipients. Male donors predominated across all groups (uDCD: 71.8%, SCD: 61.8%, ECD: 64.2%), as did male recipients (uDCD: 69.0%, SCD: 59.3%, ECD: 65.8%).

As can be observed in **Table 1**, the donor cause of death in uDCD group was mainly due to cardiac arrest (97.2%), whereas the donor cause of death in ECD group was mainly due to stroke (59.4%). Preoperative serum creatinine (SCreat) was significantly higher in uDCD donors (1.2 ± 0.33 mg/dL) compared to both SCD (0.88 ± 0.33 mg/dL) and ECD (0.75 ± 0.22 mg/dL) groups. Cold ischemia time (CIT) was significantly lower in the SCD group (13.1 ± 5.8 h) compared to both ECD (14.8 ± 5.8 h) and uDCD (14.5 ± 3.5 h) groups. Kidneys from uDCD donors had a mean warm ischemia time (WIT) of 93.8 min and a mean abdominal normothermic regional perfusion (A-NRP) duration of 177.7 min.

Recipient characteristics of the three groups are presented in **Table 2**. Recipients from ECD group had an increased percentage of diabetic aetiology (20.3%) in comparison with the other two groups. On the other hand, the uDCD group's dialysis vintage (50.5 ± 20.5 months) was lower than the other two groups. As expected, PRA in uDCD group (85.9% had 0%) was lower than in the other two groups. So, according to the protocol, the recipients from uDCD group received more commonly ATG (97.8%) as induction therapy in comparison with the other two groups. No differences were observed among recipients from the three groups concerning previous kidney transplants, dialysis modality, and HLA mismatches.

## Clinical Course

**Table 3** summarizes the post-transplant outcomes across the three donor groups. PNF occurred significantly less frequently in the SCD group (4.6%) compared to the uDCD (16.9%) and ECD (13.4%) groups. No significant difference in PNF rates was observed between uDCD and ECD. Surgical complications were the leading cause of PNF in all groups, particularly in uDCD (9.2%) and ECD (10.2%).

Regarding patient outcomes following PNF, in the SCD group, 5 out of 9 patients (55.6%) were relisted and all underwent re-transplantation. In the ECD group, 15 of 25 patients (60.0%) were relisted, and 7 (28.0%) received a second transplant. Among the 24 uDCD recipients with PNF, 19 (79.1%) were relisted, and 9 (37.5%) underwent re-transplantation during the study period. Among the 19 patients who were not relisted—either due to medical contraindications or personal decision—all were older than 60 years, with 11 (57.9%) being over 65. Finally, the overall rate of PNF following re-transplantation was 10.6% in the uDCD group (15 cases), 2.1% in the SCD group (4 cases), and 9.6% in the ECD group (18 cases).

As expected, DGF in recipients from the uDCD group (69.7%) was significantly higher than in the other two groups. In addition, DGF in the ECD group (43.9%) was non-significantly higher than that of the SCD group (37.2%). Accordingly, uDCD recipients had a higher median number of HD sessions and days of hospital stay in comparison with the other two groups. On the other hand, no significant difference was observed in the number of BPARs among the three groups.

As can be observed in **Table 4**, in univariable analysis, both preoperative serum creatinine (OR 5.042; p = 0.048) and

**TABLE 4 |** Risk factors for PNF and DGF (logistic regression) according to donation group.

**A. Risk factor for uncontrolled Donor after Circulatory Death (n = 142)**

| Univariate logistic analysis | PNF | | | DGF | | |
|---|---|---|---|---|---|---|
| | Odds Ratio | 95% CI | p value | Odds Ratio | 95% CI | p value |
| Donor age | 1.025 | 0.983–1.075 | 0.272 | 0.993 | 0.953–1.031 | 0.739 |
| Preoperative Cr, mg/dL | 5.042 | 1.082–27.46 | **0.048** | 1.059 | 0.268–4.087 | 0.933 |
| Recipient Age | 1.003 | 0.964–1.045 | 0.876 | 0.988 | 0.949–1.028 | 0.580 |
| Recipient Age >=50 | 0.863 | 0.356–2.156 | 0.746 | 1.222 | 0.494–2.938 | 0.655 |
| Original nephropaty, DM + HTA | 0.623 | 0.138–2.018 | 0.475 | 1.891 | 0.578–8.554 | 0.338 |
| Dialysis vintage. mo | 1.011 | 0.989–1.031 | 0.355 | 1.009 | 0.988–1.034 | 0.392 |
| Warm ischemia time (only for uDCD) | 1.021 | 1.004–1.039 | **0.016** | 1.009 | 0.991–1.028 | 0.334 |
| Cold ischemia time, h | 1.009 | 0.886–1.148 | 0.884 | 1.194 | 1.047–1.379 | <**0.001** |
| Multivariate logistic analysis | Odds Ratio | 95% CI | p value | Odds Ratio | 95% CI | p value |
| Preoperative Cr, mg/dL | 4.796 | 0.797–36.982 | 0.104 | NA | | |
| Warm ischemia time | 1.018 | 0.096–1.987 | **0.047** | NA | | |

**B. Risk factor for Brain Donor Death groups (n = 381)**

| Univariate logistic analysis | PNF | | | DGF | | |
|---|---|---|---|---|---|---|
| | Odds Ratio | 95% CI | p value | Odds Ratio | 95% CI | p value |
| Donor age | 1.055 | 1.022–1.092 | **0.001** | 1.020 | 1.003–1.036 | **0.017** |
| Polytraumatism cause of death | 0.723 | 0.310–1.547 | 0.424 | 0.757 | 0.478–1.189 | 0.230 |
| Preoperative Cr, mg/dL | 0.348 | 0.059–1.512 | 0.201 | 1.606 | 0.787–3.319 | 0.194 |
| Recipient Age | 1.036 | 0.999–1.078 | 0.067 | 1.000 | 0.981–1.021 | 0.898 |
| Recipient Age >=50 | 1.684 | 0.719–4.617 | 0.264 | 0.901 | 0.561–1.464 | 0.683 |
| Original nephropaty, DM + HTA | 0.842 | 0.227–2.092 | 0.733 | 1.192 | 0.677–2.091 | 0.539 |
| Dialysis vintage, mo | 0.999 | 0.988–1.008 | 0.948 | 1.002 | 0.996–1.007 | 0.593 |
| Cold ischemia time, h | 1.082 | 1.013–1.161 | **0.021** | 1.095 | 1.054–1.140 | <**0.001** |
| ATG-based protocols | 0.942 | 0.329–2.192 | 0.827 | 1.172 | 0.677–2.121 | 0.639 |
| PRA (%) | 1.006 | 0.976–1.028 | 0.595 | 0.991 | 0.972–1.007 | 0.313 |
| Multivariate logistic analysis | Odds Ratio | 95% CI | p value | Odds Ratio | 95% CI | p value |
| Donor age | 1.056 | 1.018–3.044 | **0.002** | 1.013 | 0.997–1.031 | 0.091 |
| Cold ischemia time, h | 1.065 | 0.037–1.799 | 0.072 | 1.091 | 1.050–1.136 | <**0.001** |

*Bold p values indicate significant variables. ATG, Anti-thymocyte globulin; DGF, delayed graft function; HD, PNF, primary nonfunction; NA, not applicable.*

WIT >60 min (OR 1.021; p = 0.016) were significant risk factors for PNF in the uDCD group. In contrast, in the BDD group (SCD + ECD), donor age (OR 1.055; p = 0.001) and CIT (OR 1.082; p = 0.021) were significantly associated with PNF. In multivariable analysis, WIT remained a risk factor for PNF in the uDCD group (OR 1.018; p = 0.047), whereas donor age remained significant in the BDD group (OR 1.056; p = 0.002). Additionally, CIT was a significant risk factor for DGF in both uDCD (OR 1.194; p < 0.001) and BDD (OR 1.091; p < 0.001) groups (**Table 4**).

The graft survival rate, both death-censored and uncensored, was significantly higher in the SCD group than in the ECD group (**Table 5**; **Figures 2A,B**). Death-censored graft survival at 7 years was 92.2% in the SCD group, 79.9% in the uDCD group, and 73.3% in the ECD group (p = 0.003 for SCD vs. ECD; p = 0.082 for SCD vs. uDCD). Similarly, overall graft survival not censored for death was also superior in the SCD group (78.9%) compared to uDCD (64.6%) and ECD (53.5%), with statistically significant differences (p < 0.001 for SCD vs. ECD; p = 0.010 for SCD vs. uDCD).

However, when excluding cases of primary non-function (PNF), the death-censored graft survival improved notably in all groups, reaching 91.4% in uDCD, 96.2% in SCD, and 82.7% in ECD. Under these conditions, no significant difference in death-censored graft survival was observed among the three groups (p = 0.121), reinforcing the impact of early graft loss on long-term outcomes (**Table 5**; **Figure 2C**).

The longitudinal analysis of allograft kidney function in the three groups is shown in **Figure 3** and **Table 5**. As observed, mean eGFR was consistently higher in the SCD group (69.37 mL/min/1.73 $m^2$ at 12 months) than in the ECD group (50.28 mL/min/1.73 $m^2$). In the uDCD group, eGFR increased from 1 to 3 months and stabilized around 60.66 mL/min/1.73 $m^2$ at 1 year, remaining stable thereafter. This resulted in a significantly higher eGFR in the uDCD group compared to the ECD group at 1 year and beyond. No significant difference was observed in eGFR between SCD (67.80 mL/min/1.73 $m^2$) and uDCD (59.90 mL/min/1.73 $m^2$; p = 0.396)groups over the 7-year follow-up (**Table 5**; **Figure 3**) univariablemultivariablemultivariable

## DISCUSSION

The present study examined the results of the first 8 years since the beginning of our uDCD program and compared the outcomes with those of KTs from BDD, including both SCD and ECD.

**TABLE 5 |** Long outcomes according to donation group.

| | uDCD | SCD | ECD | p value | | | p value |
|---|---|---|---|---|---|---|---|
| | | | | uDCD vs. SCD | uDCD vs. ECD | SCD vs. ECD | |
| No. of patients | **142** | **194** | **187** | | | | |
| eGFR (ml/min/1.73m2), mean (SD) {number} | | | | | | | |
| 1- year | 60.66 (21.55) {113} | 69.37 (23.74) {173} | 50.28 (19.08) {155} | **0.002** | <**0.001** | <**0.001** | <**0.001** |
| 5- year | 59.01 (23.47) {58} | 67.07 (25.63) {77} | 53.01 (22.61) {56} | 0.136 | 0.381 | 0.003 | |
| 7- year | 59.90 (23.69) {26} | 67.80 (22.74) {34} | 47.99 (23.52) {25} | 0.396 | 0.167 | 0.005 | |
| Death-censored graft survival, % (SE) {number at risk} | | | | | | | |
| 1- year | 85.1 (0.03) {101} | 94.8 (0.016) {162} | 87.4 (0.025) {132} | 0.082 | 0.283 | **0.003** | 0.014 |
| 5- year | 79.9 (0.038) {43} | 92.2 (0.022) {58} | 85.4 (0.028) {38} | | | | |
| 7- year | 79.9 (0.038) {8} | 92.2 (0.022) {12} | 73.3 (0.071) {15} | | | | |
| Graft survival (death not censored),% (SE) {number at risk} | | | | | | | |
| 1- year | 82.8 (0.032) {101} | 93.8 (0.017) {162} | 84.2 (0.027) {132} | **0.010** | 0.311 | <**0.001** | 0.007 |
| 5- year | 73.9 (0.042) {43} | 82.4 (0.035) {58} | 69.7 (0.040) {38} | | | | |
| 7- year | 64.6 (0.094) {8} | 78.9 (0.048) {12} | 53.5 (0.068) {15} | | | | |
| Death-censored graft survival excluding PNF, % (SE) {number at risk} | | | | | | | |
| 1- year | 97.4 (0.015) {98} | 98–9 (0.008) {161} | 98.6 (0.010) {132} | 0.200 | 0.810 | 0.310 | 0.121 |
| 5- year | 91.4 (0.032) {43} | 96.2 (0.017) {58} | 96.3 (0.019) {38} | | | | |
| 7- year | 91.4 (0.032) {8} | 96.2 (0.017) {12} | 82.7 (0.077) {15} | | | | |
| Patient survival, % (SE) {number at risk} | | | | | | | |
| 1- year | 96.7 (0.016) {101} | 99.0 (0.007) {162} | 96.3 (0.0149) {133} | 0.801 | 0.070 | **0.010** | 0.309 |
| 5- year | 89.9 (0.034) {43} | 88.9 (0.032) {58} | 81.6 (0.039) {38} | | | | |
| 7- year | 78.6 (0.109) {8} | 85.1 (0.048) {12} | 72.8 (0.0596) {15} | | | | |

*Bold p values indicate significant variables.*
*uDCD, uncontrolled donation after circulatory death; SCD, standard-criteria brain-dead donors; ECD, expanded-criteria brain-dead donors; SD, standard deviation; SE, standard error.*

Our uDCD allograft function and survival results agree well with other published series in the literature, mainly of uDCD KTs in Europe [10–15]. In addition, the longitudinal analysis of our uDCD KTs showed that both the functional and the survival outcomes were comparable to those observed with KTs from BDD namely SCD. This increased the kidney donor pool in our institution by 14%–38%, thus representing an excellent additional source of organs for transplantation.

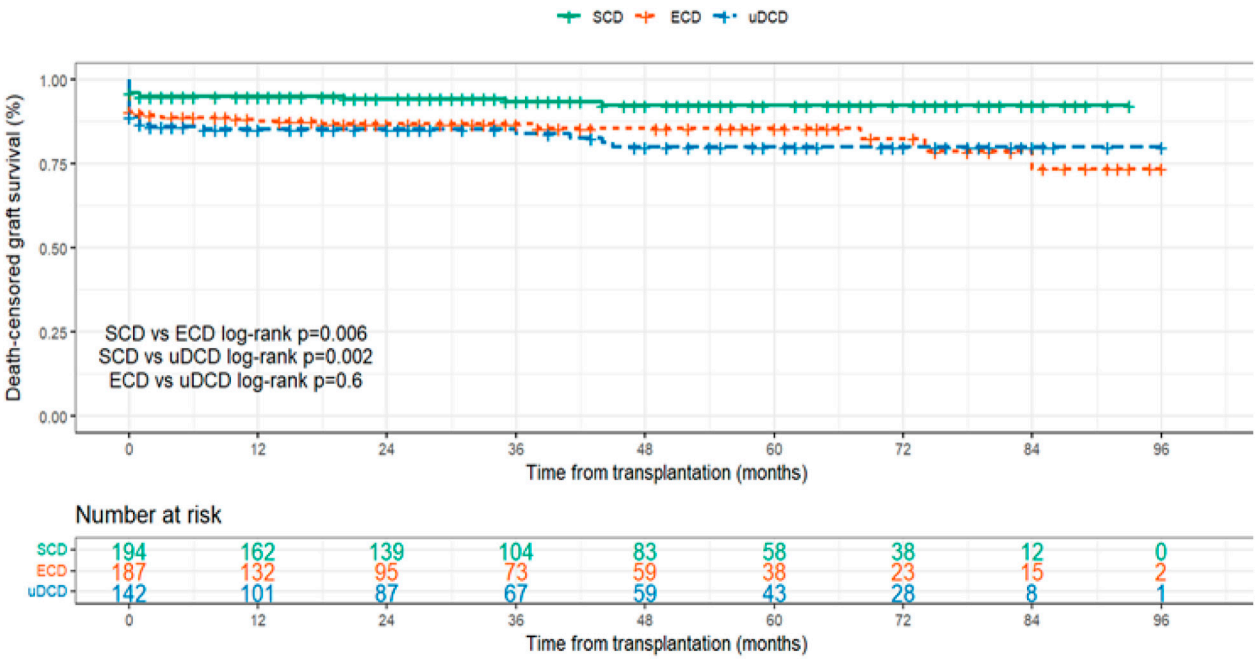
We found that PNF was a very relevant cause of KT failure in both uDCD and ECD groups, occurring in 16.9% and 13.4% of patients, respectively. Surgical complications were the most relevant cause in all donor types, but especially in uDCD and ECD. In fact, our previous experience published by Manso et al. [16] had already identified vascular complications—such as arterial or venous thrombosis—as the leading cause of PNF in uDCD recipients, particularly when combined with prolonged ischemia. These findings are consistent with the surgical etiology observed in the current cohort.

The elevated rate of PNF in the ECD group (13.4%) may be explained by the intrinsic characteristics of expanded criteria donors, namely advanced donor age and comorbidities such as hypertension and cerebrovascular disease. These factors are known to negatively affect graft viability. In our cohort, the ECD group also presented with significantly higher pre-retrieval serum creatinine and prolonged cold ischemia times, both associated with poor early graft outcomes.

Although the underlying causes of PNF should be further investigated, the incidence of PNF in the uDCD group was within the range reported by other studies (14.7%–19.6% [10]), including both controlled and uncontrolled DCDs. In adjusted analysis, serum creatinine and a WIT >60 min were the only parameters significantly associated with PNF in uDCD KTs. Of note, no PNF episodes were observed in the uDCD group during 2020, which coincided with a lower mean WIT of 54 min, further supporting the implementation of a WIT-based allocation policy to reduce the risk of PNF in this population.

Abdominal normothermic regional perfusion (A-NRP) is an organ preservation strategy consisting in a modified bypass extracorporeal circulation that re-establishes the flow of oxygenated blood to abdominal organs after cardiac arrest. This technique is being increasingly used to minimize the warm ischemic injury in uDCD, thus contributing to improved organ transplantation outcomes, including PNF, in comparison with kidneys preserved in static cold settings [17]. Since the start of our program, we have always used A-NRP in all KTs from uDCD. Therefore, this strategy cannot be used in the future to decrease the PNF rate. The absence of post-mortem machine perfusion, particularly in uDCD and ECD donors, which differs from practices in other transplant programs and could affect early graft outcomes such as PNF and we consider in future their use.

We found that our uDCD KTs showed a high rate of DGF (69.2%), as reported by other series (80.9% [12], 76% [14], and 73.7% [10]). However, the DGF rates in the uDCD group had no impact on either short- or long-term graft function and survival. This evidence, although previously reported [17–20] is not consistent in other studies [21, 22]. As we systematically used anti-thymocyte globulin induction in uDCD KTs with low immunologic risk, we propose that the lower rate of DGF observed in our uDCD population compared with other series may be explained by the immunosuppression regimen. Additionally, our immunosuppression regimen may also

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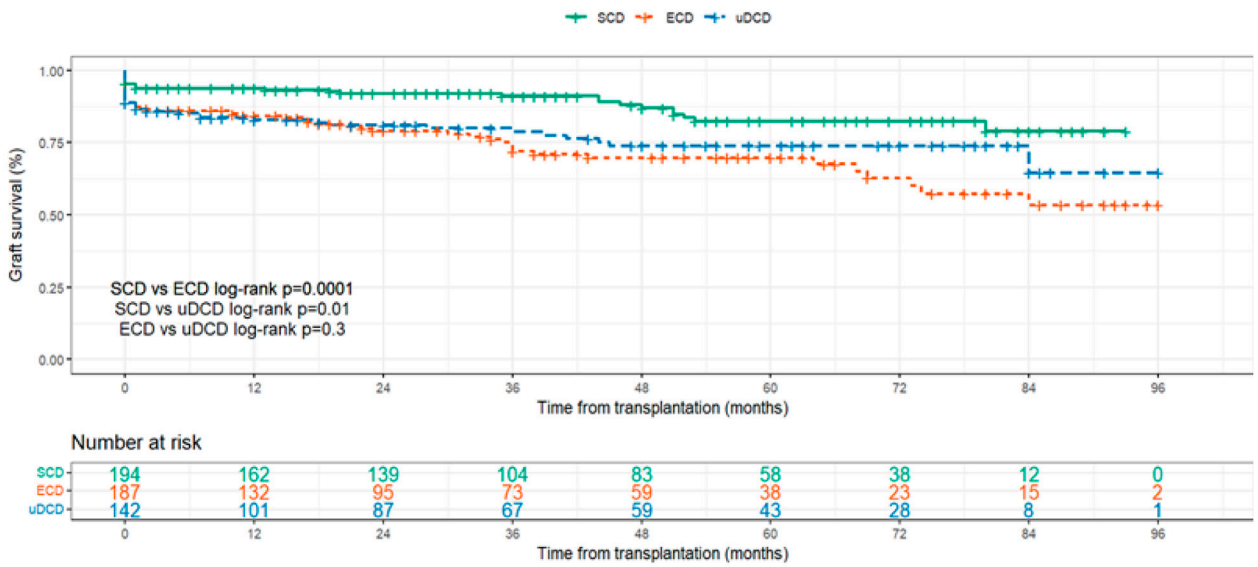
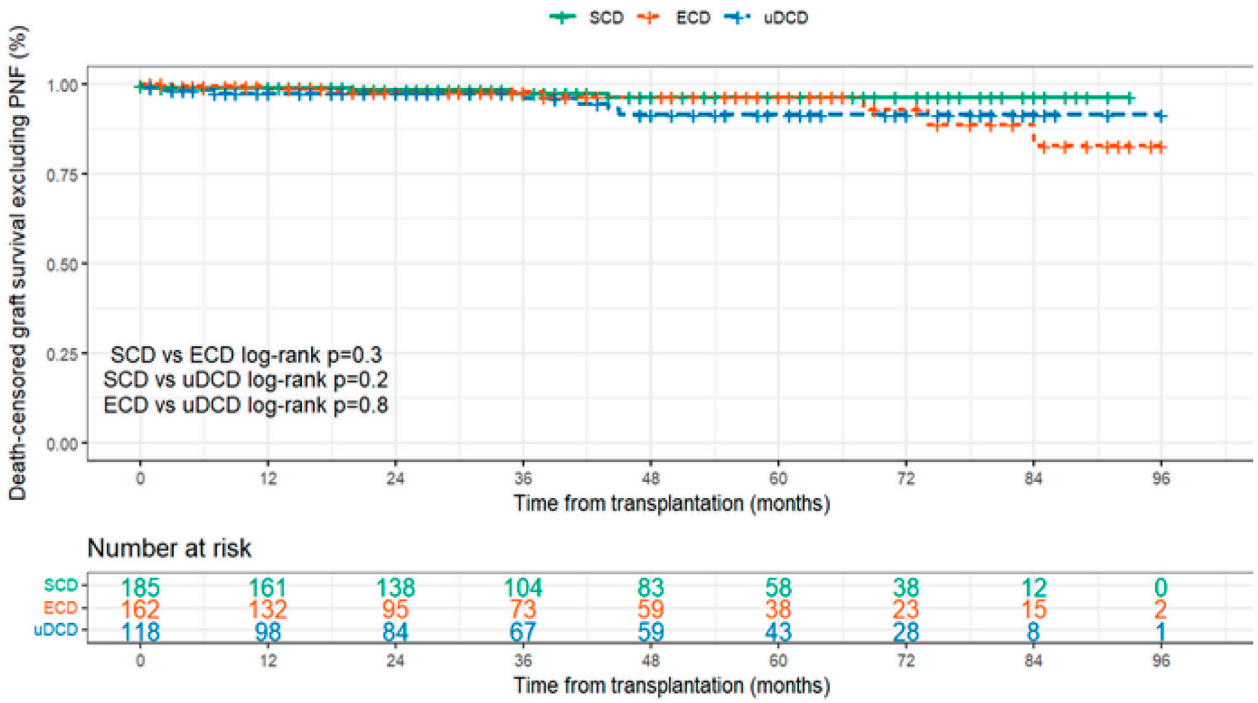



B

**FIGURE 2 |** (Continued).

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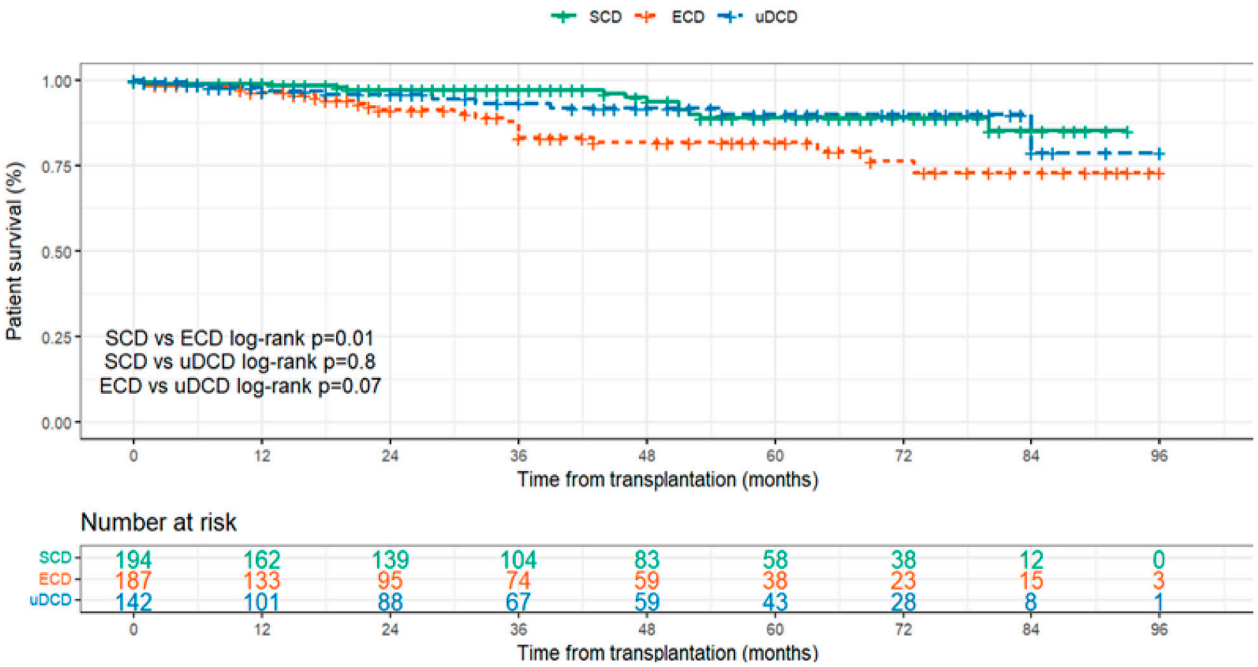


D

**FIGURE 2 |** (Continued). **(A)** Death-censored graft survival. **(B)** Graft survival (death not censored). **(C)** Death-censored graft survival excluding PNF. **(D)** Patient survival. ECD, expanded-criteria brain-dead donors; SCD, standard-criteria brain-dead donors; uDCD, uncontrolled donation after circulatory death.

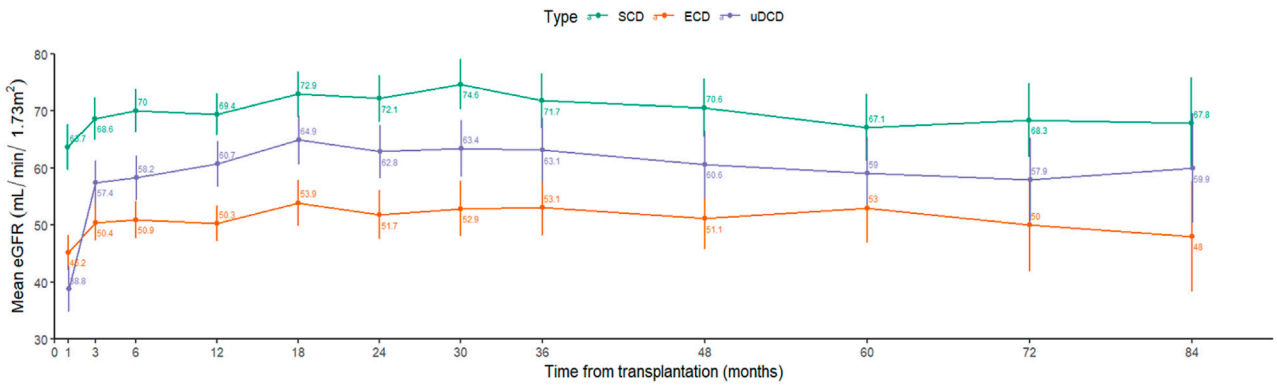


**FIGURE 3 |** Longitudinally eGFR according to donor type. eGFR, estimating glomerular filtration, using the CKD-EPI equation; ECD, expanded-criteria brain-dead donors; SCD, standard-criteria brain-dead donors; uDCD, uncontrolled donation after circulatory death.

contribute to the reduced incidence of BPAR episodes observed in the uDCD group (12.7%).

Evidence has been gathered favoring the benefit of *ex-vivo* machine perfusion techniques to improve quality and recondition organs for transplant, minimizing graft ischemia-reperfusion injury and reducing of immunogenicity while allowing the real-time monitoring of the harvested organ function, including the measurement of physiological and molecular markers, particularly in the DCD scenario where WIT may impair importantly the quality of the retrieved organs. Although criteria for kidney machine perfusion to improve graft survival/function are still pending this may come to be a useful resource to decrease both PNF and DGF in DCD KTs.

Kidney function from our uDCD KTs at 7 years (59.9 ± 23.69 mL/min/1.73 $m^2$) was comparable to other series dealing with uDCD programs [23]. In addition, the longitudinal analysis showed that from 3 months onwards, the eGFR of uDCD KTs was maintained at intermediate values to that observed in the other two groups and, at 7 years, the eGFR of uDCD KTs did not differ significantly from that observed in either SCD or ECD group. This occurred in a scenario where the renal function in the SCD group was maintained significantly higher than that of the ECD group.

Our findings are also in line with those previously reported by a French group [24], which demonstrated that the functional outcomes—particularly overall graft survival—of kidneys transplanted from uncontrolled DCD donors were comparable to those of ECD grafts. This reinforces the notion that, when properly preserved using strategies such as A-NRP, uDCD kidneys can achieve acceptable long-term performance. Historically, in our center, uDCD kidneys have been preferentially allocated to older recipients, mirroring the policy for ECD grafts. However, based on both the literature and the outcomes from our cohort, we are now considering broadening the recipient selection criteria. While our results do not support strict age restrictions, careful assessment of individual recipient risk remains essential. These findings are already influencing our allocation policy and may help optimize graft utility in future uDCD programs.

Moreover, the graft survival when censored to death was not different among KTs from the three groups. The 7-year death-censored graft survival rate of uDCD KTs in our study (79.9% and 91.4%, when excluding PNF) is very motivating and comparable to the previously cited reports [14, 15, 23, 25]. In the same way, patients' survival rates after 7 years were similar among KTs from the three types of donors.

In addition to the classic statistical analysis aiming at the comparative evaluation of the data collected in this study, we have used a linear and multivariable mixed regression model associated with graft function trajectories, where patients may drop out during the study period because of the initiation of renal replacement therapy. To our knowledge, this is the best approach to analyze graft function data longitudinally over a long follow-up period, with application of robust statistical analyses (both univariable and multivariable) to adjust for relevant confounders.

Implementing uDCD transplantation programs is highly demanding and puts significant pressure on hospital teams. Still, an increase in the number of studies from different centers across the globe may have a transforming impact on the current panorama and lead to the better use of uDCD organs for the benefit of kidney patients [18, 25, 26].

Our study has limitations stemming from the retrospective analysis in a single center despite data collected prospectively according to the unit's data management system. The retrospective and single-center nature of the analysis, which may limit generalizability, but the size of the uDCD cohort, which is one of the largest reported to date with long-term follow-up.

However, this study innovates by comparing the outcomes of uDCD KTs with those from BDD, including both SCD and ECD groups, over a 7-year follow-up period.

In conclusion, our study reports the results of the first program of uDCD KTs in Portugal and compares the outcomes of uDCD KTs vs. BDD KTs, including both SCD and ECD KTs. Overall, our results show that uDCD KTs have a long-term performance that is very similar to BDD KTs concerning renal function as well as graft and patient survival, thus representing a valuable source of organs that should be considered for the benefit of patients.

## DATA AVAILABILITY STATEMENT

Anonymized data supporting this study's findings are available from the corresponding author upon reasonable request, in compliance with institutional privacy and ethical regulations.

## ETHICS STATEMENT

The studies involving humans were reviewed and approved by the Ethics Committee of ULS São João (reference CE-385-2024). This retrospective, non-interventional study was conducted in accordance with the Helsinki and Istanbul declarations, as well as local legislation and institutional requirements. The requirement for written informed consent was waived by the Ethics Committee. No identifiable human images are included.

## AUTHOR CONTRIBUTIONS

Conception and design: AP, SS and MP. Data acquisition: AP. Analysis and interpretation of data: AP and MJP. Drafting the article: AP, IA, SS and MP. Critical revision: contribution of all authors. 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.

## GENERATIVE AI STATEMENT

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

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REVIEW
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# Liver Transplantation in the Context of Acute-On-Chronic Liver Failure (ACLF): Where Do We Stand in 2025?

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Acute-on-chronic liver failure (ACLF) is a critical condition that arises in the context of advanced liver disease, marked by rapid liver function deterioration and associated multi-organ failure. This syndrome is associated with a major short-term mortality risk, requiring aggressive and specialized clinical care. Despite ongoing efforts, effective therapeutic options for ACLF are lacking, with liver transplantation (LT) considered the only life-saving intervention, yielding acceptable outcomes in carefully selected patients. However, the place of LT for ACLF remains a matter of debate, given the high prevalence of the syndrome, the sickness of liver transplant candidates, the persistent shortage of available liver grafts, and the increasing number of indications to LT. This review aims to provide a comprehensive analysis of the role of LT in ACLF, evaluating current evidence on patient selection, optimal timing for transplantation, and ongoing debates surrounding this practice, specifically the rationale for prioritizing graft allocation for this indication. Furthermore, we will explore global management strategies for ACLF, focusing on bridging patients to LT and improving survival outcomes. Through this review, we seek to enhance understanding of the evolving role of LT in ACLF and offer insights into future directions for clinical practice and research in this critical area.

OPEN ACCESS

**Keywords: cirrhosis, acute-on-chronic liver failure (ACLF), liver transplantation, liver transplantation window, organ failure**

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## INTRODUCTION

In patients with chronic liver disease, an acute insult—whether intrahepatic or extrahepatic—can precipitate both hepatic and extrahepatic organ failures, a syndrome now recognized as acute-on-chronic liver failure (ACLF). ACLF is characterized by hepatic failure occurring in the setting of chronic liver disease, combined with extrahepatic organ failures, leading to a high risk of short-term mortality [1]. Among patients hospitalized for acutely decompensated cirrhosis, ACLF was present in 22.6% at admission and developed during hospitalization in an additional 8.3% [2]. The European Association for the Study of the Liver–Chronic Liver Failure (EASL-CLIF) Consortium defines six organ failures (OFs) relevant for ACLF diagnosis: liver, kidney, and brain function, along with coagulation, circulation, and respiration. Dysfunction and failure of organ systems are based on thresholds merged in the CLIF-C OF score [3]. As per EASL-CLIF definition, ACLF is classified into three grades (ACLF-1 to ACLF-3) based on the number of OFs, with higher grades correlating with increased mortality. Other definitions, such as that of the Asian Pacific Association for the Study of

the Liver (APASL), differ regarding the underlying stage of liver disease, prior episodes of decompensation, the severity of OFs, and the inclusion of extrahepatic failures [4]. Despite these disparities, all definitions converge on the poor prognosis associated with ACLF [5]. According to the European definition, patients with at least three OFs (ACLF-3) face a 28-day mortality rate exceeding 80% [6]. According to the APASL-ACLF Research Consortium (AARC) scoring system patients with an AARC grade III (corresponding to 3 extrahepatic organ failures), face a 28-day mortality risk of 85.9% [4].

ACLF also has a distinct pathophysiology compared to decompensated cirrhosis, with intense systemic inflammation being the cornerstone of its pathogenesis. Studies have demonstrated a direct correlation between systemic inflammation and ACLF severity: the greater the inflammatory response, the higher the number of OFs at diagnosis and the greater the short-term mortality [7]. In Western countries, bacterial infections and alcohol-associated hepatitis (AH) are the most common precipitating factors [8], similarly alcohol consumption, infections and hemorrhage were the most frequent factors in Latin America [9]. Systemic inflammation is primarily driven by hepatic cell death and inflammatory processes, which enhance bacterial translocation from the gut [10]. This translocation triggers a proinflammatory response, followed by a compensatory systemic anti-inflammatory reaction, leading to immune suppression and an increased risk of infections. This inflammatory state is marked by significantly elevated plasma levels of proinflammatory cytokines (IL-6 and TNF-α), chemokines, adhesion molecules, soluble markers of macrophage activation, and circulating white blood cells [10].

In this review, we provide an in-depth analysis of existing evidence, focusing on LT outcomes and predictive mortality factors in ACLF, patient prioritization on the LT waiting list, and future perspectives on LT for ACLF.

# OUTCOMES OF LIVER TRANSPLANTATION IN ACLF

## Short-Term Outcomes of Deceased-Donor-LT (DDLT)

In this review, we will focus exclusively on studies that included patients with ACLF at the time of LT, as defined by either the EASL or APASL criteria as most studies investigation LT outcome in ACLF setting were based on these definitions (**Table 1**).

In patients with ACLF, the sequential assessment of ACLF grade during the first days of management helps identify those with persistent severe ACLF, which is associated—regardless of the underlying liver disease etiology and the stage of cirrhosis prior to ACLF—with a very low probability of survival without transplantation (estimated between 10% and 20% at 28 days for ACLF grade 3) [2]. Moreover, in patients with persistent severe ACLF, most deaths occur within 15 days of admission, and their mortality risk is comparable to or even higher than that of patients awaiting transplantation for acute liver failure listed for transplantation. Therefore, these patients must be identified promptly to allow for LT as soon as possible. The first large multicenter study reporting LT outcomes in patients with multiple organ failure was published in 2013, demonstrating an acceptable one- and three-year survival rate of 74% and 62% respectively [23]. Liver transplantation outcomes in patients with severe ACLF, as defined by the EASL criteria, were not specifically studied until 2015. To date, 25 retrospective studies have been conducted in this population, with one-year survival rates exceeding 80% in the majority of series (**Table 1**) [6, 24–29]. Until recently, the available data were primarily derived from either single-center retrospective studies with limited sample sizes or analyses of large national registries. However, the latter were often criticized for lacking the granularity necessary to accurately identify patients with severe ACLF at the time of LT—particularly regarding the indication for mechanical ventilation (neurological vs. respiratory failure) and the absence of key variables such as the $PaO_2/FiO_2$ ratio, which is essential to assess respiratory function. As a result, the field had been anticipating robust prospective data, which became available in 2024 with the publication of the UK experience following the implementation of the ACLF-tier classification system. The results of this study confirmed initial findings with a 1-year survival rate of 81% among transplanted patients, compared to 0% in those who were listed but not transplanted [30]. In the ongoing large, prospective, international multicenter observational study "CHANCE" (Liver Transplantation in Patients With CirrHosis and Severe Acute-on-Chronic Liver Failure: iNdications and outComEs–NCT04613921), interim analyses have also confirmed this trend, reporting a 3-month mortality rate of only 9% among liver-transplanted patients with severe ACLF [31]. Notably, in large cohort studies, outcomes—while acceptable across all patients with severe ACLF—are more favorable in those with fewer organ failures. One-year survival rates are higher in patients with grade 2 ACLF compared to those with grade 3 ACLF involving three organ failures, and even lower in those with four to six organ failures. Nevertheless, even in the latter group, one-year survival has been reported to exceed 80% in the largest retrospective series [24, 25].

## Long-Term Outcomes

Few studies have assessed long-term patient and graft survival following LT in the context of severe ACLF. The most notable study, based on U.S. registry data, did not identify an increased risk of death or graft loss beyond the first year post-LT in patients transplanted with ACLF [26]. In this study, most deaths in the ACLF transplant group occurred within the first year and were primarily related to infectious complications or cardiovascular events. Long-term data from the French tricentric study published in 2025 reported no excess mortality or graft loss at 5 and 10 years in patients with ACLF grade 3 compared to matched patients with ACLF grade 1 and 2 or without ACLF. The 5-year survival rates for patients with ACLF grades 1, 2, and 3 were 73%, 71%, and 76%, respectively [32]. Notably, patients transplanted in the context of severe ACLF tend to have an increased risk of death from

**TABLE 1 |** Outcomes of studies evaluating liver transplantation for ACLF.

| Study | Study period | ACLF grades 1; 2; 3* (n) | 1-year post-LT survival for ACLF grades 1; 2; 3* | Long-term post-LT survival for ACLF grades 1; 2; 3* |
|---|---|---|---|---|
| Deceased-donor liver transplantation | | | | |
| Kwon et al. [11] | 2008–2019 | 102; 129; 140 | ACLF grade 3: 67.9% | 5-year survival : 57.6% |
| Artru et al. [32] | 2008–2014 | ACLF grade 3 : 73 | NA | 5-year survival 76.4%; 69.7%; 72.6%; 10-year survival 58.6%; 58.3%; 56.8%; |
| Bernal et al. [30] | 2021–2023 | ACLF grade 3 : 42 | ACLF grade 3 : 77% | NA |
| Alukal et al. [29] | 2005–2021 | ACLF grade 3 : 4806 | ACLF grade 3: 86.2% | NA |
| Hernaez et al. [43] | 2014–2019 | 0; 237; 284 | 84.4% for grade 2; 76.4% for grade 3 | NA |
| Zhu et al. [12] | 2018–2020 | 75; 64; 73 | 93.3%; 73.4%; 60.3% | NA |
| Xia et al. [13] | 2015–2021 | 18; 97; 47 | 83.0%; 83.2%; 69.8% | 3-year survival 83.0%; 80.3%; 69.8% |
| Sundaram et al. [28] | 2018–2019 | 61; 74; 77 | 88.5%; 87.8%; 85.7% | NA |
| Cervantes-Alvarez et al. [14] | 2015–2019 | 40; 33; 22 | 87.5%; 97.0%; 90.9% | 6-year survival 80.0%; 93.9%; 77.3% |
| Artzner et al. [39] | 2018–2019 | ACLF grade 3 : 98 | ACLF grade 3: 79% | NA |
| Goosmann et al. [15] | 2009–2014 | All grades : 98 | NA | 5-year survival 55.1% |
| Belli et al. [27] | 2018–2019 | 58; 78; 98 | 88.6% for grade 1; 78.9% for grade 3 | NA |
| Sundaram et al. [26] | 2004–2017 | ACLF grade 3 : 2744 | ACLF grade 3: 82% | NA |
| Artzner et al. [47] | 2007–2017 | ACLF grade 3 : 152 | ACLF grade 3: 67.1% | NA |
| Agbim et al. [16] | 2006–2013 | 50; 32; 19 | 86%; 81%; 74% | NA |
| Sundaram et al. [26] | 2004–2017 | 8757: 9039; 7981 | 89.5%; 88.6%; 80.6% | 5-year survival 75.2%; 74.9%; 67.7% |
| Sundaram et al. [41] | 2002–2014 | ACLF grade 3 : 2349 | ACLF grade 3: 79.8% | NA |
| Marciano et al. [17] | 2010–2016 | 34; 18; 8 | 82.3%; 100.0%; 62.5% | NA |
| Sundaram et al. [25] | 2005–2016 | 7375; 7513; 6381 | 89.1%; 88.1%; 81.8% | NA |
| Thuluvath et al. [24] | 2002–2016 | 4330; 3557; 3556 | 88%; 88%; 83% | 5-year survival 74%; 74%; 70% |
| Huebener et al. [48] | 2009–2014 | 24; 45; 29 | 3-month survival 72.4% | 2-year survival : 60.2% |
| Artru et al. [6] | 2008–2014 | ACLF grade 3 : 73 | ACLF grade 3: 83.6% | NA |
| Levesque et al. [18] | 2008–2013 | 68; 42; 30 | 76.5%; 78.6%; 43.3% | NA |
| Michard et al. [19] | 2007–2014 | All grades : 55 | 60% | NA |
| Finkenstedt et al. [20] | 2002–2010 | All grades : 33 | 87% | 5-year survival 82% |
| Xing et al. [21] | 2001–2009 | All grades : 133 | 75.9% | 5-year survival 72.1% |
| Living-donor liver transplantation | | | | |
| Kwon et al. [11] | 2008–2019 | 261; 147; 75 | ACLF grade 3: 72% | 5-year survival : 67.5% |
| Kulkarni et al. [22] | 2019–2021 | All grades : 55 | 72.7% | NA |

**Overall results across all ACLF grades if individual grade-specific data are unavailable.*
*Abbreviations ACLF, Acute-on-chronic liver failure; NA, not available.*

infectious or cardiovascular events compared to patients transplanted in other settings [32]. A recent report from King's College Hospital confirmed favorable long-term outcomes in patients transplanted while in the ICU, with a 5-year survival rate exceeding 80% (81.9%) [30].

## SHORT-TERM OUTCOMES OF LDLT

Few studies have investigated survival after LT from a living donor in patients with ACLF at the time of transplantation. These studies have been conducted exclusively in Asia, are all single-center and retrospective, and involve small cohorts. Nevertheless, in these expert LDLT centers, short-term outcomes appear favorable, with survival rates even surpassing those of DDLT (**Table 1**) [33–36]. These findings are important and even promising, as in the context of LDLT, donor screening is rapid, allowing for timely graft allocation to the recipient. Furthermore, the transplant team can choose the optimal timing for LT based on the recipient's clinical evolution, unlike in DDLT, where graft availability is entirely dependent on donor organ availability. This may partly explain the favorable outcomes observed with LDLT.

## LEARNING CURVE AND SELECTION PROCESS

Over the past decade, LT for patients with ACLF has benefited from a significant institutional learning curve, leading to enhanced patient selection and perioperative management. A recent study based on UNOS data analyses highlighted that increasing experience with LT in critically ill patients—defined as those in the intensive care unit with one or more of the following at the time of LT: (i) grade III/IV hepatic encephalopathy, (ii) mechanical ventilation, (iii) dialysis, and/or (iv) vasopressor support—has significantly grown over the past 15 years (4.3% of total LTs in 2005–2008 vs. 7.9% in 2017–2020, $p < 0.001$) [37]. This trend has been associated with improved candidate selection: the proportion of patients transplanted while on dialysis or vasopressors increased, whereas those requiring mechanical ventilation decreased over the study period. Additionally, the waitlist time for these patients shortened [37]. These changes have translated into better post-transplant outcomes, with 1-year survival rising from 72.5% in 2005–2008 to 89.5% in 2017–2020 ($p < 0.0001$) [37]. Similarly, in a monocentric experience, an optimized pre-transplant intensive care unit management (ICU) and timely intervention has led to better post-transplant outcomes [38]. Indeed, in this study, one-year post-LT survival among patients

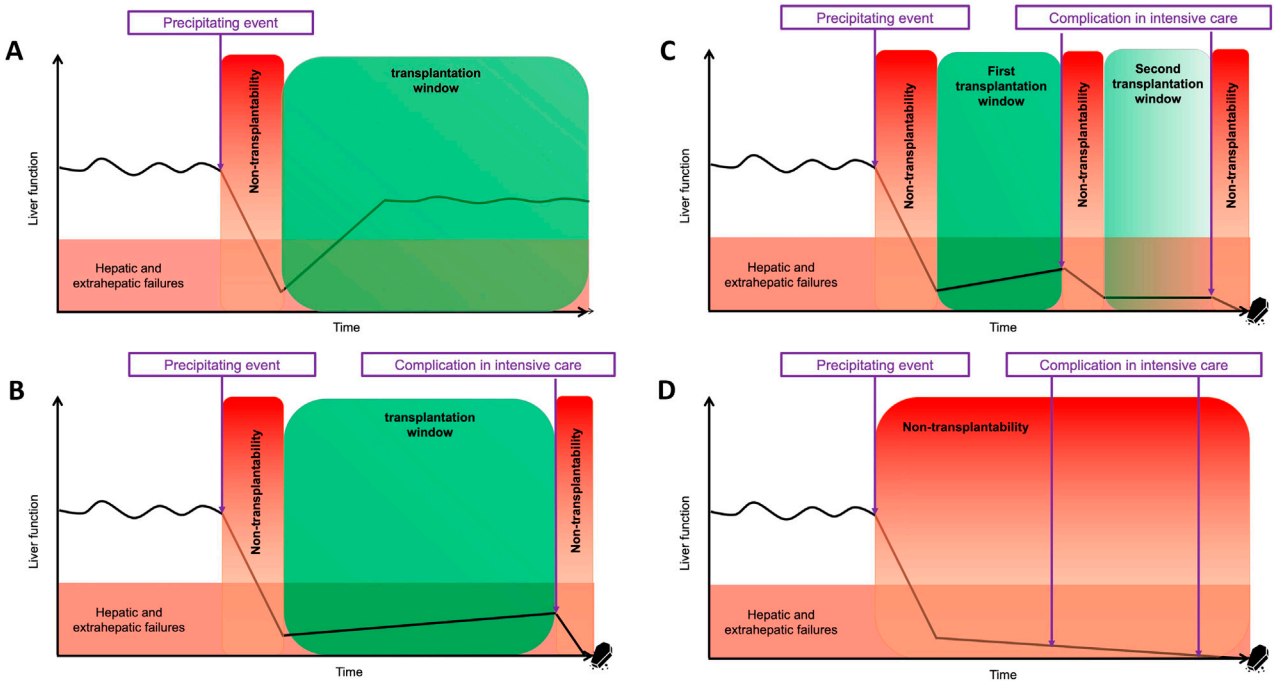


**FIGURE 1 |** Concept of the "transplantation window" during a critical care hospitalization in severe ACLF with different scenari. **(A)** Rapid improvement of severe ACLF: patients must be referred to a LT center for evaluation as 1-year transplant-free survival is between 20% and 60%. **(B)** One transplantation window. **(C)** Two transplantation windows. The first "transplantation window" is likely more favorable ("greener") than the second, as the patient presents with fewer complications related to hospitalization (e.g., deconditioning, colonization by multidrug-resistant bacteria, etc.). **(D)** Absence of transplantation window commonly due to uncontrolled sepsis and organ failure. *Adapted from Artru, Trovato et al., Lancet Gastroenterol. Hepatol. 2024.* [64].

with ACLF grade 3 at the time of transplantation increased from 66% in the 2007–2015 period to 86% in the 2015–2019 period [38]. These advancements underscore the progressive refinement of LT practices for ACLF, culminating in improved survival rates and patient care.

While there is substantial variability across liver transplant centers in the proportion of patients with severe ACLF ultimately placed on the waiting list, estimates suggest this figure ranges between 15% and 30% of patients initially considered as potential LT candidates [39]. Approximately 50% of patients are deemed unsuitable for transplantation after the initiation of the evaluation process, primarily due to comorbidities or issues related to addiction [40]. Following placement on the waiting list, an additional 30%–40% of patients with severe ACLF die before transplantation, contributing to a highly selective process [31, 41]. As a result, the outcomes presented in **Figures 1A–C** reflect those of a very carefully selected subgroup of patients who ultimately underwent LT in the setting of severe ACLF.

## PREDICTIVE FACTORS OF MORTALITY AFTER LIVER TRANSPLANTATION FOR ACLF

### General Considerations

First, several patient-related factors are predictive of mortality after transplantation in the context of severe ACLF (**Box 1**). Among them, age stands as one of the most important factors. Indeed, in patients with severe ACLF, the risk of mortality after LT progressively increases with age beyond 50 years. At the threshold of 60 years, this risk is increased by 70%–100% [27, 42]. The presence of diabetes has also been reported as a mortality risk factor, with a 40% higher post-transplant mortality rate [26, 43]. Body mass index (BMI), as a continuous variable, also appears to be associated with an increase in post-transplant mortality [43]. Although no dedicated study has been conducted in the population of patients with severe ACLF who are candidates for transplantation, it is suggested that the presence of cirrhotic cardiomyopathy (with a prevalence of approximately 30% in this population according to the revised 2019 criteria), particularly with a septal e' <7 cm/s, and a history of atrial fibrillation are factors associated with an increased risk of mortality after transplantation [44]. While the underlying cause of cirrhosis does not seem to impact LT outcomes, the Karnofsky index and malnutrition, assessed through sarcopenia, have both been independently associated with post-LT mortality [25, 45]. These parameters, along with frailty indices such as the Clinical Frailty Scale, which evaluate the patient's overall condition before transplantation, play a crucial role in the transplant team's decision-making process [46]. Finally, the presence of portal vein thrombosis, regardless of its extent and location, may also be associated with an increased risk of complications and post-transplant mortality [28].

**BOX 1 | Predictive factors of mortality after liver transplantation for ACLF. *Limited scientific evidence. Abbreviations: ICU, intensive care unit; PVT, portal vein thrombosis; DRI, donor risk index.**

**Patient-related factors on admission to ICU**

- Age (especially when ≥60 years)
- Diabetes mellitus
- Body mass index
- Cardiac risk factors (arrhythmias, severe valvular disease, coronary artery disease)
- Cumulative comorbidities as expressed in Charlson Comorbidity Index
- Frailty, malnutrition – sarcopenia
- PVT*
- Cirrhotic cardiomyopathy*

**Factors related to a patient's stay in ICU**

- Respiratory failure as per EASL definition (PaO2/FiO2 ≤200)
- Worsening organ failure, elevated arterial lactate (>4 mmol/L)
- Vasopressor use and multiple vasopressors requirement
- Infection with multidrug-resistant organisms during hospitalization
- Prolonged time in ICU to transplantation (>15 days)*

**Donor-related factors**

- High Donor Risk Index (e.g. DRI ≥1.7)
- Age of the donor
- Diabetes mellitus of the donor

Regarding variables dependent on the ICU stay, three key factors have been identified as robust predictors of LT outcomes (**Box 1**). (i) Presence of respiratory failure, defined by a $PaO_2/FiO_2$ ratio ≤200 or the need for intubation with mechanical ventilation, regardless of the underlying cause, is a major predictor of post-LT mortality [6, 25, 27, 47]. Consequently, in a patient considered for LT, successful weaning from mechanical ventilation before transplantation strongly supports proceeding with the procedure. While data on other organ failures are less robust, evidence suggests that renal replacement therapy and severe hemodynamic failure are also associated with a higher risk of post-transplant mortality. (ii) Evolution of OFs in the ICU. Stabilization or improvement of OFs before transplantation—particularly within the 48 h preceding LT—has been associated with better post-transplant prognosis. This is reflected in low arterial lactate levels on the day of LT (≤4 mmol/L) [6, 26, 48]. The presence of hemodynamic failure on the day of LT, illustrated by the use of one or two inotropes, also appears to be associated with 1-year post-LT mortality [43]. Currently, no specific norepinephrine dose threshold at the time of LT has been established in the literature beyond which transplantation would be considered unreasonable. Since they are frequently associated with acute deterioration of organ function, uncontrolled sepsis and active gastrointestinal bleeding are considered as definitive contraindications to LT [6, 46]. (iii) Time to transplantation. Favorable LT outcomes have been observed in studies where the median time from ICU admission or waitlist registration to transplantation ranges from 7 to 15 days [6, 27, 42]. Prolonged ICU stays not only increase the risk of mortality while on the waitlist but also would raise the likelihood of multidrug-resistant infections and further deterioration of the patient's nutritional and muscular condition advocating against proceeding LT in patients with prolonged ICU stay outside of some specific cases [49].

Finally, regarding donor-dependent variables associated with mortality, the Donor Risk Index (DRI) is a reliable predictor of both patient and graft survival in the U.S., regardless of the indication for transplantation (**Box 1**). In the context of LT for severe ACLF, a high DRI (≥1.7) has been associated with increased short- and long-term mortality risk [25, 26]. However, these findings have not been confirmed outside the U.S., particularly in Europe, even when using a score better suited to the European graft allocation system [27, 32]. For instance, in the French retrospective tricentric study, graft characteristics did not differ between ACLF grade 3 recipients and other patients (non-ACLF and ACLF grades 1 and 2), suggesting that the favorable outcomes observed were not related to superior graft quality. In practice, the combination of a limited number of organ offers and the urgent need for transplantation imposes constraints that prevent optimal graft selection for these patients.

Potential absolute contra-indication for LT in ACLF based on a Delphi of expert in the field has been highlight in **Box 2**.

**BOX 2 | Proposed absolute contraindications to liver transplantation in the context of ACLF. *Based on Delphi consensus from Weiss et al. Transplantation 2020. [34]. #Based on the consensus document on UK ACLF Tier Bernal et al., Lancet Reg Health 2024. [18]. Abbreviations: LT, liver transplantation; ARDS, acute respiratory distress syndrome; ECMO, extra corporeal membrane oxygenation.**

**Potential absolute contraindications to LT in the context of ACLF**

**Non modifiable – related to the patient**

- Frailty with Clinical Frailty Scale ≥7 before admission*

**Modifiable – related to the ICU stay**

- Norepinephrine requirement >1 γ/kg/min*
- ARDS with PaO2/FiO2 ratio < 150*
- Arterial lactate >9 mmol/L*
- Active bacterial or fungal sepsis*
- Severe irreversible neurological injury#
- Patient under Extra Corporeal Membrane Oxygenation (ECMO) device#
- Severe acute pancreatitis or intestinal ischemia#

## Concept of the Transplantation Window

The modifiable risk factors related to the ICU stay help to define a period known as the "transplantation window". The precise definition and prospective validation of its boundaries are still lacking. These boundaries identify periods of non-transplantability surrounding the transplantation windows. However, depending on the clinical context, the number and timing of potential transplantation windows, as well as periods of non-transplantability, may vary significantly and remain currently unpredictable (**Figures 1A–D**). It is crucial to discuss these boundaries within the multidisciplinary team for each patient individually taking into account the non-modifiable (patients-related) risk factors and to reassess them daily to optimize access to LT and improve post-transplant outcomes. Over the past 5 years, significant efforts have been made to better define specific boundaries linked to organ failures, which help delineate the optimal transplantation window and are now integrated into the scoring systems described below.

## Scoring Systems

The UCLA-FRS (University of California Los Angeles Futility Risk Score) has been specifically developed to identify predictors

of short-term mortality in patients with MELD score above 40 [44]. It incorporates the Charlson Comorbidity Index adjusted for age, the presence of septic shock prior to transplantation, and cardiovascular risk factors (including a history of arrhythmias, severe valvular disease, or significant coronary artery disease) [44].

The transplantation for ACLF-3 model (TAM) has been developed in patients with ACLF-3 at time of LT [47]. The TAM integrates age (cutoff: 53 years), respiratory failure (P/F ratio ≤200), lactatemia (cutoff: 4 mmol/L), and circulating leukocyte count (which is inversely associated with post-LT survival, with a cutoff of 10 G/L) [47]. It appears to be more predictive when calculated on the day of LT [47]. However, when recently evaluated in the French tricentric cohort, this score failed to retrospectively identify patients at risk of one-year mortality following LT [50]. Indeed, in this cohort—with a one-year survival rate of 84%—approximately 60% of patients had either an age ≥53 years or a leukocyte count ≤10 G/L, suggesting that these parameters might require further optimization.

A second specific prognostic model of patients with severe ACLF is the SALT-M score (Sundaram ACLF Liver Transplantation Mortality Score). It was published in 2023 to predict mortality after LT in patients with severe ACLF [43] and combines patient-related factors (age ≥50 years, diabetes, and body mass index) with modifiable ICU-related variables (respiratory failure and the need for vasopressors). Its predictive accuracy has been validated in an external French bicentric cohort, and it may contribute to defining the transplantation window [43].

However, while potentially useful, these scores should be considered within a comprehensive clinical approach rather than as a definitive "ultimate" decision-making tool, given their inherent uncertainty and the immediate, high-impact nature of therapeutic decisions in these critically ill patients.

## GENERAL MANAGEMENT IN POTENTIAL CANDIDATES TO LT ADMITTED TO ICU

Effective management of OFs is crucial to stabilizing patients with ACLF in anticipation of LT. Kidney failure, whether resulting from hepatorenal syndrome or other causes such as acute tubular injury, is the most frequently encountered organ failure in ACLF. In the intensive care setting, continuous renal replacement therapy is the preferred approach and can also aid in lowering ammonia levels in cases of severe hepatic encephalopathy as well as participating in hemodynamic stabilization [3, 51]. Circulatory failure should be addressed similarly to non-cirrhotic patients and guided by dynamic assessments, such as bedside echocardiography, to optimize fluid resuscitation and vasopressor use with norepinephrine as the first-line vasopressor and a target mean arterial pressure of 60–65 mmHg [52, 53]. Consideration of adrenal insufficiency, and cautious use of albumin (when indicated) or crystalloids are essential, while beta-blockers should be discontinued in cases of shock or renal failure [53, 54]. Patients with a Glasgow Coma Score of ≤8 require airway protection to prevent hypoxia and hypercapnia [55]. The use of benzodiazepines for sedation should be avoided. Due to the absence of specific mechanical ventilation guidelines for ACLF, management should adhere to general critical care principles, prioritizing lung-protective strategies (low tidal volumes and appropriate positive end-expiratory pressure (PEEP), particularly in ARDS patients) [53, 56]. For patients awaiting LT or receiving corticosteroids, screening for invasive fungal infection is essential; however, there are no formal recommendations for initiating empiric antifungal therapy. Any suspected bacterial infection warrants immediate and thorough investigation, followed by broad-spectrum empirical antibiotic therapy tailored to local epidemiology [57]. Antibiotic de-escalation should be considered based on clinical progression, microbiological findings, and the presence of multidrug-resistant organisms [3]. Finally, liver failure requires close monitoring of blood glucose levels and appropriate nutritional support, preferably via the enteral route. Nutritional goals should include an energy intake of 20–30 kcal/kg/day and a protein intake of 1.2–1.5 g/kg/day [3].

Furthermore, from the moment a patient with severe ACLF is admitted to ICU, the question of LT arises, along with the need for an addiction assessment in cases of alcohol-related cirrhosis. This assessment is crucial but can be challenging, particularly due to severe encephalopathy and/or orotracheal intubation. Information from the patient's primary physician and family is essential. However, there is currently limited data in the literature to predict the risk of alcohol relapse after LT in the context of severe pre-transplant ACLF.

Based on these considerations, a proposed algorithm for managing severe ACLF patients who may be eligible for LT is illustrated in **Figure 2**.

## PATIENT PRIORITIZATION

While OFs are the key determinant of outcomes in patients with ACLF, LT allocation policies worldwide are still largely based on MELD-derived systems, which do not account for extrahepatic organ dysfunctions. As a result, these patients are often disadvantaged compared to others with similar MELD scores but without OF outside the liver [58, 59]. These findings have been corroborated by preliminary analyses from the CHANCE study, which reported excess waitlist mortality among patients with a MELD score <30 and severe ACLF [31]. In countries where the median MELD score for access to LT is relatively low, this may be less problematic. However, in most allocation systems—especially in the context of increasing organ shortages—this may necessitate prioritization strategies to ensure graft allocation within a few days. In this regard, both Spain and the United Kingdom have taken the lead with either national or regional priority in patients with severe ACLF [30, 60]. In the UK, national priority listing can be requested for patients meeting specific high-acuity criteria. Eligible candidates include those with cirrhosis and liver failure characterized by jaundice and coagulopathy, in association with severe organ dysfunction necessitating intensive care support and an

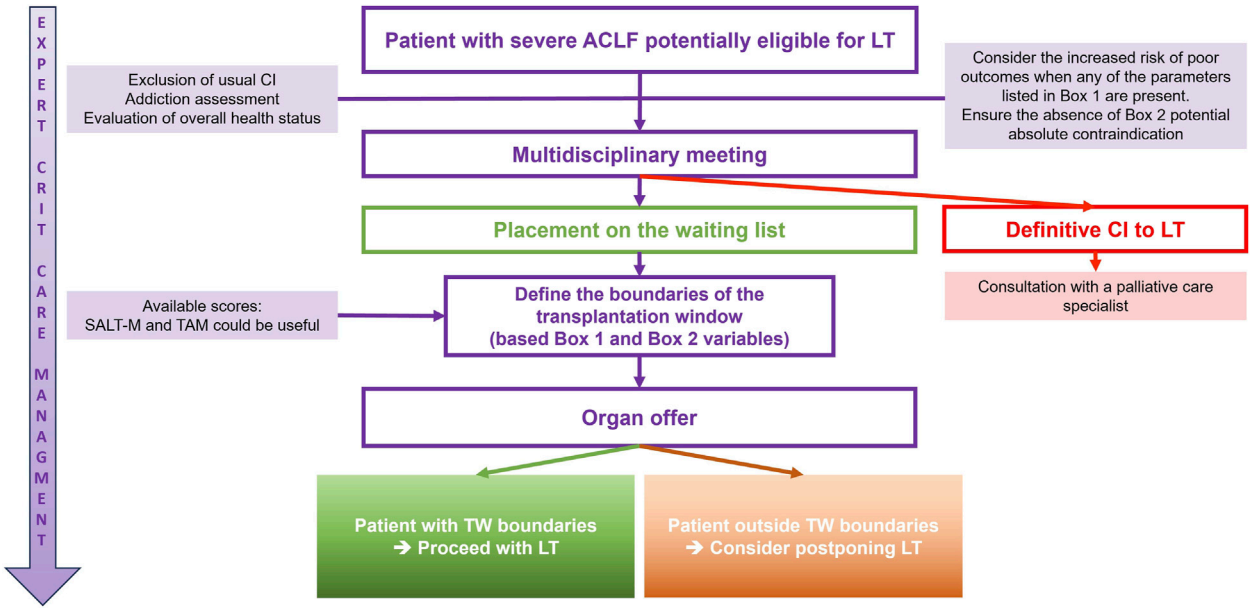


**FIGURE 2 |** Proposal for a management algorithm for a cirrhotic patient with severe ACLF and potential eligibility for liver transplantation (LT). Abbreviations: ACLF, Acute-on-chronic liver failure; CI, contraindication; LT, liver transplantation; SALT-M, Sundaram ACLF Liver Transplantation Mortality; TAM, Transplantation for ACLF-3 model *Adapted from Artru, Trovato et al., Lancet Gastroenterol. Hepatol. 2024* [64].

anticipated 28-day survival of less than 50%, typically corresponding to ACLF grade 3. Exclusion criteria comprise age over 60 years, comorbid conditions or ongoing alcohol use incompatible with standard LT, prior LT, active bacterial or fungal sepsis, CMV viraemia, severe irreversible neurological injury, advanced multi-organ failure with a poor trajectory, use of ECMO, significant frailty limiting rehabilitation potential, active malignancy, and severe acute pancreatitis or intestinal ischemia. In their recently published prospective studies, patients listed with national priority for ACLF received a transplant within a median of 3 days. Nevertheless, approximately 20% of candidates were not transplanted due to clinical deterioration—rates that are consistent with global reported dropout rates, even in regions with greater organ availability and in other liver transplant indications [30, 61].

Despite the growing body of evidence, ongoing debates persist regarding the inherent risks of prioritizing ACLF patients, as doing so could limit access to LT for patients with more classical indications [62]. This concern is particularly relevant in the current era, where cutting-edge research is expanding transplant eligibility to new oncological populations [63]. Although the utility of liver grafts in carefully selected oncological patients has been demonstrated, short-term outcomes for ACLF recipients—especially those transplanted from the ICU—remain inferior to those of patients with decompensated cirrhosis transplanted outside of critical care. This raises concerns about the overall impact on transplant outcomes should the indication for ACLF broaden significantly. Moreover, ACLF is a relatively common syndrome, and the potential surge in eligible candidates remains largely unexplored and difficult to predict. It is therefore crucial to identify and address the candidate-, ICU stay-, and graft-related factors contributing to poorer outcomes in this setting, with the aim of standardizing both short- and long-term outcomes. Doing so will help ensure graft utility and bridge the gap between clinical judgment and evidence-based science in candidate selection [62].

## UNMET NEEDS AND FUTURE PERSPECTIVES

### Organ Failure Severity and the Role of Unmeasured Variables

The limited number of studies with granular data has thus far hindered the identification of well-defined thresholds for OF severity associated with unacceptable outcomes in LT candidates with severe ACLF. For example, while the use of two vasopressors has been associated with a 3.6-fold increase in 1-year mortality [43], translating this finding into clinical practice remains challenging due to variability in vasopressor use thresholds across centers. Similarly, although a $PaO_2/FiO_2$ (P/F) ratio ≤200 is strongly linked to poor prognosis, no studies have formally investigated whether further deterioration (e.g., P/F ≤ 150) worsens outcomes—or conversely, whether improvement to a P/F ratio >250 meaningfully improves prognosis. Moreover, beyond the P/F ratio itself, it would be informative to investigate whether the specific causes of hypoxemia—such as pleural effusion, pulmonary edema, or infectious consolidation—are associated with clinical outcomes. Prospective data from the CHANCE study will certainly help address some of these critical knowledge gaps. However, given the relatively low

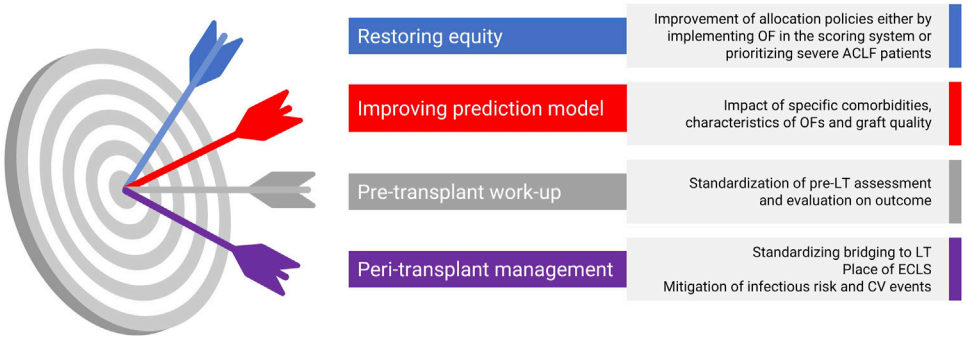


**FIGURE 3 |** Key unmet needs and future directions in liver transplantation for ACLF. Summary of four priority areas to improve LT outcomes in ACLF: (1) restoring equity via adapted allocation policies; (2) refining prediction models by integrating comorbidities, OF profiles, and graft quality; (3) standardizing pre-LT evaluation; and (4) optimizing peri-transplant management, including bridging strategies, ECLS use, and mitigation of infectious and CV risks. Abbreviations: ACLF, acute-on-chronic liver failure; LT, liver transplantation; OF, organ failure; ECLS, extracorporeal liver support; CV, cardiovascular.

mortality rate observed in this population, a very large sample size of transplanted patients would be required to achieve adequate statistical power. Moreover, the exhaustivity of collection of variables potentially influencing outcomes in this setting will be critical. This will be specifically the case of factors such as comorbidity scores, sarcopenia, frailty, the clinical trajectory in the final hours preceding transplantation or the experience and expertise of the transplant center. Finally, even with the collection of detailed data, it is important to note that the SALT-M score—despite being derived from a large and granular dataset—has an $R^2$ value below 20%, meaning that it explains less than one-fifth of the variability in outcomes [43]. Although the CHANCE study may improve the predictive performance of existing models, it remains essential to stay cautious and acknowledge the limitations of scoring systems to predict mortality risk following LT. At this stage and given the major implications of the decision to proceed with LT, such systems should not be regarded as the final arbiter but rather serve as supportive tools to inform and guide clinical judgment.

## Pre-transplant Assessment

In patients with ACLF undergoing LT, two clinical scenarios are typically encountered. About one-third experience ACLF while already listed for transplantation, having undergone standard pre-transplant assessments [30, 39, 64]. In contrast, many patients are first considered for transplantation only after ICU admission, necessitating a rapid evaluation during ongoing critical illness—a process for which specific guidelines are currently lacking. Recent data have shown that the pre-transplant work-up in ACLF patients is often abbreviated compared to other transplant candidates, typically limited to transthoracic echocardiography, body CT, upper gastrointestinal endoscopy, and PSA testing in male patients [65]. While this streamlined work-up allows for rapid listing, it may come at the cost of reduced sensitivity in identifying contraindications—particularly cardiovascular ones—given the elevated risk of cardiovascular-related mortality in this population. The potential role of non-invasive approaches—such as coronary CT angiography or newly developed tools like the Coronary Artery Disease in Liver Transplantation (CAD-LT) score—to identify patients at highest risk for significant coronary artery disease requiring invasive angiography prior to LT warrants further investigation, particularly in this unstable population for whom transport are often challenging [66]. Finally, thorough assessment of the addiction profile warrants dedicated investigation to help standardize access to LT, especially when direct discussion is not possible due to encephalopathy and/or intubation. In particular, the relative importance of input from relatives, the general practitioner, and the addiction specialist who followed the patient prior to hospitalization appears crucial and should be formally investigated.

## Peri-Transplant Management

A major unmet need in the management of ACLF lies in the standardization of the management of the peri-transplant period, particularly as a bridge to transplantation. Extracorporeal liver support systems, such as plasma exchange (PLEX), represent a promising area of investigation. The ongoing APACHE phase 3 randomized trial (NCT03702920) is currently evaluating high-volume plasma exchange with albumin 5% (PE-A5%) in combination with standard medical therapy in patients with moderate-to-severe ACLF. Building on encouraging pilot data, APACHE is designed to assess whether PE-A5% can improve survival and organ function in these high-risk patients, with the potential to enhance transplant eligibility and post-transplant outcomes. Renal replacement therapy (RRT), often necessary in ACLF due to frequent kidney involvement, must also be integrated into a multimodal support strategy, alongside tailored nutritional support to address the severe catabolic state of these patients. The integration of Enhanced Recovery After Surgery (ERAS)-inspired bundles of care—including early mobilization, optimized hemodynamic monitoring, and protocolized organ support—could contribute to better peri-transplant conditioning and reduce postoperative complications however their applicability to this specific population remains uncertain [67]. In parallel, particular attention should be paid to mitigating cardiovascular risk

during the perioperative period. While optimizing pre-transplant cardiovascular assessment is essential, early and comprehensive post-transplant evaluation should also be systematically considered. This delayed but targeted approach may help detect silent coronary artery disease and reduce the risk of early post-transplant cardiac complications. Furthermore, infection remains a leading cause of mortality in ACLF and post-transplant periods, necessitating a more personalized immunosuppression strategy. Emerging approaches based on immune functional markers, such as monocytic HLA-DR expression, may allow for dynamic risk stratification and individualized immunomodulation [68]. These strategies represent critical components in the development of a comprehensive, patient-centered peri-transplant management paradigm in ACLF.

Unmet needs and future directions have been summarized in the **Figure 3**.

## CONCLUSION

LT remains a cornerstone of treatment for well-selected patients with ACLF. Accumulated data suggest that LT in ACLF leads to an acceptable survival rate at 1 year (often exceeding 80%) and at 5 years (76%). These outcomes are influenced by several factors, including the grade of ACLF, the timing of transplantation, and patient-specific characteristics such as age, diabetes, and comorbidities like cirrhotic cardiomyopathy. Predictive factors for post-transplant mortality are crucial in identifying patients who are most likely to benefit from LT, with the severity of OFs during ICU admission playing a key role.

However, many questions remain unresolved regarding the management of ACLF patients in the transplant setting. Specifically, the impact of various thresholds for OF severity on outcomes and the role of unmeasured variables, such as frailty and sarcopenia, require further exploration. Additionally, the challenge of managing patients who rapidly deteriorate and require urgent LT underscores the need for tailored pre-transplant assessment protocols and guidelines.

Further research is essential to optimize patient selection, refine prediction models, and better understand the long-term outcomes for these high-risk transplant recipients. The evolving landscape of LT in ACLF calls for a balanced approach, ensuring equitable access to liver transplants while maintaining graft utility and improving overall patient survival.

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FA, Conceptualization: Lead; Writing – original draft: Lead; Writing – review and editing: Lead. SL'H and VC, Conceptualization: Supporting; Writing – original draft: Lead; Writing – review and editing: Supporting. All authors contributed to the article and approved the submitted version.

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

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

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REVIEW
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# The Progress and Challenges of Implementing HLA Molecular Matching in Clinical Practice

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HLA molecular matching in solid organ transplantation in the form of eplets, solvent-accessible amino acids or PIRCHE-II has been proposed as a more granular method than HLA matching on the antigen level. While many studies have shown the association between molecular mismatches and *de novo* donor-specific antibody formation, rejection and graft loss, evidence for prospective molecular matching in allocation is currently lacking, and the actual practical implementation and feasibility of molecular matching remains unclear. In this review the various potential applications of molecular matching in transplantation are discussed, including 1) organ allocation in deceased donor programs, 2) living donor selection, 3) increasing the transplantability of highly sensitized patients and 4) risk stratification to facilitate personalized immunosuppressive management, along with the challenges and gaps in current knowledge regarding these approaches. While clinical application of molecular mismatch analysis in solid organ transplantation holds promise, the fundamentals of HLA-specific antibody biology and epitope-paratope interactions should be further elucidated. This will aid in unraveling the factors that affect the relative immunogenicity of HLA molecular mismatches in order to start using molecular matching in clinical transplantation.

**Keywords: HLA, eplet, kidney transplantation, molecular mismatch, organ allocation**

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## HIGHLIGHTS

- Molecular mismatch analysis can be used to increase the chance of finding a suitable donor for highly sensitized patients.
- Data regarding eplet frequencies in different populations are required to explore the consequences of eplet-based allocation algorithms for ethnically diverse populations.
- Antibody verification is essential to identify clinically relevant molecular mismatches to be used for deceased donor allocation.
- For deceased donor allocation, high resolution HLA typing is required at the time of allocation.
- Clinical studies with clear pre-defined outcome measures are required to investigate the application of molecular mismatch analysis in kidney paired exchange programs for living donation.
- The application of molecular mismatch analysis for post-transplant risk stratification to guide tailored immunosuppression should be investigated in prospective clinical trials.
- The fundamentals of HLA-specific antibody biology and epitope-paratope interactions need to be further elucidated in order to unravel the factors that affect the relative immunogenicity of molecular mismatches.

## INTRODUCTION

After the discovery of the HLA system in the 1950s, Paul Terasaki demonstrated the correlation between HLA matching and kidney allograft survival in 1966 [1]. Since then, HLA matching has been one of the cornerstones of transplantation and transplant organizations across the world have included HLA matching in their algorithms for organ allocation [2], aiming to minimize the chance of rejection and the development of *de novo* donor-specific antibodies (DSA). In Eurotransplant, current HLA matching occurs on HLA-A, -B (broad antigen level), and -DR (split antigen level) only, with priority for full-house matches, and a point system for all other instances [3]. However, due to the high polymorphism of HLA and the scarcity of donor organs, the majority of kidney transplant recipients receive a graft with a certain degree of HLA antigen mismatch [4]. Refinement in HLA typing techniques and amino acid sequence alignments have demonstrated that the high level of polymorphism of HLA can be explained by a few hundred polymorphic amino acid configurations, which are often referred to as epitopes [5, 6], despite this actually being a faulty use of nomenclature [7]. While these amino acid configurations can be shared between different HLA molecules, every individual HLA molecule is comprised of a unique set of these polymorphic sites (**Figure 1**). In theory, this means that an HLA antigen mismatched graft could be actually fully matched on the level of amino acid configurations (**Figure 2**), which is referred to as molecular matching. In 2006, Rene Duquesnoy introduced the term "HLA eplet" to describe a configuration of antibody accessible polymorphic amino acids within a 3.5 Ångstrom radius on the HLA molecule, that can be recognized by a B cell receptor through interaction with the CDR-H3 region [9]. Since then, various methods have been developed for HLA molecular match analysis, but HLA eplets, as described in the HLA Eplet Registry and incorporated in HLAMatchmaker [10], remain most well-known. HLA molecular matching has been proposed as a more feasible method to prevent *de novo* DSA (dnDSA) formation than HLA matching on the antigen level [6, 11, 12], and many studies have shown the association between eplet mismatches and dnDSA formation, rejection and graft loss [13–17]. However, since evidence for a beneficial effect of prospective eplet matching in allocation is currently lacking, the actual practical implementation and feasibility of molecular matching remains unclear. In fact, the application of eplet matching in transplantation has even been deemed premature and the question has been raised whether molecular matching will actually reduce the complexity of HLA matching [7]. In this review the various potential applications of molecular matching in transplantation will be discussed, including 1) organ allocation in deceased donor programs, 2) living donor selection, 3) increasing the transplantability of highly sensitized patients and 4) risk stratification to facilitate personalized immunosuppressive management, along with the challenges and gaps in current knowledge regarding these approaches.

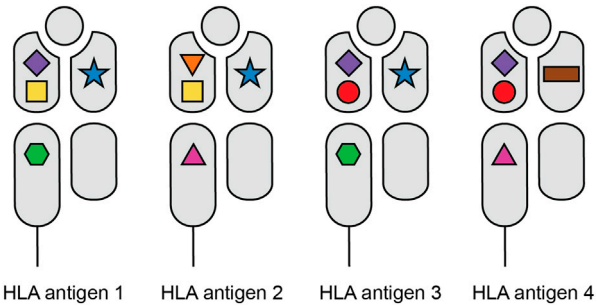


**FIGURE 1 |** HLA polymorphism on the level of amino acid configurations. The various shapes depict the different amino acid configurations on the HLA molecules. While each HLA antigen expresses a unique set of these polymorphic sites, the individual amino acid configurations can be shared between different HLA molecules.

## IDENTIFICATION OF CLINICALLY RELEVANT EPLETS IS REQUIRED FOR CLINICAL APPLICATION OF HLAMATCHMAKER

The lack of empirical evidence for clinically relevant eplets is the one of the main obstacles for implementation of eplet matching in transplantation. As the current eplet repertoire has been theoretically defined based on HLA amino acid sequences and not on proven immunogenicity, the question remains which eplet mismatches are immunogenic and which are permissible [7, 18]. The identification of clinically relevant eplets is a crucial condition for the modification of allocation algorithms towards eplet-based matching, as denial of an organ offer based on eplet mismatches which are not clinically relevant would be unacceptable.

Antibody verification is currently the only method to validate that specific polymorphic amino acids (translated to eplets) can actually be bound by antibodies. With the lack of better techniques, this measurement of antigenicity is translated to presumed immunogenicity. Much experimental evidence has been gathered over the years, which has been summarized in the HLA Eplet Registry, an online database of HLA eplet data [19–21]. A recent review of the HLA Eplet Registry provides insight in the different methods that have been used for antibody verification, and demonstrates that not all eplets considered antibody-verified by the HLA Eplet Registry were verified based on high quality peer-reviewed research [22], which has prompted an adaptation of the Registry. It also showed that especially for HLA class II, there are several theoretical eplets for which no antibody verification has been performed yet. Therefore, antibody verification of eplets using human HLA-specific recombinant monoclonal antibodies (mAbs), and adsorption/elution studies is still an ongoing and useful effort [23–27].

Antibody verification of eplets using mAbs and eluted antibodies after adsorption from serum is based on the

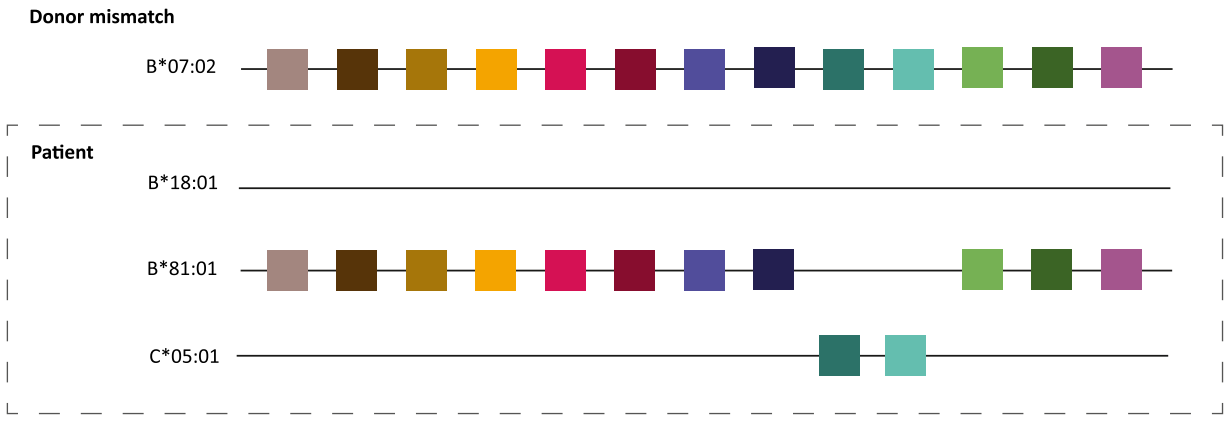


**FIGURE 2 |** HLA molecular matching principle. The colored squares depict amino acid configurations or eplets present on the donor B*07:02 that are lacking on patient B*18:01. Although there is one HLA antigen mismatch between donor and recipient, there are no mismatches on the molecular level due to shared amino acid patches between the donor allele and the recipient's HLA B*81:01 and C*05:01 alleles. Adapted with permission from "Principle of HLAMatchmaker is inter and intra-comparison of triplets. In this particular patients all triplets mismatched between HLA-B18 and HLA-B7 are shared by the other HLA alleles of the patient. Final interpretation: no foreign triplets on B18 and therefore no alloantibody induction" by Claas et al. [8], licensed under CC-BY-NC-ND.

identification of uniquely shared amino acids present on the reactive HLA alleles in the single antigen bead (SAB) assay. However occasionally, even for mAbs, the reactivity analysis in the SAB assay leads to the identification of multiple uniquely shared amino acids that are not located within 3.5 Ångstrom from each other, and therefore cannot be part of a single eplet. Since in these cases the amino acid residues involved are simultaneously present on all reactive HLA alleles in the SAB kits, it is not possible to determine which of these residues are truly crucial for antibody binding. Site-directed mutagenesis of wildtype HLA molecules is an elegant strategy to tackle this problem and has been recently used to narrow down an HLA-A1-specific antibody reactivity pattern consisting of three distant amino acids to a single amino acid [28]. Additionally for HLA class II, CRISPR-Cas9 modified cells have provided insight in crucial regions for the binding of an HLA-DQ specific antibody with its target HLA [29].

One of the exciting next steps in further understanding the fundamental biology of HLA antigen and antibody interaction, is the actual visualization of antibody binding to its target HLA using crystal structures or cryogenic electron microscopy (cryo-EM). Such studies can further inform on the correctness of eplet definition and verification. Moreover, these approaches will increase our understanding of the electrostatic and physiochemical properties of amino acids that are relevant for immunogenicity [30–32]. Recently, the first crystal structure of an HLA-A*11:01-specific antibody bound to its target HLA has been reported [33]. The characterization of the epitope-paratope interaction demonstrated that the single amino acid that was predicted to be crucial for antibody binding, was indeed part of the epitope. Importantly, while this study showed the power of crystal structure analysis, the described HLA-A*11:01-specific antibody was generated using a phage library, and may not represent an antibody developing during a human immune response to mismatched HLA. Currently, studies are ongoing to characterize the binding of fully human HLA-DR and -DQ-specific mAbs using cryo-EM, which allows for visualization of the binding of these mAbs to their target molecules. A comprehensive overview on epitope-paratope biology and the impact on antibody binding has been published elsewhere [34].

Since antibody verification of eplets using human mAbs and adsorption/elution studies on serum samples is a slow and laborious process, ideally a prediction algorithm of HLA epitope immunogenicity should be developed. Preferably, this algorithm would incorporate all existing evidence generated by the reactivity patterns of HLA-specific mAbs, the identified crucial amino acids by mutagenesis and crystallography or cryo-EM, and the physiochemical and electrostatic properties of the amino acids involved. Additionally, the role of T cell epitopes in the humoral alloimmune response has to be investigated further, as T cell help is required for the initiation of a long-lived antibody response [35]. Although predicted T cell epitopes (PIRCHE-II) have been associated with graft failure in kidney transplantation [36], the approach of predicting HLA-derived T cell epitopes presented in recipient HLA class II molecules is at the moment merely based on the underlying amino acid differences between HLA alleles and relatively low peptide binding affinity to HLA class II. Currently, there is no experimental evidence on whether the predicted peptides derived from allogeneic HLA are actually generated in the lysosomal compartment. Furthermore, by setting a relatively low binding strength threshold, it remains to be determined which predicted peptides are actually presented by HLA class II molecules *in vivo*.

Ultimately, a comprehensive model of HLA immunogenicity for the induction of a humoral immune response should incorporate the antigen-specific crosstalk between B cells and T cells. Molecular mismatch scores on the B and T cell level have been combined in several studies, hinting towards clinical relevance of taking both arms of the immune system into consideration [37–39]. However, these studies have merely combined molecular mismatch scores, irrespective of which specific allele these disparities occurred. Studies where true

linked recognition between B cells and T cells is taken into consideration are currently lacking.

Finally, the level of immunogenicity of individual eplets is not merely based on amino acid mismatches, their physiochemical properties and the availability of T cell help, but also dependent on the population that is investigated. This calls for studies that investigate the differential immunogenicity of eplets in large datasets of various and ethnically diverse populations. The differential immunogenicity of molecular mismatches across transplant populations worldwide is one of the topics of the 19th International HLA & Immunogenetics Workshop that will be organized in 2026.

## DECEASED DONOR ALLOCATION

To date, only one small pilot study from 2016 has prospectively investigated incorporating eplet mismatch loads into deceased donor allocation for 19 pediatric kidney transplant patients. By determining individual eplet mismatch thresholds for each patient at the time of listing, recipients received a significantly lower number of HLA Class II eplet mismatches compared to the general allocation scheme [40]. While the numbers were small, low rates of dnDSA within the first 12 months are promising. However, a major limitation in the chosen strategy is that high-resolution typing of deceased donors at the time of allocation was not available, which poses one of the major obstacles on the road towards implementation of molecular matching in transplantation. For proper molecular mismatch analysis for the individual patient, high-resolution HLA typing of both the donor and potential recipient(s) is required. While many transplant centers have introduced high-resolution typing for the living donor transplantation setting, high-resolution typing is not yet routinely performed for deceased donor transplantation in most transplant centers. Importantly, HLA typing on the second field level is not only more costly than low-resolution typing, but also takes more time to complete. Most commercial kits offering high-resolution typing based on next-generation sequencing take 1–5 days for completion [41], making it an unsuitable technique for the typing of deceased donors. Nonetheless, a recent study described a high-resolution typing method using Nanopore sequencing, which resulted in high-resolution typing for 11 loci within 4 h, indicating that second field typing for deceased donors within the timeframe of allocation is within reach [42]. Several commercial companies are currently optimizing the Nanopore sequencing workflow for deceased donor typing.

A provisional solution for the lack of high-resolution typing is imputation of second field typing based on low-resolution haplotypes [43]. This method has been applied frequently in retrospective cohort studies investigating molecular mismatch association with transplant outcomes. In the absence of true high-resolution HLA typing data, this approach is justifiable for large retrospective studies. However, as this method can lead to inaccuracies in molecular mismatch estimations, it is not deemed suitable for individual patients in the clinical setting [44, 45].

Secondly, as HLA allele frequencies vary considerably amongst different populations in the world [46], this inherently means that frequencies of specific molecular mismatches will differ across different populations. Eplet repertoire variation has to be taken into account in studies that investigate differential immunogenicity of individual eplets, because a very high or very low frequency of a given eplet in a population can skew immunogenicity scores, which may consequently not be applicable to other populations [47]. The fact that this issue needs consideration is illustrated by the situation in the United States, where it became clear that African Americans were disadvantaged regarding access to kidney transplantation due to HLA matching requirements [48]. Subsequently, priority for HLA-A and HLA-B matching was eliminated in the kidney allocation system of the United Network of Organ Sharing [49, 50]. Data regarding eplet frequencies in different populations are required, so that the consequences for the implementation of eplet-based allocation algorithms for ethnically diverse populations can be investigated. In this light, a recent study investigated eplet frequencies across six different ethnic groups using HLA alleles included in the Common, Intermediate and Well-Documented (CIWD) 3.0.0 catalogue and demonstrated that 98.6% of eplets are present on the common HLA alleles in all ethnic groups [51]. It should be noted however that HLA allele frequencies (and thus eplet frequencies) may be different in donor and recipient populations.

Related to the issue of equity is the concern that molecular matching would lead to longer waiting times on the transplant waiting list, especially for ethnic minorities. As quality of life is poor for patients on dialysis, and the survival benefit of transplantation as compared with dialysis is significant [52], it would not be acceptable that improved HLA compatibility by utilizing molecular matching would be at the expense of longer waiting times. Currently, data are scarce on the consequences of molecular matching for the kidney transplant waiting list regarding waiting times and equity for ethnic minorities. A recent simulation study from the United States suggested that eplet-based allocation prioritizing HLA-DQ eplet matching was equitable for Black and Hispanic candidates, but this was not the case for Asian patients [53]. Additionally, a Canadian simulation study in a highly ethnically diverse population demonstrated that eplet matching would allow for better matched grafts by converting alleles to eplets, and that eplet matching would be feasible even within a waiting list of only 250 patients [54]. However, neither of these studies included simulations regarding waiting time. An allocation simulation study investigating the effect of T cell epitope matching on waiting times in the Eurotransplant region found that this approach did not significantly impact waiting times [55].

## HIGHLY SENSITIZED PATIENTS AND RETRANSPLANTATION

Although the road to implementation of molecular matching in deceased donor allocation algorithms still seems long, molecular mismatch analysis can already be utilized to increase the chance of finding a suitable donor for highly sensitized patients. Since the chance of finding a donor for these patients is very slim due to the large number of unacceptable antigens, the Acceptable Mismatch (AM) Program was launched in the Eurotransplant region in 1989 [56]. Unlike regular allocation, the AM program is based on finding a donor that is compatible with the patient's HLA type

plus acceptable antigens, which are defined as antigens to which the patients has not developed antibodies [57]. Defining additional acceptable antigens based on triplet sharing was in fact the primary concept behind HLAMatchmaker, as described in the early 2000s by Rene Duquesnoy and Frans Claas [58, 59]. In the present day of SAB technology, eplet analysis can be utilized for the definition of acceptable antigens: by analyzing SAB data from the highly sensitized patient, eplets present on antigens towards which no antibodies have been formed can be extrapolated to antigens not tested in SAB assays, to maximize the number of acceptable antigens defined [58]. Furthermore, eplet analysis can assist in determining which of the unacceptable antigens as established by SAB analysis can be explained by a previous immunizing event (such as a previous transplantation, pregnancy or blood transfusion), and are therefore truly unacceptable. If the reactivity cannot be explained by an immunizing event or a shared eplet thereof, this bead reactivity might be background or non-specific binding. Such information should prompt further investigation of the reactivity (for instance using flow-cytometry cross match), in order to decide whether this antigen should be listed as unacceptable, or rather be considered as a risk factor, taking into account MFI value of the reactive antigen [34]. A recent study from Portugal showed that calculation of an eplet-based virtual PRA increased the transplant probability for highly-sensitized patients [60].

In line with the aforementioned, a recent study investigated patients with pre-existing so-called "donor epitope-specific antibodies (DESA)" and showed that clinically relevant DESA were associated with increased risk on graft loss in deceased donor transplantations [61]. Analysis of "molecular mismatch-specific HLA antibodies" might be of particular interest in patients that undergo retransplantation, as shown in a recent study where retransplant patients with pre-existing DSA that target a repeated molecular mismatch (i.e., antibody verified eplets), had lower graft survival and higher ABMR rates than patients with DSA that were not directed against a repeated molecular mismatch [62]. Data on the clinical relevance of repeated HLA mismatches in retransplant candidates are scarce. A recent study demonstrated that repeated HLA-DRB1 and HLA-DQB1 mismatches on the split antigen level in the absence of pre-existing DSA affected DSA formation, rejection and graft survival [63]. Unfortunately, no analysis on the molecular mismatch level was performed. A case report of a female transplant recipient that was highly sensitized by three pregnancies described that she developed ABMR due to a repeated eplet mismatch between her husband and her donor [64]. More studies investigating the clinical relevance of repeated mismatches on the molecular mismatch level are required.

## LIVING DONATION

While the previous section was predominantly related to deceased donor transplantation, determining the molecular mismatch level can also be of value in living donor transplantation. Primarily in patients that are likely to require a retransplantation later in life (*e.g.*, pediatric patients), it is critical to limit the chance of dnDSA formation at the first transplantation, so that there are no pre-existent HLA-specific antibodies that can impede a repeat transplantation at a later timepoint. In cases where there are multiple potential (otherwise comparable) donors, molecular mismatch analysis can inform on which mismatched donor HLA has the lowest chance of inducing an antibody response (**Figure 3**). Furthermore, molecular mismatch analysis of prospective transplant recipients patients that have a compatible potential living donor could inform clinicians to explore whether entering a kidney paired exchange program might offer a lower immunological risk. To date only one study has prospectively investigated eplet matching in a kidney paired exchange program. In this study, results from seven pediatric patients included in the Australian Kidney Exchange were reported. According to the authors, all patients were transplanted with a lower immunological risk compared to their registered donors. However, three patients did develop DSA within a median follow-up period of 12 months [65]. Furthermore, the National Kidney Registry in the United States, an organization that facilitates hundreds of paired exchange transplantations annually, has been promoting eplet matching on their website for several years [66]. The first results of this program are expected to be published soon.

With such limited data, the use of molecular mismatch analysis in kidney paired exchange programs should further be methodically investigated in clinical studies. Regardless, other factors besides HLA will contribute to the definitive selection of a living donor, including blood group, age, medical history, and psychological and social factors. Which method of molecular mismatch calculation (i.e., eplets, solvent-accessible amino acids or PIRCHE-II) will be most appropriate for donor selection will have to be demonstrated in clinical studies.

## POST-TRANSPLANT RISK STRATIFICATION

Identification of immunogenic and permissible eplet mismatches is essential for the implementation of molecular matching in deceased donor allocation schemes and molecular mismatch analysis for living donor selection. However, deciphering immunogenicity of individual eplet mismatches may be less relevant for utilizing eplet mismatch levels for post-transplant risk stratification, as eplet mismatches would be merely used as a tool to assess the risk for immunological rejection after transplantation, rather than being incorporated into organ allocation and donor selection.

Many studies have demonstrated that eplet mismatch loads are associated with the risk of DSA formation, rejection and graft loss after transplantation on the cohort level [13–15, 17, 67, 68]. Furthermore, Wiebe et al. have shown that HLA class II eplet mismatch load was associated with the tacrolimus trough levels that are required to prevent DSA formation [69]. In a cohort of 596 kidney transplant recipients, HLA-DR/DQ eplet mismatch was a predictor of dnDSA development and patients with a high

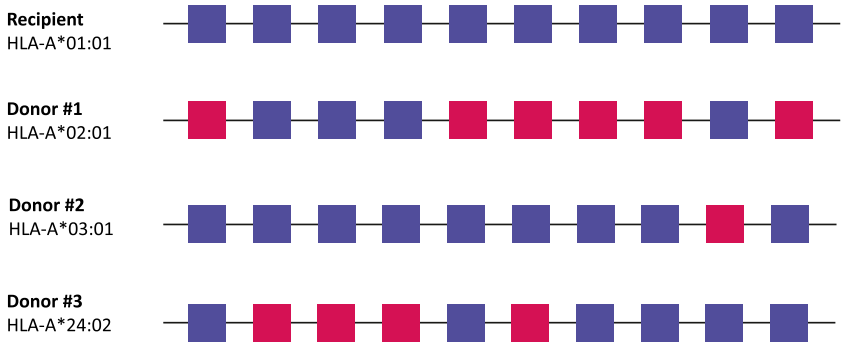


**FIGURE 3 |** Living donor selection based on molecular mismatch levels. In this example, every potential donor has a single HLA antigen mismatch with the recipient, but a different number of antibody-verified eplet mismatches. In this case, donor #2 would be the best option for the recipient, as there is only one antibody-verified eplet mismatch with the recipient. Antibody-verified eplet mismatches are assessed taking into account the full HLA typing of the recipient. Squares: antibody-verified eplets. Blue, matched eplets; red, mismatched eplets.

**TABLE 1 |** Methods for HLA molecular mismatch analysis.

| | |
|---|---|
| HLAMatchmaker | Excel program that compares the amino acid sequences of donor and recipient HLA alleles to identify mismatched eplets. An eplet is defined as a cluster of polymorphic amino acid residues within a 3.0–3.5 Ångstrom radius.[a] eplets have been incorporated in several applications, such as the eplet Registry, EpVix, and commercial software from SAB vendors |
| Amino Acid Mismatch Score (AAMS) | Online application that compares HLA sequences from the extracellular domain to calculate the number of polymorphic amino acids for a given donor HLA mismatch in the context of recipient HLA[b] |
| Electrostatic Mismatch Score (EMS3D) | Online application that quantifies differences in tertiary structure and surface electrostatic potential between donor and recipient HLA molecules[c] |
| HLA-EMMA | Software that calculates the number of solvent-accessible amino acid mismatches between donor and recipients. Solvent-accessible amino acids are defined as residues that are accessible for the B cell receptor and could therefore potentially interact with the B cell receptor, as well as with antibodies[d] |
| Snowflake | Algorithm that analyses the surface area of mismatched amino acids while taking HLA protein-specific structural disparities into consideration. Recently extended with an algorithm (Snowball) that predicts local ellipsoid protrusion ranking aiming to enhance the accuracy of prediction of solvent accessibility (together "Snow")[e] |
| PIRCHE-II (predicted indirectly recognizable HLA epitopes presented by HLA class II) | Online-available *in silico* model that predicts HLA-derived peptides from mismatched HLA that can be presented in self-HLA class II molecules. While HLAMatchmaker, EMS3D, HLA-EMMA and Snow are based on epitopes recognized by B cells, PIRCHE-II quantifies the number of theoretical T cell epitopes presented in HLA class II[f] |

[a]*RJ, Duquesnoy. Hum Immunol (2002) 63(5):339–52.*
[b]*Kosmoliaptsis et al. Transplantation (2009) 88(6):791–8.*
[c]*Mallon et al. J Immunol (2018) 201(12):3780–92.*
[d]*Kramer et al. HLA (2020) 96(1):43–51.*
[e]*Niemann et al. Front Immunol (2025) 16:1,548,934.*
[f]*Geneugelijk et al. Front Immunol (2018) 9:321.*

number of eplet mismatches were less likely to tolerate low tacrolimus levels without developing dnDSA. As there still is an unmet need for tools that could guide personalized immunosuppressive therapy in transplantation [70, 71], HLA molecular mismatch level in the form of amino acids, eplets or PIRCHE-II could potentially be used as a parameter in post-transplant risk stratification. However, there are several challenges considering the application of molecular mismatch for this purpose.

Firstly, it is unclear which HLA loci should be considered in post-transplant risk stratification. The majority of studies have reported data on HLA class II, since most dnDSA are directed at HLA-DQ mismatches. However, it is not clear if only HLA class II, or even only HLA-DQ should be considered, or that all classical HLA loci should be taken into account (Meziyerh et al., submitted).

Secondly, as eluded above, there are several methods for calculating HLA molecular mismatch loads [34] which all have been associated with transplant outcomes like DSA formation and graft survival. The use of different computer programs, including HLAMatchmaker, PIRCHE-II [36], the Electrostatic Mismatch Score (EMS) [32] and HLA-EMMA [72], makes it

difficult to compare studies, because each method will result in different optimal cutoff values and ranges (see **Table 1** for an overview of the most used methods). Moreover, the eplet repertoire as analyzed by HLAMatchmaker has changed over the last years, which resulted in different eplet mismatch thresholds in different studies [69]. This heterogeneity impedes the ability to draw conclusions leading to thresholds that can be validated in other cohorts. The vast diversity in the primary outcomes of the aforementioned retrospective studies (ranging from allograft survival at 10 years [73], to ABMR and DSA at 5 years [17] and ABMR, TCMR and dnDSA at 10 years [15]) emphasizes the need for prospective studies with clear pre-defined outcome measures.

Lastly, the definition of a cutoff value that divides a study population between low risk and high risk, results in the possibility that when this threshold will be applied in a general population, a patient who received a graft bearing a molecular mismatch level below the threshold still received an organ containing a highly immunogenic mismatch that could lead to DSA formation. In fact, even a single amino acid mismatch on foreign HLA is sufficient to induce antibody formation [47, 74, 75]. Additionally, optimal thresholds for risk stratification likely will be population- and transplant center-specific. This is illustrated by a recent study that suggested that previously defined eplet mismatch thresholds need to be adjusted to be applicable to a patient cohort with different immunosuppression (cyclosporin vs. tacrolimus-based) [76]. Therefore, identification of immunogenic - and thus clinically relevant - molecular mismatches in diverse populations remains important for the refinement of post-transplant risk stratification.

In order to take post-transplant risk stratification based on molecular mismatch levels forward, studies are required to investigate whether molecular mismatch load can identify individual patients that can benefit of reduction of immunosuppression. In the retrospective analysis of the CTOT-09 study, HLA-DQ eplet mismatch load >16 predicted dnDSA formation after tacrolimus withdrawal in 5 out of 8 patients. None of the patients with a HLA-DQ eplet mismatch load below the predefined threshold of 16 developed dnDSA [77]. In the CELIMINN trial, HLA class I and HLA-DQ eplet mismatches predicted dnDSA formation in patients that received tacrolimus monotherapy after discontinuation of prednisone and mycophenolate mofetil [78]. Likewise, a recent study demonstrated that in patients treated with mesenchymal stromal cell (MSC) therapy to facilitate tacrolimus withdrawal, HLA-DQ eplet mismatch levels of ≥11 predicted dnDSA formation in 11 out of 21 patients, versus 0 out of 8 patients with an HLA-DQ eplet mismatch load below the threshold [79]. These studies indicate that the negative predictive value of eplet mismatch load for prediction of dnDSA formation after drug weaning is high, but the positive predictive value is low.

Although previous tacrolimus withdrawal trials selected ''immune-quiescent'' or long term stable kidney transplant patients [77, 80], molecular mismatch analysis was not a criterium to select low risk patients. A next step in the investigation of molecular mismatch for risk stratification should be a prospective study that will randomize patients with low immunological risk based on molecular mismatch analysis to a predefined reduction of immunosuppression at a specific timepoint, such as lower tacrolimus trough levels or complete withdrawal of an immunosuppressive drug. As described above, with a high negative predictive value for dnDSA formation, low eplet mismatch load is expected to be a safe biomarker to guide immunosuppressive drug weaning. Primary and secondary outcomes should include dnDSA formation, rejection, fibrosis, graft loss, and adverse effects of immunosuppression such as infections and malignancies, measured during a follow-up period of at least 5 years. Additionally, therapeutic drug monitoring data such as tacrolimus trough levels should be reported to account for potential bias due to differences in immunosuppressive exposure.

Lastly, as opposed to identifying low risk patients for immunosuppression weaning trials, molecular mismatch load could also be used to select high risk patients for studies with rare endpoints, such as antibody-mediated rejection (AMR). Currently, as the incidence for AMR is low, clinical studies investigating this outcome need to include very large numbers of patients to generate enough power for conclusive results. By selecting patients based on high molecular mismatch load, the study population for intervention studies could be enriched for high risk patients, which would facilitate smaller study cohorts [81].

## CONCLUSION

The clinical application of molecular mismatch analysis in transplantation is promising and has several approaches. However, it is of importance that the fundamentals of HLA-specific antibody biology and epitope-paratope interactions are further elucidated, to unravel the factors that affect the relative immunogenicity of HLA (molecular) mismatches. Excellent quality research in this field requires further development of human HLA-specific monoclonal antibodies, recombinant (and potentially modified) HLA molecules, as well as the curation and analysis of informative sera and cell lines. The collection of large datasets with high-resolution HLA typed transplant recipients and donors, the availability of SAB data for DSA analysis and kidney biopsy data for rejection will aid in further clinical implementation. Furthermore, the time has come for the initiation of prospective studies that investigate the value of HLA molecular mismatch analysis in post-transplant risk stratification for reduction of immunosuppression.

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# Advancements in Cytomegalovirus Management Among Solid Organ Transplant Recipients: Insights From the ESOT CMV Workshop 2023

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Cytomegalovirus (CMV) infection poses significant challenges in solid organ transplant (SOT) recipients, impacting graft outcomes, morbidity, and in some cases survival. The ESOT CMV Workshop 2023 convened European experts to discuss current practices and advances in the management of CMV with the aim of improving the quality of life of transplant recipients. Discussions covered crucial areas such as preventive strategies, diagnostic challenges, therapeutic approaches, and the role of cell-mediated immunity (CMI) monitoring. Despite advances, ambiguity persists in optimal CMV management across European transplant centers. Preventive strategies, including universal prophylaxis and pre-emptive therapy, are effective but consensus is lacking with respect to the preferred approach. Diagnostic challenges such as standardization of viral load thresholds and detection of end-organ disease complicate timely intervention. While newer therapies like maribavir hold promise for treating complicated CMV infections, sustaining viral clearance remains a challenge. Integrating CMI monitoring into CMV management could personalize treatment decisions but has limitations in in terms of predictive value and accessibility. Further research is needed to fill these gaps and optimize CMV management. The collaborative efforts, led by the European Society for Organ Transplantation (ESOT), aim to standardize and improve CMV care, ensuring better outcomes for SOT recipients.

**Keywords: cytomegalovirus, solid organ transplant recipients, universal prophylaxis, pre-emptive therapy, cell-mediated immunity**

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## INTRODUCTION

Cytomegalovirus (CMV) is a widespread herpes virus [1]. CMV seroprevalence affects approximately 80% of the global population, and tends to increase with age [2]. However, there is considerable inter-country variation. France has reported a CMV seroprevalence point estimate of 41.9% among individuals aged 15–49 years, whereas Croatia has reported an overall rate of 74.4% [3, 4]. Although CMV infection is usually asymptomatic or results in only mild disease in the general population, it can lead to severe outcomes in patients who are immunosuppressed, particularly solid organ transplant (SOT) recipients, where latent CMV infection may reactivate and lead to CMV disease [1, 5]. Post-transplant CMV disease may also result from transmission through an infected transplanted organ in seronegative patients [1, 5], significantly impacting graft loss, morbidity and occasionally mortality [6–8]. CMV disease typically occurs within 3 months of transplant (early-onset), although onset may be delayed when antiviral prophylaxis is preferred (late-onset) [5, 9]. Invasive disease can result as a direct cytopathic effect of CMV in organs, manifesting as pneumonia, gastrointestinal (GI) tract disease, hepatitis, encephalitis, and retinitis. CMV infection can also indirectly impact graft function and exacerbate the risk of opportunistic infections [5].

The management of CMV disease in SOT recipients varies considerably across different European centers, highlighting the absence of standardized care protocols [10, 11]. The European Society for Organ Transplantation (ESOT) organised a 1-day workshop on the "Management of CMV in solid organ transplant recipients" in Milan, Italy, on 17 November 2023 with the primary objective of discussing strategies to harmonize CMV management practices across Europe. Experts in the field discussed historic and current diagnostic and therapeutic approaches to the management of CMV. The workshop provided an opportunity for delegates involved in CMV management to share country-specific insights and explore strategies aimed at improving treatment outcomes in SOT settings. Consisting of five expert-led sessions covering CMV prevention, testing, diagnosis, management, and immune monitoring, and complemented by interactive case study sessions, the workshop aimed to elucidate key insights and strategies for improving treatment outcomes in SOT settings. This meeting report summarizes clinical cases analyzed during the workshop, focusing on opportunities to improve outcomes for transplant recipients through cell-mediated immunity (CMI) and the management of resistant or difficult-to-treat CMV disease and reviews the highlights and emerging trends discussed during the workshop, offering valuable insights into the evolving landscape of CMV management in SOT recipients. The Scientific Leads determined the three topics for the case studies: CMV disease, CMV resistance, and immune monitoring for CMV. The three case studies were then independently developed by the faculty.

## MANAGEMENT OF CMV INFECTION AND DISEASE

### The Relationship Between CMV and Patient Outcomes

CMV is the most common pathogen detected after SOT and is associated with significant morbidity and in some cases may lead to death or graft loss [12]. Therefore, understanding the relationship between CMV and patient outcomes post-transplantation is critical. CMV infection has complicated SOT since the first procedure [13]. In a 1964 study by Hill RB et al, among the 61 SOT recipients included, 32 died (mainly kidney recipients), with a median survival post-transplant of only 36.5 days. Notably, autopsy findings revealed that 26 of these patients had active pulmonary infection, with CMV identified as the predominant pathogen in 58% of cases, suggesting a possible association between CMV pneumonitis and mortality [14]. In addition to direct effects related to organ-specific infections, later reports showed an association between CMV infection and acute or chronic graft rejection. In a pivotal study from Grattan MT et al, CMV infection was found to be associated with acute rejection and coronary artery disease in heart transplant recipients [15]. More recently, in a retrospective cohort study involving 192 kidney transplant recipients, patients with CMV disease had a significant likelihood of developing acute rejection after CMV infection or reactivation [16]. Additionally, in 2014 Stern and colleagues conducted a study involving 1414 recipients of heart, kidney, liver, or lung allografts, revealing an increased risk of biopsy-proven graft rejection within 4 weeks after CMV replication was detected [17].

Advances in screening, prophylactic antiviral therapy, and pre-emptive treatment have mitigated the impact of CMV disease on morbidity and mortality following SOT. However, although significant improvements have been made, in the current era, morbidity and mortality data related to CMV disease during organ transplantation remain variable despite advancements in antiviral treatments and the use of newer immunosuppressive drugs [18].

Likelihood of CMV infection in patients undergoing SOT is influenced by several factors. The most significant risk factor is the serological status of the donor (D) and recipient (R), determined by the presence or absence of anti-CMV antibodies. The highest risk of CMV infection occurs when an organ from a CMV-seropositive donor (D+) is transplanted into a CMV-seronegative recipient (R-), designated (D+/R-). Consequently, pre-transplant screening is widely acknowledged to be of paramount importance [19]. Additionally, the type of organ transplanted also affects the CMV risk, with lung transplant recipients facing the highest susceptibility, followed by heart, kidney, and liver recipients (**Figure 1**). Thus, the riskiest scenario regarding CMV infection involves lung transplantation from a seropositive donor to a seronegative recipient (D+/R-). The level of immunosuppression is also important to consider, with the administration of lymphocyte-depleting antibodies (i.e., anti-thymocyte globulin [ATG]) as induction and/or anti-rejection therapy also being associated with increased incidence of CMV, in seropositive recipients. Of note, risk stratification based on

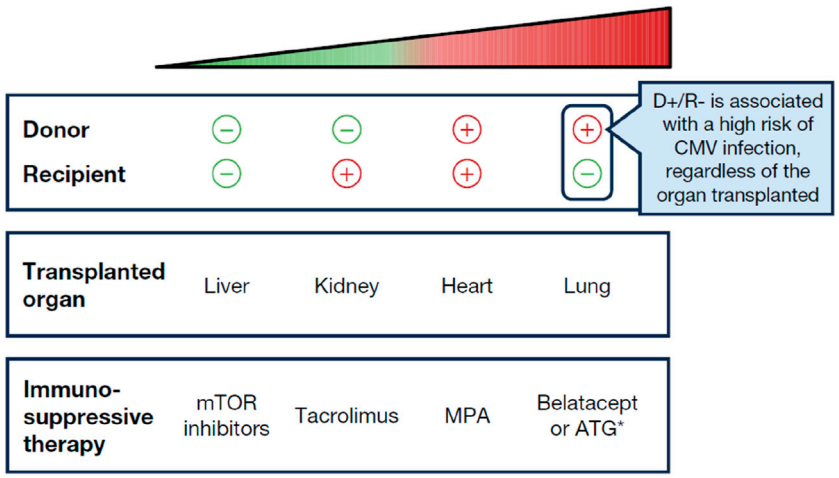


**FIGURE 1 |** The "hierarchy" of risk with respect to CMV infection, from lowest to highest risk. *Only a higher risk in R+ patients. The choice of immunosuppressant therapy may vary depending on the organ transplanted as certain immunosuppressive therapies may not be suitable for all types of transplants. MPA, mycophenolic acid; mTOR, mammalian target of rapamycin.

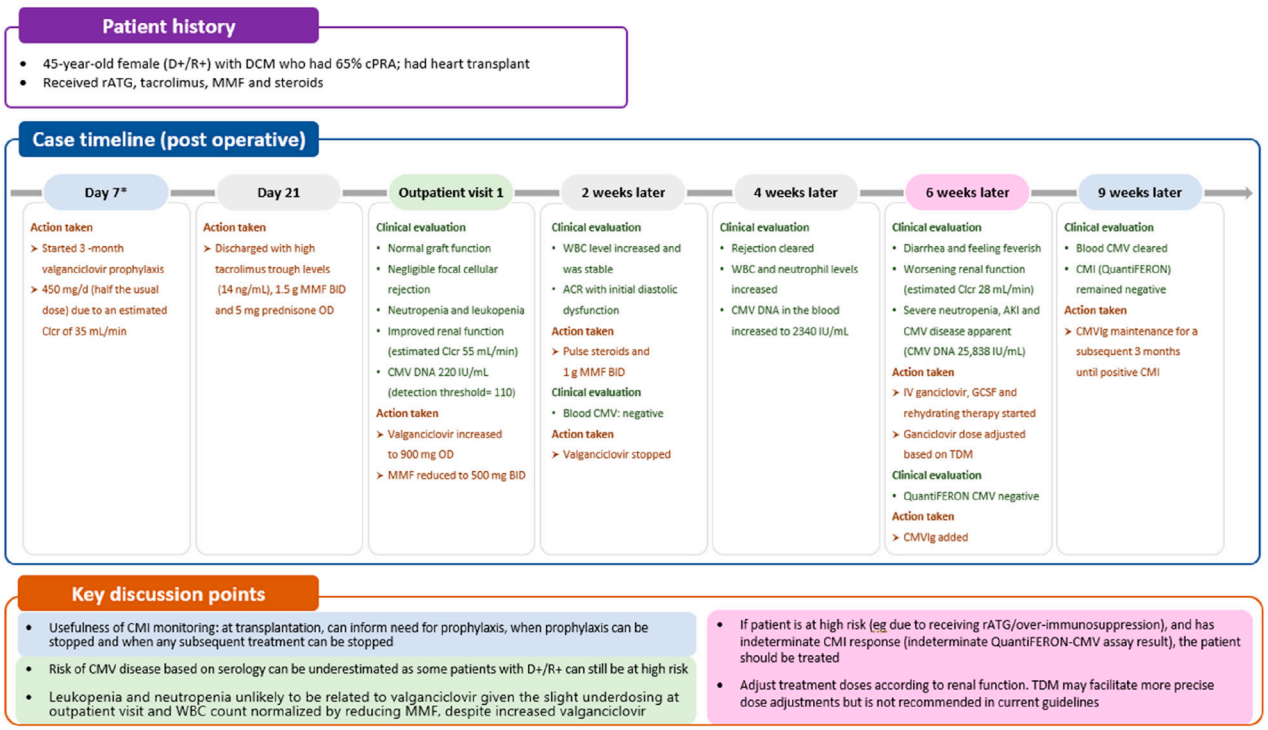


**FIGURE 2 |** Case 1: immune monitoring for CMV. *First available evaluation. ACR, acute cellular rejection; AKI, acute kidney injury; BID, twice daily; Clcr, creatinine clearance rate; CMI, cell-mediated immunity; CMV, cytomegalovirus; CMVIg, cytomegalovirus immunoglobulin; cPRA, calculated panel reactive antibody; DCM, dilated cardiomyopathy; GCSF, granulocyte colony-stimulating factor; IV, intravenous; MMF, mycophenolate mofetil; OD, once daily; rATG, rabbit antithymocyte globulin; TDM, therapeutic drug monitoring; WBC, white blood cell.

donor and recipient serology may only partially estimate the risk for CMV disease. Case 1 underscores the importance of considering all the factors associated with CMV infection (**Figure 2**), such as in a D+/R+ scenario in the presence of additional risk factors like the need for increased immunosuppression.

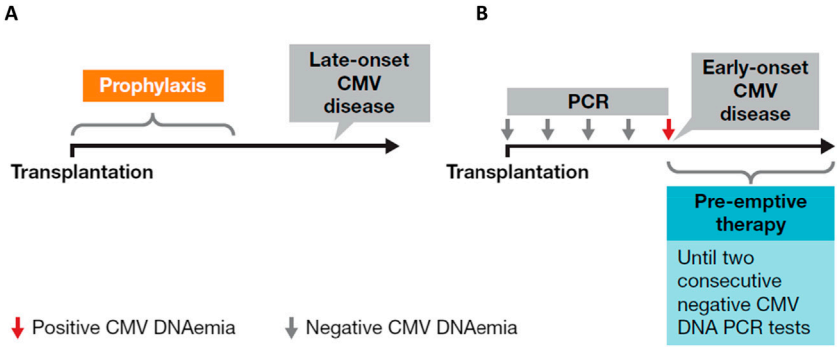


**FIGURE 3 |** Two available preventive strategies for CMV: **(A)** universal prophylaxis and **(B)** pre-emptive approach. Figure was created by Oriol Manuel. Adapted and reproduced with permission from [20].

**TABLE 1 |** An overview of authors' consensus on preventive measures for CMV.

| Preventive strategy | Universal prophylaxis | Pre-emptive approach |
|---|---|---|
| Criteria | • Given to all patients at risk | • Weekly monitoring of viremia during the first 3 months and then twice a month from months 3–6<br>• Antivirals continued until at least two consecutive negative DNAemia tests are achieved or according to center-specific thresholds[a] |
| Frequent preferred indications | • High-risk serostatus (D+/R-)[b]<br>• Lung transplant recipients<br>• Potent immunosuppression (such as the use of belatacept or induction with ATG in R+ patients)<br>• History of CMV reactivation<br>• Limited monitoring capabilities<br>• Individual patient factors | • Lower risk patients (CMV seropositive recipients and those not receiving ATG) [24]<br>• May be given to D+/R- patients only in centres capable of performing strict and reliable monitoring of DNAemia[c] |
| Benefits [25] | • Easy to implement<br>• Potential to prevent other herpes viruses (in the case of valganciclovir), other opportunistic infections and rejection<br>• May influence graft function and may reduce the impact of indirect effects of CMV<br>• Prevention of severe CMV disease<br>• May be more appropriate in resource-limited settings where close monitoring is unavailable | • Reduced drug exposure<br>• Preservation of immune response<br>• Lower overall drug cost<br>• Targeted treatment with individualized approach<br>• Early detection of CMV reactivation<br>• Patient-centered care |
| Challenges | • Associated with a higher incidence of late-onset CMV, so needs clinical monitoring after discontinuation of antivirals<br>• High costs (for letermovir)<br>• Drug toxicity (for valganciclovir)<br>• Higher risk of antiviral resistance<br>• Reduces immunobiological control<br>• Difficulty in determining optimal duration of prophylaxis<br>• Risk of drug interactions with other medications/immunosuppressives | • Requires close monitoring (risk of missed reactivation events)<br>• No universal value for the initiation of treatment and cut-off values are center specific<br>• Risk of over-treatment or under-treatment<br>• Impact on indirect effects of CMV unknown<br>• Does not address other herpes viruses<br>• Higher logistic costs<br>• Patient education and engagement |

[a]*There is no consensus on a specific threshold but rather on a significant increase of viral load.*
[b]*Preference is based on opinions at the workshop and is in line with the results of a survey conducted by ESOT in 2022 (in which 90% of respondents reported use of prophylaxis in D+/R- patients) [26] and current guidelines that support prophylaxis in kidney and cardiothoracic patients [19]. The situation is different for patients undergoing liver transplantation.*
[c]*Grossi PA, et al. Transpl Int. 2022;35:10332 [26].*

## Strategies to Improve CMV Infection and Disease Outcomes

Given the profound implications of CMV disease in SOT recipients, effective CMV prevention strategies can enhance the success and improve the outcomes of transplant procedures. Two preventive strategies are available (**Figure 3**): universal prophylaxis (administration of antivirals to all patients at risk, starting within 10 days after transplant and continuing for at least 3 months and up to 12 months in D+/R- lung transplant recipients [duration dependent on organ transplanted and D/R serostatus]) or pre-emptive therapy (monitoring for DNAemia every week, followed by the administration of antivirals until at

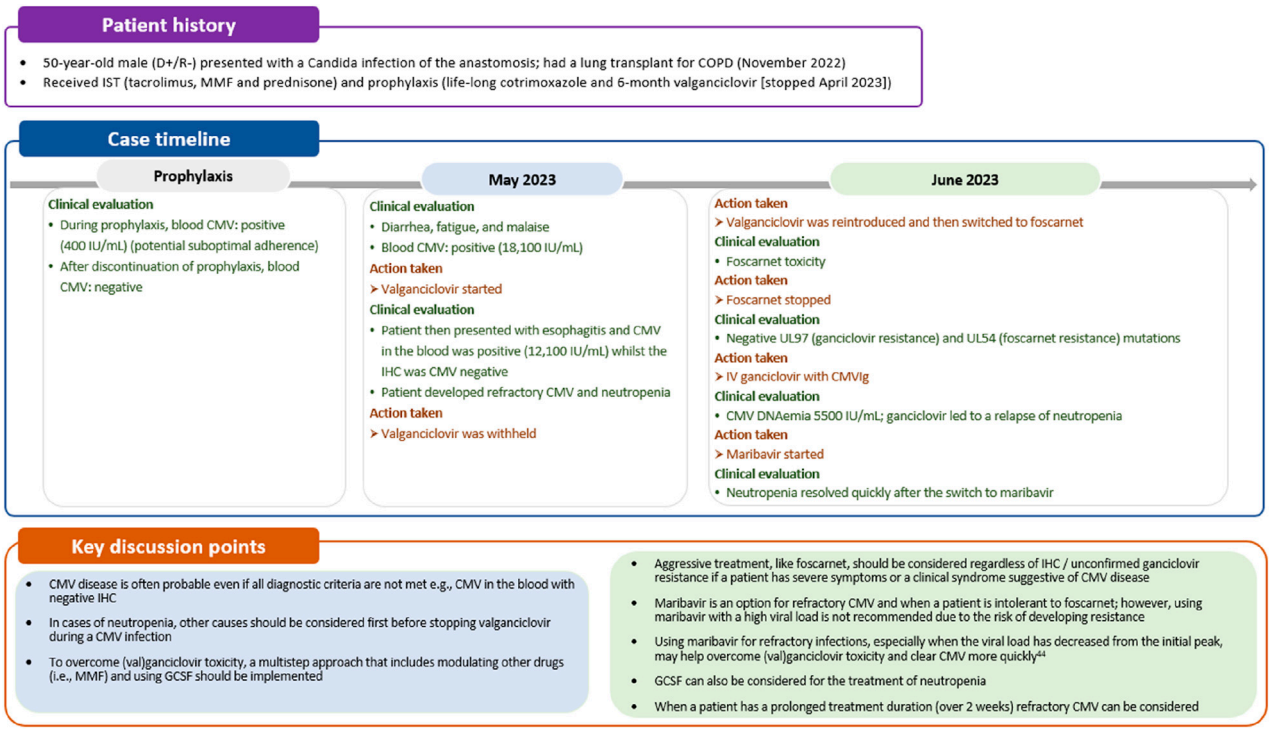


**FIGURE 4 |** Case 2: Managing difficult to treat CMV disease. CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; GCSF, granulocyte-colony stimulating factor; IHC, immunohistochemistry; IST, immunosuppressive therapy; MMF, mycophenolate mofetil.

least two consecutive negative DNAemia tests are achieved or according to center-specific thresholds) [6]. Both strategies are effective in preventing CMV disease, with no consensus on the superiority of one over the other, but with prophylaxis preferred in lung transplants and pre-emptive therapy in liver [21–23]. The choice between universal prophylaxis and pre-emptive therapy will be driven by the expected relative benefits of each strategy (**Table 1**) as well as the clinical situation in the individual patient. As an illustration, in case 1, a patient (D+/R+) undergoing a heart transplant, who based on serology and transplanted organ could be considered to be at lower risk of CMV infection than a seronegative recipient and a candidate for pre-emptive therapy, received universal prophylaxis with valganciclovir due to the increased risk of CMV disease associated with potent immunosuppression (**Table 1**; **Figure 2**).

Valganciclovir is the standard of care for CMV prophylaxis in the most at-risk donor–recipient category (D+/R-) [27]. However, prolonged exposure in the setting of universal prophylaxis can lead to drug toxicity, in particular leukopenia [27]. A recent study by Limaye and colleagues demonstrated that letermovir is non-inferior in effectiveness to valganciclovir for CMV prophylaxis in D+/R- kidney transplant recipients, but with lower rates of leukopenia or neutropenia, suggesting its potential as a preferred option in D+/R- kidney transplant patients [27]. Furthermore, the use of CMV immunoglobulin (CMVIg) in combination with antivirals in CMV prophylaxis may be beneficial in specific circumstances, especially in D+/R- recipients of thoracic organs [28–30]. A meta-analysis assessed CMV infection rates in SOT patients who received prophylactic CMVIg, revealing a lower incidence of CMV infection in this cohort. Specifically, the average CMV infection rate was 35.8% (95% CI: 33.4%–38.2%) among patients who received CMVIg, compared with 41.4% (95% CI: 38.6%–44.2%) in the control group not receiving CMVIg ($p = 0.003$) [28]. Despite these promising results, the use of CMVIg remains controversial due to the lack of recent interventional data on efficacy.

## Challenges With Testing and Diagnosis Techniques for CMV and Strategies for Improvement

Unlike universal prophylaxis, a pre-emptive approach to CMV prevention requires regular monitoring for CMV viremia [6]. The quantitative nucleic acid amplification test detects and quantifies CMV DNA and is preferred over antigenemia. It has become the standard of care for diagnosing and monitoring post-transplant CMV infection [31]. Post-transplant monitoring typically occurs weekly during the first 3 months and then twice a month from months 3–6 as patients stabilize. Despite the international standardization of reporting all viral load values in IU/mL during the QNAT, a consensus around viral load thresholds remains a challenge because laboratory assays and matrix choices differ between centers. This leaves individual centers

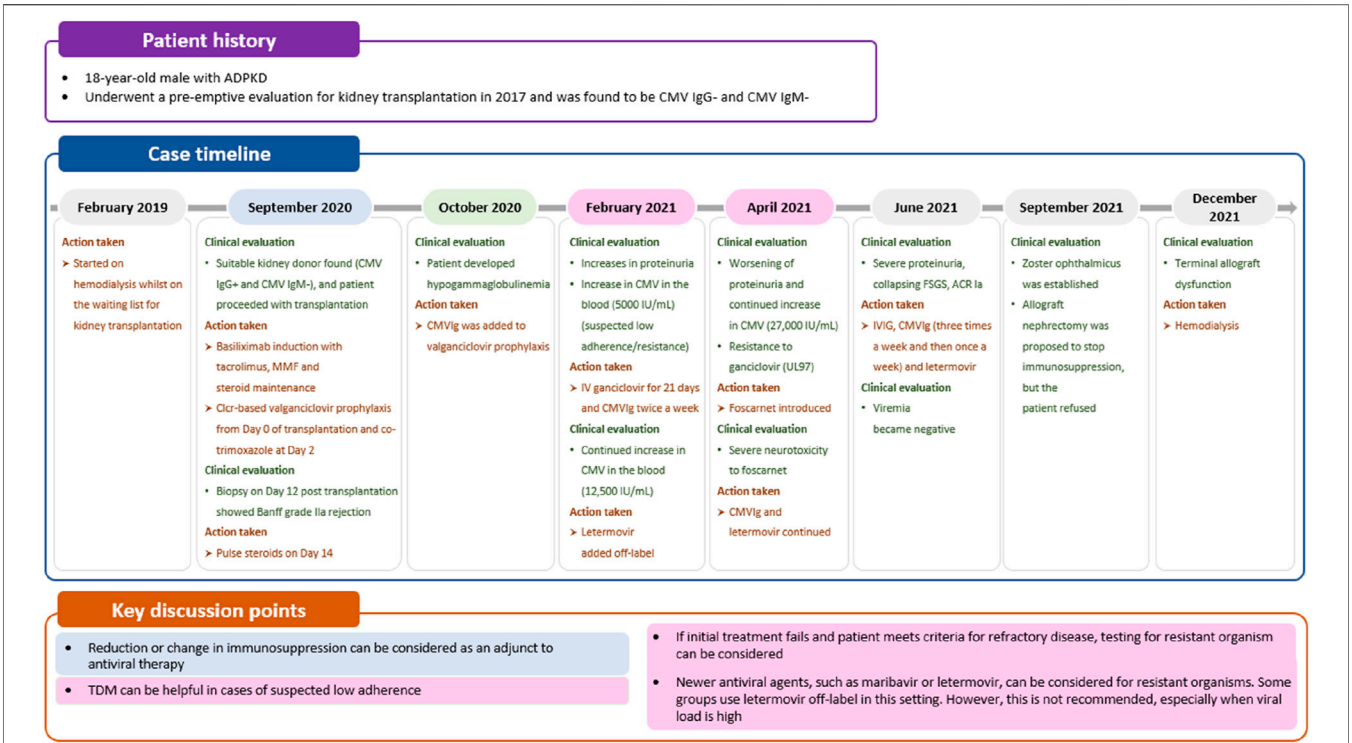


**FIGURE 5 |** Case 3: Managing CMV resistance. ACR, acute cellular rejection; ADPKD, autosomal dominant polycystic kidney disease; CMV, cytomegalovirus; CMVIg, cytomegalovirus immunoglobulin; Clcr, creatinine clearance; FSGS, focal segmental glomerulosclerosis; IVIG, intravenous immunoglobulin; MMF, mycophenolate mofetil; TDM, therapeutic drug monitoring.

with the task of determining specific thresholds in their laboratories [26].

Studies have shown that an increase in viral load correlates with the occurrence of CMV disease [5]. Therefore, focusing on viral load trends over time is more useful and important for predicting disease development and guiding therapeutic decisions than using center-specific absolute viral load values, which lack standardization [5]. To illustrate this, in case 1, CMV DNA in the blood increased 10-fold in 2 weeks, which prompted treatment with an antiviral, granulocyte-colony stimulating factor (GCSF) and CMVIg. It is important to underline that DNAemia values from plasma and whole blood are not comparable [5], so it is crucial not to change tests or matrix choices during treatment and follow-up. Additionally, in our opinion, distinguishing the clinical significance of a viral load increase is complicated by free DNA release into plasma from infected cells, potentially leading to low-level or persistent DNAemia, which could be mistaken as an indication of active replication. In this context, assessment of late mRNAemia in the plasma could help identify episodes of active viral replication and could have the potential to shorten the duration of pre-emptive or prophylaxis strategies and aid the management of long-term infections, in particular when using drugs that inhibit CMV replication steps downstream of DNA polymerase, such as letermovir and maribavir [32]. Nevertheless, this tool is still undergoing investigation and validation.

The presence of CMV disease is possible even if all diagnostic criteria are not met and treatment should be initiated in such situations. Diagnostic criteria may vary depending on the organ involved, and relying solely on QNAT may not always be sufficient, particularly in GI tract-related CMV disease [33]. In the GI tract, end-organ disease can be evident (positive immunohistochemistry [IHC]) despite negative DNAemia [34]. Therefore, diagnosis of CMV GI disease requires the presence of upper and/or lower GI symptoms along with endoscopic evidence or laboratory confirmation of CMV infection [33]. Additionally, it is possible for biopsy samples to be taken from unaffected parts of the intestine in individuals with CMV GI disease. Confirmation of CMV pneumonia typically involves clinical signs and/or symptoms suggestive of pneumonia combined with laboratory confirmation of CMV in lung tissue [33]. In case 2, a lung transplant recipient exhibited CMV viremia but negative IHC results from an esophageal biopsy (**Figure 4**). Despite this negative IHC result for esophagitis, treatment for CMV disease was initiated in the patient. This decision was based on the fact that the patient had a clinical syndrome suggestive of CMV disease.

## Treatment of CMV Infection and Disease

First-line treatment of CMV infection or disease is oral valganciclovir or intravenous ganciclovir, with the latter often preferred in cases of life-threatening CMV, very high viral loads, or when oral absorption of medication is a concern [6]. Valganciclovir is a prodrug of ganciclovir, with both inhibiting viral DNA polymerases. In addition, these drugs can lead to

varying degrees of bone marrow suppression and subsequently, neutropenia [35]. Oral valganciclovir has a longer half-life than intravenous ganciclovir meaning prolonged exposure (or slower clearance) of the drug and its metabolites [36]. Therefore, oral valganciclovir would be expected to be associated with a greater degree of neutropenia than intravenous ganciclovir, which was evident in study WV15376 (11% vs. 13%, respectively) [36, 37]. Higher incidences of neutropenia may be observed in patients with decreased kidney function, as this leads to reduced clearance of ganciclovir and a prolonged terminal half-life [36]. Neutropenia is one of the most common adverse event associated with valganciclovir, as was reported in case 2 (**Figure 4**) [38]. In cases of valganciclovir-induced neutropenia, dose adjustments or discontinuation of therapy, as well as management of other drugs, may be necessary, particularly if neutrophil counts fall below pre-defined thresholds or clinical symptoms of infection arise. Close monitoring of blood counts, particularly neutrophil levels, is crucial during valganciclovir therapy to manage neutropenia-related complications promptly.

Of note, as highlighted in case 2, neutropenia should not automatically prompt discontinuation of valganciclovir. Bone marrow suppression and neutropenia can also be caused by CMV infection itself [39], making it essential to assess whether neutropenia is a direct consequence of CMV replication. This may involve evaluating CMV viral load by DNAemia or other diagnostic tests to confirm active CMV infection. Furthermore, it is imperative to consider and investigate other potential underlying conditions or factors that could contribute to neutropenia. These may include medications commonly used in transplant recipients, such as immunosuppressive or anti-infective agents (e.g., mTOR inhibitors, mycophenolic acid, trimethoprim sulfamethoxazole). Additionally, concomitant viral infections (e.g., Epstein–Barr virus, Human Herpesvirus-8 [although relatively infrequent], and Parvovirus B19, adenovirus), hematologic disorders, or nutritional deficiencies need to be excluded. Once other potential causes are excluded, the initial consideration can be granulocyte-colony stimulating factor (GCSF) administration, followed by a possible switch to an alternative drug with a more acceptable safety profile (**Figure 4**) [35]. It is not recommended to use foscarnet or cidofovir to overcome valganciclovir toxicity, given their less acceptable safety profiles. Although it is preferred to only use these drugs as an alternative to valganciclovir in case of documented ganciclovir resistance, guidelines recommend considering foscarnet in refractory CMV cases with severe clinical symptoms or life-threatening disease [6]. Where available, maribavir can be considered for refractory or ganciclovir resistant infection in cases of intermediate viral loads, or as second step approach after an initial short course with foscarnet [6]. This approach is likely to minimize the toxicity of foscarnet and the risk of maribavir resistance that may occur when treating high viral loads.

Tailoring treatment approaches to each clinical scenario is essential for optimizing patient care. Impaired kidney function can lead to decreased drug clearance and increased drug concentrations, potentially increasing the risk of drug toxicity [40]. Therefore, in cases of kidney impairment, treatment doses should be adjusted according to kidney function to minimize adverse events, as highlighted in case 1 (**Figure 2**). However, despite these recommendations, data from kidney transplant recipients suggest that as many as one-third of patients may be receiving a dose of valganciclovir that is too high [41]. Immunosuppressive drugs, including corticosteroids, calcineurin inhibitors (e.g., tacrolimus and cyclosporine), and in particular antimetabolites (e.g., mycophenolate mofetil or mycophenolic acid), inhibit the immune response by suppressing the activity of immune cells, including T cells and natural killer cells [42], thus hindering immune surveillance and the ability to combat CMV infection. Switching or reducing immunosuppressive therapy should be considered as an adjunct to antiviral therapy to improve treatment outcomes, as demonstrated in case 1 (**Figure 2**).

Monitoring CMV viral load and complete blood count should be conducted on a weekly basis to guide the duration of therapy. Treatment should continue for a minimum of 2 weeks, until DNAemia falls below the detection threshold and signs and symptoms of CMV disease are resolved [6]. As introduced above, DNAemia may not accurately reflect CMV disease status in all clinical situations and longer courses of treatment may be needed, for example, in the treatment of tissue-invasive GI disease and pneumonitis in lung transplant recipients [6]. If a patient fails to respond after the recommended treatment duration with (val)ganciclovir, maribavir could be considered as an alternative option (**Figure 4**). However, as outlined earlier, we advise caution when using maribavir in patients with a high viral load due to the potential risk of selecting a resistant mutant [43]. Treatment failure may result from a resistant/refractory CMV infection or low adherence. Therapeutic drug monitoring, although not generally recommended in current guidelines, can be helpful in cases of suspected low adherence, or to ensure optimal drug levels in cases of kidney insufficiency, although a valganciclovir concentration clearly predictive of CMV clearance has not been determined [44]. If treatment fails in an adherent patient who meets the criteria for refractory disease, testing for resistant CMV should be considered.

## Treatment of Refractory/Resistant CMV Infection

Despite preventive strategies and well-established antiviral therapies, managing refractory/resistant CMV infection in patients undergoing SOT remains a significant challenge. Resistant/refractory CMV infection is defined as the failure to respond after 14 days of appropriate treatment [45]. **Table 2** provides an overview of the definitions for refractory and resistant CMV infection. Ensuring appropriate dosing of antivirals is essential in the management of CMV infection, as suboptimal dosing can lead to an increased risk of treatment failure and resistance development [6].

In case of resistant CMV infection, mutations in the UL97 gene are most frequent, while UL54 mutations typically arise after prolonged pre-treatment and may lead to cross-resistance with cidofovir and foscarnet [45]. A laboratory study conducted in 2023 revealed CMV drug resistance in

**TABLE 2 |** Summary of definitions of refractory and resistant CMV [46].

| Term | Definition |
|---|---|
| Refractory CMV infection[a] | CMV virema (DNAemia or antigenemia) that either:<br>1. Has a >1 $\log_{10}$ increase in CMV DNA levels in the same blood compartment from the highest level previously measured in the same laboratory and/or with the same commercial assay)<br>OR<br>2. Persists (≤1 $\log_{10}$ increase or decrease in CMV DNA levels) after ≥2 weeks of appropriate antiviral therapy. |
| Resistant CMV infection | Refractory infection with the presence of genetic mutations correlating to antiviral resistance, which leads to treatment failure |

[a]*Refractory and probable refractory CMV infection are classified as one category.*

approximately 30% (n = 826/2750) of samples from transplant recipients sent for genotyping [47]. The most common resistance mutations in the UL97 gene were for ganciclovir and maribavir accounting for 27.64% and 9.96% of samples, respectively [47]. However, reported rates of CMV drug resistance may vary across publications. The annual reported incidence rate of ganciclovir resistance was less than 1% in 80% of transplant centers but reached up to 10% in some, according to the 2022 ESOT survey [26] and a recent trial of maribavir in patients with refractory or resistant CMV infection has reported a resistance rate in the region of 25% [43, 48]. Risk factors for resistant/refractory CMV infection include younger age, D+/R- serostatus, lung transplant, recurrent CMV infection, ongoing viral replication, prolonged antiviral treatment, subtherapeutic antiviral levels, high viral loads, and severe immunosuppression [45, 49]. Additionally, administering belatacept increases the risk of refractory CMV infection compared with other immunosuppressants. Belatacept was unable to sustain viral control relative to tacrolimus in high-risk recipients (n = 60) [50].

The latest international treatment recommendations for managing resistant CMV infection, as outlined in the 2025 guidelines, involves first reducing immunosuppression if feasible, followed by administering foscarnet, cidofovir, or high-dose ganciclovir depending on disease severity and genetic mutation type [6]. However, there is limited evidence supporting the use of high-dose ganciclovir. Additionally, older antivirals pose significant toxicity concerns, with ganciclovir linked to neutropenia, and foscarnet and cidofovir associated with a high risk of acute kidney injuries and increased mortality [51, 52].

Maribavir, an oral benzimidazole riboside antiviral, inhibits the CMV UL97 protein kinase involved in viral maturation and egress [53]. It was approved for the treatment of resistant/refractory CMV infection in the UK and Europe in 2022, with approval in the USA granted in 2021 [54, 55]. Maribavir is considered a valid alternative treatment for resistant/refractory CMV due to its more favorable safety profile [52]. A phase 2 study showed that ≥400 mg of maribavir twice daily achieved CMV clearance in SOT patients with resistant/refractory CMV [56]. The results from this study led to a large prospective phase 3 study in SOT and hematopoietic stem cell transplantation recipients (n = 352) with refractory CMV infection: after 8 weeks of therapy, maribavir showed greater CMV DNAemia clearance and fewer treatment discontinuations due to treatment-emergent adverse events compared with investigator-assigned therapy (valganciclovir/ganciclovir, cidofovir, or foscarnet) [52]. The viral response rate was 55.7%, compared with 23.9% in the investigator-assigned therapy group [52]. However, among the patients who achieved CMV clearance by Week 8 in the maribavir group, 66.4% of patients experienced a loss of response by Week 16 [52]. Alternative strategies, such as a longer treatment duration, should be evaluated, while also acknowledging the continued relevance of the conventional drugs, foscarnet and cidofovir, depending on the individual patient situation. However, it is important to note that from 6 weeks, maribavir can lead to CMV mutations and resistance in recurrent infections [57], and resistance to valganciclovir and maribavir in the same patient has been reported [58].

Letermovir disrupts the viral terminase complex (pUL56) and is currently approved for prophylaxis in patients undergoing hematopoietic stem cell transplantation or high-risk (D+/R-) kidney transplantation [59, 60]. Due to its more favorable safety profile and reduced risk of CMV resistance compared with valganciclovir, letermovir is sometimes used off-label for the treatment of resistant CMV, as observed in case 3 (**Figure 5**) [27]. However, there are concerns regarding the higher risk of resistant mutations, especially in patients with high viral loads, making letermovir potentially unsuitable in such patients [61].

Further "proof of concept" studies are needed to determine the role of letermovir in treating refractory infections and whether CMVIg can enhance T-cell response. In specific cases, combining CMVIg with antivirals may present a more viable approach. CMVIg can provide an additional mechanism of action by modulating the immune response through various mechanisms, including CMV neutralization, dendritic cell maturation modulation, decreased T-cell activation, and decreased cytokine production [62]. Although it is only licensed for prophylactic use, some clinicians use CMVIg off-label to support the treatment of resistant CMV infection. For example, in case 3, CMVIg was added to off-label letermovir treatment for a patient with hypogammaglobulinemia and ganciclovir-resistant CMV infection (**Figure 5**). Despite the potential benefits of CMVIg, there is limited evidence supporting its off-label use in the treatment of CMV infections [63, 64].

## The Role of Cell-Mediated Immunity (CMI) Monitoring in CMV Disease

The integration of CMI monitoring into the care pathway for CMV disease has the potential to revolutionize the management of CMV infection by offering a personalized approach to CMV

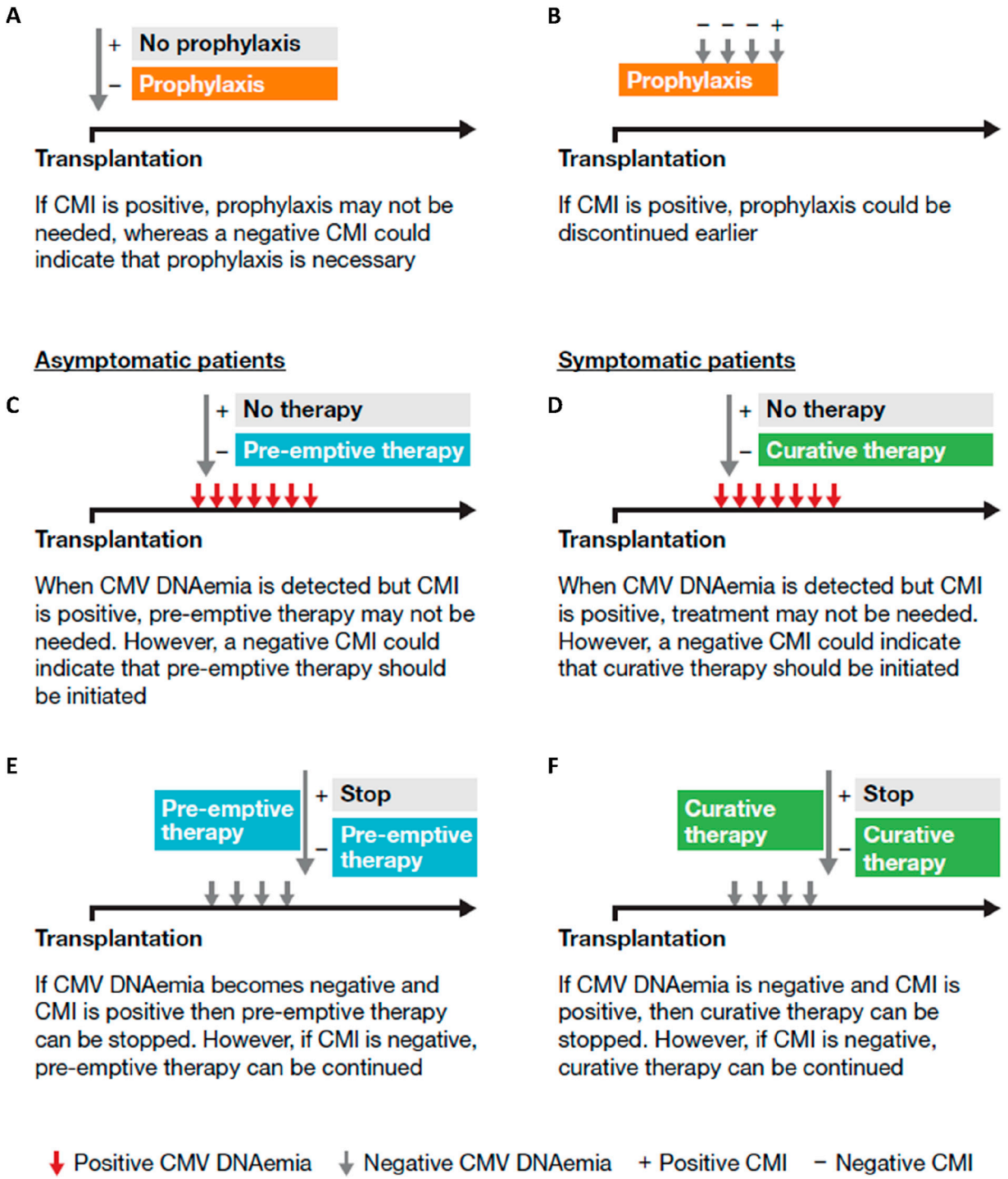


**FIGURE 6 |** The potential use of CMI monitoring: **(A)** at transplantation; **(B)** during prophylaxis; at the onset of the infection in asymptomatic **(C)** and symptomatic patients **(D)**; **(E)** during pre-emptive therapy in asymptomatic patients; **(F)** at the end of the infection in symptomatic patients [69]. Based on Kaminski H, et al. Immunol Rev. 2020; 298:264–88.

management and enhanced care for individual patients [65]. CMI monitoring measures the production of interferon gamma, or other cytokines, produced by T cells in response to CMV antigens [66]. The level of CMI is commonly quantified using the ELISPOT or QuantiFERON-CMV assay [55]. Typically, a high CMI response indicates protection against CMV disease, whereas

a low CMI response increases the risk of CMV reactivation or progression [66]. The impact of immunosuppressants on CMV-specific T-cell functionality varies [67], and by closely monitoring the immune response, preventive and curative strategies may be tailored appropriately [66, 68].

CMI monitoring can be used as a decision-making tool at various stages of the patient journey (**Figure 6**) [65]. Unlike serology, which may misrepresent the risk of CMV infection in some patients, particularly D+/R+ patients, CMI is primarily driven by T cells and does not rely on B-cell antibody production [70]. CMI monitoring can be useful for stratifying the risk of CMV infection [31, 71, 72]. with the absence of pre-existing CMV-specific CMI in the recipient increasing the risk of CMV infection [31, 73]. In a prospective multicenter study in D+/R+ kidney recipients deemed to be at high risk for CMV based on pretransplant CMI significantly higher CMV infection rates were observed compared with those considered to be at lower risk, regardless of whether prophylaxis or pre-emptive protocols were followed [74]. However, with some immunosuppressive regimens additional comprehensive profiling of cytokine and chemokine responses may improve the performance of CMV-specific CMI [67, 68].

In case 1, CMI monitoring was used to assess the necessity of CMVIg treatment. An indeterminate QuantiFERON-CMV result, typically interpreted as negative, suggested low or no overall immunity, including against CMV, prompting the initiation of CMVIg. Additionally, CMI monitoring could have guided the decision on universal prophylaxis initiation or earlier initiation of valganciclovir (**Figure 2**). This approach allows low-risk patients to avoid unnecessary CMV preventive therapy and minimize their exposure to antivirals, consequently decreasing the associated risk of adverse effects [66].

If CMI is positive, prophylaxis could be discontinued earlier as was demonstrated in a recent study in D+/R- kidney and liver transplant recipients receiving prophylactic valganciclovir [75] and a similar study in CMV seropositive kidney transplant recipients receiving ATG [76]. Thus, CMI measurements could be used to tailor the duration of prophylaxis, particularly in high-risk patients, aiming to reduce the risk of toxicity [66]. However, although no difference in CMV disease and replication has been shown in some studies [76], others have been unable to establish non-inferiority of this CMI-guided approach on CMV infection [75]. In patients with low-level DNAemia, CMI testing serves as an adjunctive tool to guide the decision to initiate curative treatment in symptomatic patients, and to determine its optimal duration, or to guide pre-emptive therapy in asymptomatic patients [66]. Interestingly, patients with an indeterminate result appear to be over-immunosuppressed and have a greater risk of CMV reactivation than those with a negative result [66], as observed in case 1 where the patient, a high-risk individual post-rabbit ATG (rATG) administration, exhibited an indeterminate result (**Figure 2**).

Despite the promising findings from several studies, the integration of CMI monitoring into routine clinical practice still faces challenges due to certain limitations. These include the lack of a clearly defined protective threshold, timings, and accessibility. Where CMI is not available, clinicians can refer to alternative indicators of global immunity, such as lymphocyte count or hypogammaglobulinemia. Low levels of lymphocytes and immunoglobulins may indicate the need for additional interventions in patients at risk of CMV disease.

Before CMV-specific CMI monitoring can be integrated into routine clinical practice, several questions regarding immune-guided CMV management must be addressed. These include understanding why current CMI monitoring has a poor predictive value for D+/R- patients, explaining the reasons behind the occurrence of CMV infections in some R+ patients despite a positive QuantiFERON test result, and exploring the mechanisms enabling certain CMI−CMV patients to control CMV infection following curative treatment. Addressing these questions is essential for optimizing the utility of CMI monitoring in personalized CMV management strategies.

## CONCLUSION

The management of CMV presents complex challenges, underscoring the necessity to standardize CMV management through an evidence-based approach. The workshop highlighted the need for further close collaboration between experts in the field to continue optimizing CMV management. Newer antivirals, such as maribavir, could reduce antiviral-associated toxicity in resistant/refractory CMV infections, but limitations remain. CMI is increasingly being employed to make key decisions throughout the patient's treatment journey however, more information is required before CMI becomes a part of routine practice. ESOT will continue to try to streamline and optimize the management of CMV infection and disease in this challenging population.

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

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## CONFLICT OF INTEREST

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MEETING REPORT
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# Updates on Donor-Derived Infection in Solid Organ Transplantation, Report from the 2024 GTI (Infection and Transplantation Group) Annual Meeting

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The annual meeting of the French GTI (Transplantation and Infection Group) focused on donor-derived infections (DDIs) in solid organ transplant (SOT) recipients. Given the ongoing organ shortage, rigorous donor screening is essential to detect potential infectious risks. Donor evaluation should include medical history, travel, vaccination status, serologies, and exposures. Various pathogens are of concern, including viruses (HIV, hepatitis, BK polyomavirus), multidrug-resistant bacteria, fungi, and emerging arboviruses like West Nile virus and dengue. HIV-positive donor to HIV-positive recipient (D+/R+) transplantations are increasingly accepted, with promising outcomes. Hepatitis E (HEV) is now the most common viral hepatitis and may lead to chronic infection in SOT recipients, requiring ribavirin treatment. Non-Candida fungal infections, though rare, are associated with high mortality and demand early recognition. Climate change and

globalization are expanding the range of vector-borne infections, necessitating seasonal and regional screening. BK polyomavirus remains a major complication in kidney transplant recipients, and monitoring viral load is critical. Bacterial infections from donors are uncommon but should be evaluated based on site, organism, resistance profile, and treatment history. Overall, maintaining safety in transplantation requires constant vigilance, updated knowledge, and personalized risk-benefit analysis to adapt to emerging infectious threats—especially amid ongoing organ scarcity.

**Keywords: donor-derived infection, chronic hepatitis, Bk virus, HIV, non-candida derived fungal infection**

## INTRODUCTION

The French annual meeting of the GTI ("Groupe Transplantation et Infection") focused this year on donor-derived infections in solid organ transplant recipients. We summarize in this report the presentations and discussions around the covered topics, highlighting current challenges and expert opinions. Each topic includes new insights/perspectives on clinical presentation, diagnostic and prevention strategies, and risk management protocols for those at-risk for donor-derived infections.

## OVERVIEW OF DONOR-DERIVED INFECTIONS

In Europe in 2022, 27,952 organ transplants were performed, but over 40,000 patients were on a waiting list. The challenge is to strike a balance between availability and need, as transplant candidates may die while waiting, and donor-derived infections are possible complications of solid organ transplantation (SOT). The 8th edition of the EDQM Guide on Quality and Safety of Organs for Transplantation has been published in 2022 and is available online[1] [1]. To mitigate the infectious risk, it is suggested to collect [2]: medical history including prior infections, vaccinations (with an emphasis on live attenuated vaccines), occupational exposures, travel history without limit time before transplantation, receipt of transfusions of blood or blood products, human immunodeficiency virus (HIV, hepatitis B virus (HBV), hepatitis C virus (HCV) serostatus or other transmissible diseases, tattooing, ear or body piercing, drug use, sexual behavior, jail incarceration, pets or zoonotic contacts. Basic screening for infections in deceased organ donors is summarized in [1]. Depending on specific risk factors identified in the donor, other tests may be required.

Blood HIV, HBV, HCV nucleic acid amplification tests may be used in the "window period," when serology is still negative. Globalization (migration, tourism, global trade) is an important factor underlying the emergence and re-emergence of specific infectious diseases. The 8th edition of the Guide on the quality and safety of organs intended for transplantation has published a table of possible risks of transmissible infections according to geographical areas. These tests should be considered for screening symptomatic donors who have lived and/or traveled to one of several of these endemic areas and whom may have been exposed or at risk of vertically acquired infection. Additionally, receipt of live attenuated vaccine within 4 weeks prior to transplantation by the patient or his/her close contacts should be investigated because of the risk of reactivation or transmission upon immunosuppression.

Some specific guidelines are summarized below:

In cases of herpes simplex virus (HSV 1 or 2) serological mismatch, severe hepatitis have been reported, and given that transmission may occur with any organ, the Swiss guidelines advise that all transplant recipients with HSV serological mismatch should receive a 6-month course of antiviral prophylaxis [3].

Disseminated toxoplasmosis after transplantation is rare but can be fatal due to delayed diagnosis and treatment. The U.S. Organ Procurement and Transplantation Network now recommends screening of all deceased donors.

Donor-derived HTLV-1 is another challenge in solid organ transplantation, but the approach is heterogeneous across countries. In France, Spain, and the UK all donors are screened regardless of risk factors, while in other countries screening is limited to donors who have lived and/or traveled to endemic areas. In Europe, Romania is reported to be the only country with a high prevalence of HTLV-1.

Five arboviruses require our attention in view of their emergence in different parts of the world: yellow fever, dengue (DENV), Zika, West Nile (WNV), chikungunya, transmitted by blood and organs [4], and will discussed later in a specific chapter.

Romagnoli R, et al. [5] published the results of the first 10 liver transplants with proven SARS-CoV-2 positive donors, suggesting a very low risk of transmission with liver transplantation. Nevertheless, the comparison of the 2 populations of transplanted livers from COVID-19 donors with non-COVID 19 donors showed more frequent hepatic artery thrombosis, which would deserve to be explored in the future. Data are awaited on the risk associated with the use of organs (apart from lungs, which are systematically rejected) from donors with proven SARS-CoV-2 infection, with emphasis on the timing of the procedure in relation to the onset of immunological protection.

## TRANSPLANTATION IN HIV

Dr. Victoria Manda, from Saint-Louis/Lariboisière University Hospital, Paris, France, presented an overview of current

[1]https://freepub.edqm.eu/publications

practices worldwide regarding solid-organ transplantation in people living with HIV (PLWH).

As of 2022, the global population of PLWH was estimated at around 39 million, with 1.3 million new infections, mainly in South America and Africa [UNAIDS 2023]. The risk of chronic kidney disease is higher in PLWH compared to the general population, becoming a significant public health concern by accelerating disease progression and complicating treatment. Kidney transplantation (KT) is the standard treatment for end-stage renal disease [6], but access remains limited for PLWH compared to HIV-negative patients.

The first KT from an HIV-positive deceased donor (D) to an HIV-positive recipient (R) was performed in South Africa in 2010. The first liver transplant from an HIV-positive living donor to an HIV-positive recipient took place in the United States in 2017 [7]. In South Africa, between 2008 and 2014, 27 PLWH received KT. All recipients had $CD4^+$ T-cell counts above 200/$mm^3$ and were on highly active antiretroviral therapy (HAART) with undetectable HIV viral loads. Some donors had not previously received ART, while others had only received first-line combinations.

Patient survival rates were 84%, 84%, and 74% at 1, 3, and 5 years post-transplant, respectively. Graft survival rates were 93%, 84%, and 84% over the same time periods. Overall rejection rates were 8% at 1 year and 22% at 3 years. HIV viral loads remained undetectable post-transplantation [8].

Following the passage of the HIV Organ Policy Equity (HOPE) Act in 2013, HIV D+/R+ transplants can be performed under research protocols in the U.S. To inform policy and practice—especially regarding whether this approach should continue—multicenter pilot studies were conducted to assess the feasibility and safety of liver or kidney transplantation involving HIV-positive donors (D+/R+) versus HIV-negative donors to HIV-positive recipients (D-/R+) [9, 10]. Donor selection required no current or prior opportunistic infections, recipient use of HAART, and favorable results from a donor pre-implantation biopsy.

A prospective multicenter pilot study specifically examined the safety and risks associated with HIV+ donors for KT by directly comparing HIV D+/R+ and HIV D-/R+ cases [9]. From 2016 to 2019, across 14 centers, 75 HIV+ kidney transplants were performed (25 D+ and 50 D-), with a median follow-up of 1.7 years. There were no deaths or differences in 1-year graft survival, estimated glomerular filtration rate, HIV breakthrough, infectious hospitalizations, or opportunistic infections. However, delayed graft function occurred significantly more often in D+ cases than in D-. One-year rejection rates were also higher in D+ recipients but did not reach statistical significance. Lymphocyte-depleting induction therapy was associated with lower rejection rates. The authors noted that the trend toward higher rejection in D+ cases raised concerns and recommended further research [9].

Of note, passive HIV strain transfer from a viremic HIV-positive donor to an HAART-treated HIV-positive recipient was detected in blood and urine for up to 16 days post-transplantation, but not beyond [10].

In cases of liver failure, only a few case reports exist of HIV D+/R+ liver transplantation (LT), all with limited follow-up. Therefore, as with KT, a prospective multicenter pilot study was conducted comparing HIV D+/R+ LT to HIV D-/R+ LT [11]. Between 2016 and 2019, 45 LT recipients (including 8 simultaneous LT-KT cases) were enrolled across 9 centers. The cohort included 24 HIV D+/R+ and 21 HIV D-/R+ patients, with a median follow-up of 23 months. The median CD4 count was 287 cells/μL, and all recipients were on antiretroviral therapy. Additionally, 56% were hepatitis C virus (HCV) seropositive, and 13% were HCV-viremic.

One-year weighted survival was significantly higher in the D- group than in the D+ group (100% vs. 83%, $p = 0.04$). There were no significant differences in 1-year graft survival, rejection, HIV breakthrough, or serious adverse events. However, the D+/R+ group experienced more opportunistic infections, infection-related hospitalizations, and cancer cases, warranting further investigation [11].

In France in 2023, 325 PLWH were on the waiting list for KT and 20 for LT [12]. A program was launched in 2022 to evaluate HIV+ donors for PLWH candidates. For deceased donors, criteria are stringent: brain death must be confirmed, the donor must be on HAART with a viral load below 50 copies/mL for at least 1 year, and antiviral therapy must be stable. No opportunistic infections should be present at the time of donation. No minimum CD4 count is required. HIV proviral DNA genotyping is expected to be feasible through biocollection of the donor's plasma. French guidelines do not specify antiviral resistance criteria, as an undetectable viral load is mandatory for donor eligibility.

For living donors, the decision is made on a case-by-case basis by an independent multidisciplinary expert committee. HIV-positive individuals with well-controlled infections and no comorbidities may be considered low-risk kidney donors. Recipient eligibility criteria are the same as those for HIV-negative organs. No data is currently available on HIV D+/R– transplantation.

## DONOR-TRANSMITTED INFECTIONS - VIRAL HEPATITIS

Nassim Kamar, Toulouse University Hospital, France, presented an overview about donor-transmitted viral hepatitis.

### Hepatitis E Virus (HEV)

Currently, HEV infection is the most common viral hepatitis. HEV is an RNA virus that is still underdiagnosed. Genotypes 1 and 2 (G1 and G2) are strictly human, of orofecal transmission, G3 & 4 are a zoonosis. Chronic HEV is defined by replication beyond 3 months after infection.

In case of HEV infection in SOT, around two-thirds developed chronic hepatitis. The use of tacrolimus rather than cyclosporin A is one of the main predictive factors for chronic hepatitis [13]. Immunosuppressant dose reduction may be a first-line therapeutic option. The successful use of ribavirin (RBV) for the treatment of chronic HEV infection was described in 59 immunosuppressed recipients of SOT [14] at a median dose of 600 (29–1200) mg/day, and a median therapy duration

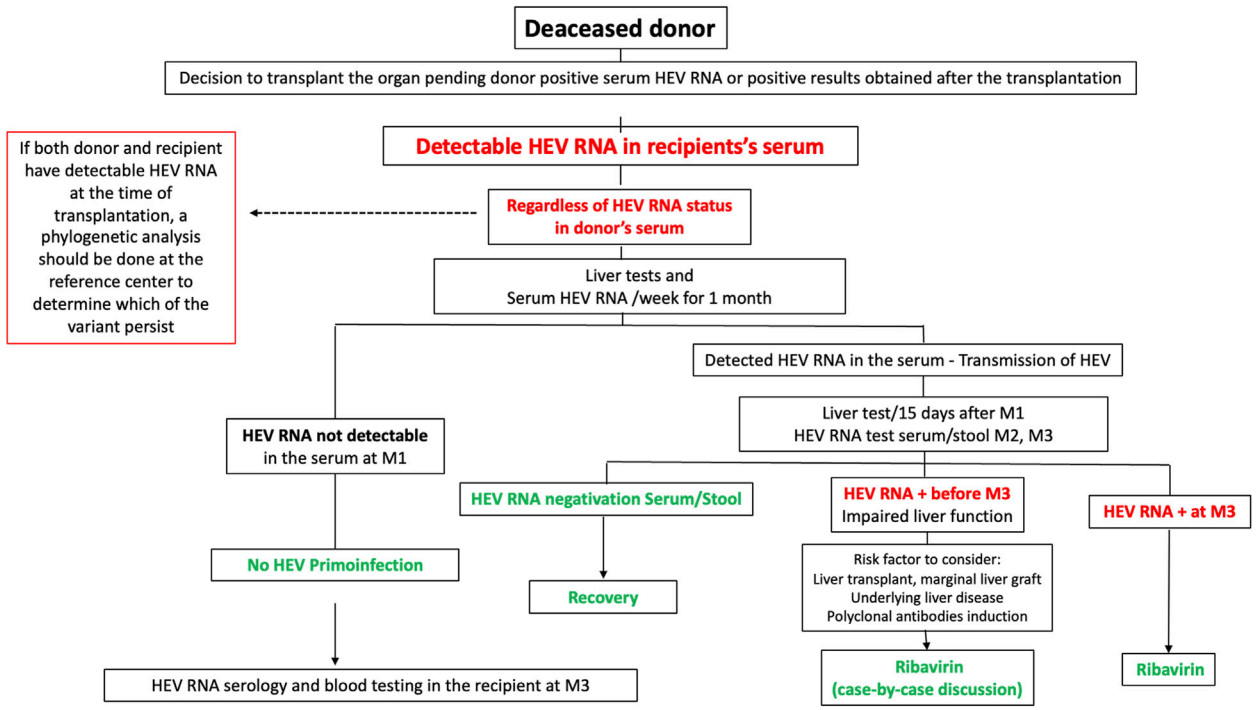


**FIGURE 1 |** Recommendations in France for cases where HEV RNA is detected in the recipient's blood following transplantation from a HEV-positive deceased organ donor.

3 (1–18) months. The duration of therapy can be optimized according to HEV fecal shedding [15]. Indeed, before stopping ribavirin, HEV RNA should be negative both in the serum and the stools. In case, HEV RNA is not detected anymore in the serum but still detected in stools, ribavirin should be pursued and HEV RNA should looked for monthly until both become negative which allow stopping ribavirin.

In case of failure, PEGylated-interferon-α has been successfully used in liver-transplant patients, but it is contraindicated in other organ recipients because it increases the risk of acute rejection [16].

Human-to-human transmission of HEV 3&4 is possible but rare, through the blood or the organs. HEV RNA is detected in roughly 1 out of 1200 blood donors. Systematic screening has been implemented in the UK; the incidence of HEV in the organs of deceased donors is 0.94 per 1,000, which is approximately four times higher than in their blood [17].

In France, HEV RNA is now screened in donors' sera. If HEV RNA is detected in a living donor, transplantation is delayed. In deceased donors, if this information is available before harvesting, the transplantation team should inform the recipient. Sometimes, donor HEV RNA positivity information may only become available after transplantation. In both cases, hepatic surveillance is recommended. If the recipient becomes positive for more than 1 month, a treatment by RBV is discussed individually, and if positive for 3 months, RBV is given. **Figure 1** summarizes the recommendations in France for cases where HEV RNA is detected in the recipient's blood following transplantation from a HEV-positive deceased organ donor.

## Hepatitis C (HCV)

The lastest KDIGO Guidelines recommend that all chronic renal disease (CRD) and KT patients with HCV infection are evaluated for direct-acting antiviral (DAA)-based therapy [18]. DAA therapy should be administered to all HCV-infected transplant candidates, either before or after transplantation. Factors guiding timing of HCV treatment (before vs. after kidney transplantation) include donor type (living vs. deceased donor), anticipated waiting-list time by donor type, severity of hepatic fibrosis, and willingness of the patient and program to accept an organ from an HCV-infected donor. The KT from HCV deceased donor into HCV recipients followed by early post-transplant treatment with DAA agents successfully shortened the waiting time for HCV-infected kidney transplant candidates [19].

In France, since 2023, the eligibility criteria for recipients have been expanded to include all organ recipients, regardless of whether the donor is HCV serology positive or negative, as long as the donor's RNA is negative. The risk of HCV transmission from a donor with a positive anti-HCV antibody and negative viral genomic screening (VGM) is estimated to be between 1% and 9% in liver transplantation'.

A systematic PCR monitoring in post transplantation and DAA enable a 100% sustained virologic response.

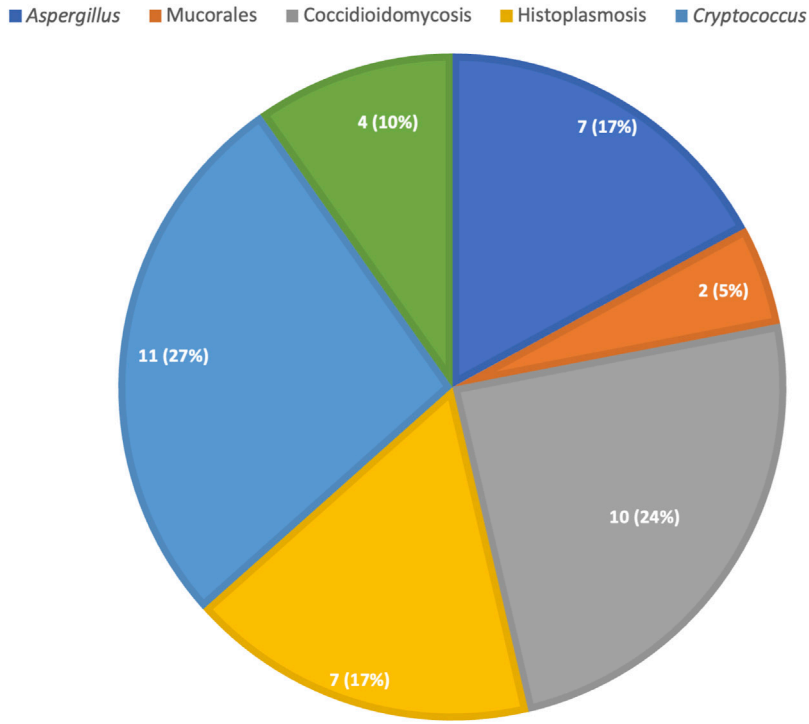


**FIGURE 2 |** Distribution of proven/probable donor derived non-candida fungal infections, absolute count (percent).

## Hepatitis B (HBV)

In France since 2023, the transplantation of kidneys from deceased donors who are hepatitis B surface antigen (HBsAg)-positive and/or viremic (HBV DNA-positive) has been possible for recipients who are HBsAg-positive and/or viremic. Recipients must be treated in post transplantation with anti-HBV therapy during at least 6–12 months and longer as HBsAg or viremia remain positive.

The utilization of HBV NAT+ allografts into seronegative recipients is not authorized in France. It has been investigated in US [20]. KT and LT patient and allograft survival were not different between HBV NAT+ and HBV NAT- recipients whereas HBV NAT+ KT recipients had shortened waitlist time and pretransplant duration on dialysis.

# DONOR DERIVED NON-CANDIDA FUNGAL INFECTIONS IN SOLID ORGAN TRANSPLANT (SOT) RECIPIENTS

Olivier Lortholary, Necker University Hospital, France, then addressed donor derived non-*Candida* fungal infections.

Data from the Transplant-Associated Infection Surveillance Network (TRANSNET), a prospective study performed in 23 transplant centers throughout the U.S, demonstrated that the most frequent non-Candida invasive fungal infections (IFI) were invasive aspergillosis (19%), cryptococcosis (8%), non-*Aspergillus* molds (8%) and endemic fungi (5%) [21].

Donor derived fungal infections are very rare but may have a poor prognosis. Thus, an early unusual or severe infection after SOT should suggest graft transmitted IFI.

In the 10 years analysis of donor-derived infections in the U.S, the mortality rate was 15% for all infections but it was considerably higher for certain fungi, particularly *Coccidioides* [22]. Delay in diagnosis likely contributed to the high mortality as these diseases may present with diffuse, difficult-to-recognize symptoms in the posttransplant period. In this study, coccidioidomycosis, aspergillosis, cryptococcosis and histoplasmosis were the most frequent non-*Candida* IFI. The distribution of proven/probable donor-derived non-*Candida* fungal infection is recapitulated in **Figure 2**. As the number of donors coming from endemic areas increases, travel to endemic areas should be investigated, when possible, in donors.

## Donors With Active or Latent Invasive Fungal Disease (IFD)

The circumstance is rare but potentially severe for graft and or recipient. The graft can be contaminated by an infected donor or by an organ contaminated at the time of sampling/transportation (for example, the presence of *candida* in the preservation fluid). The criteria for classification of donor-derived fungal infections were defined in 2012 [23].

To avoid such complications, sampling is contraindicated when donors are known to have an active fungal infection. However, donor active infections may be unknown at time of transplantation: in potential donors with unexplained meningoencephalitis, cryptococcosis screening should be considered. Descriptions of donor-derived *cryptococcosis* also showed reactivation of a latent infection in the transplanted organ [24]. Mold infections as *Aspergillus fumigatus and Scedosporium apiospermum* have also been acquired through unrecognized infections in the donor. Transplant tourism represents another concern: IFI, frequently originating at the graft site have emerged as a devastating complication and are associated with high rates of graft loss and death [25].

Regarding endemic mycoses, few cases have been described [26].

A prospective study performed in 15 transplant centers throughout the U.S, found only 33 cases of dimorphic mycoses among 16,806 patients who received a SOT during the 5-year study period; 23 histoplasmosis, 6 coccidioidomycosis, and 4 blastomycosis, most of them were primary infections, not transmitted by the donor [27].

*Histoplasmosis* occurs in only 0.1%–0.5% of transplant recipients from endemic areas and 1%–5% of healthy subjects have positive tests for Histoplasma antigen or antibodies, primary infection being the dominant mode of acquisition. When disease is transmitted via an infected allograft, the infection is most likely transmitted via the liver allograft. Itraconazole treatment, until antigen clearance is met, is recommended for the management of histoplasmosis in living donor. *Coccidioidomycosis* is another endemic mycosis, mainly in the southwest of United States, and may lead to vasculitis and meningoencephalitis in the recipient. Its first-line therapy in donors is fluconazole [28].

### The Contamination of Preservation Fluid

While *Candida* spp are from far the most frequent fungal source of contamination fluid, *Aspergillus* contamination has also been described [29].

Both infections may lead to devastating abscesses and arteritis, with high transplant removal and death rates. Thus a systematic fungal culture of preservation fluid could be discussed and is already recommended in France [30].

In the future, increasing rates of travels and migrations, as well as changing climate, may increase specific risks for transplant recipients, especially those of transmitted or primary acquired endemic mycoses.

## EMERGING ARBOVIRUSES IN TRANSPLANTATION

Dr. Carole Eldin, infectious disease physician and member of the UVE research unit (Unité des Virus Emergents), Marseille, France, presented an update on emerging arboviruses in SOT settings. Climate change is associated with an expansion of the density and distribution area of many vectors (mainly mosquitoes) and an increase in vectorial capacity, leading to the emergence of arboviruses.

### West Nile Virus (WNV)

West Nile virus is an Orthoflavivirus transmitted by Culex mosquitoes from animals (mostly birds, which are the reservoir, or horses) to humans. The only way of human-to-human transmission is through SOT or blood transfusion. The incidence of WNV infection is increasing in Europe [31]. In France, a cluster was described in the South-West (Gironde) in 2023, where 32 cases were reported, including 4 blood donors.

WNV infections in solid organ transplant (SOT) recipients can occur following SOT from an infected donor or via a mosquito bite after SOT. Infections are more severe in SOT recipients and can lead to neuroinvasive disease, mainly encephalitis, in 40%–60% of infected patients [32, 33].

Prevention of the infection is based on the screening of organ donors (PCR and serology), which has been recommended by the French High Council of Public Health [34] since 2020 for donors who have been exposed to risk areas during the virus circulation period up to 28 days before organ donation. The list of at-risk areas is regularly updated [35]. The result of WNV screening should be known before transplantation, and the donation should be postponed in case of a positive result, particularly in the case of living donation. However, improving access to screening techniques is still in progress to allow for real-time decision-making.

In case of a diagnosis of infection in a SOT recipient, there are no established treatment guidelines, but some cases have reported the use of immunomodulation, intravenous immunoglobulins [36], interferon, or prophylaxis with ribavirin or WNV antibodies from convalescent plasma [37, 38]. We summarize the current French National Biomedicine agency in **Figure 3**.

### Tick-Borne Encephalitis (TBE)

This arbovirus is mainly transmitted to humans by an Ixodes tick bite.

Transmission of TBE from a deceased donor to three SOT recipients (two kidneys and one liver) has been reported in Poland [39], leading to death.

Since 2020, the HCSP has recommended the inclusion of a history of tick bites within the past 28 days in the organ pre-donation questionnaires. In case of a positive response in an at-risk geographical area and period, specific molecular and serological tests should be performed. No treatment is currently available.

### Dengue (DENV)

The world has seen a historic increase in cases of dengue in recent years [40]. In France, in 2022, 35 autochthonous cases were reported, and an autochthonous cluster of 3 cases in the Paris area confirmed the emergence of this virus [41]. Some cases of dengue after renal transplantation have been reported, notably in French overseas territories (Réunion Island) [42, 43]. The particularity of some cases is that the negative blood test results from donors were not sufficient to prevent transmission to the recipient. This report suggests that the kidney may be a potential viral reservoir for dengue virus. To date, there is no specific prevention measure in mainland France regarding transplantation.

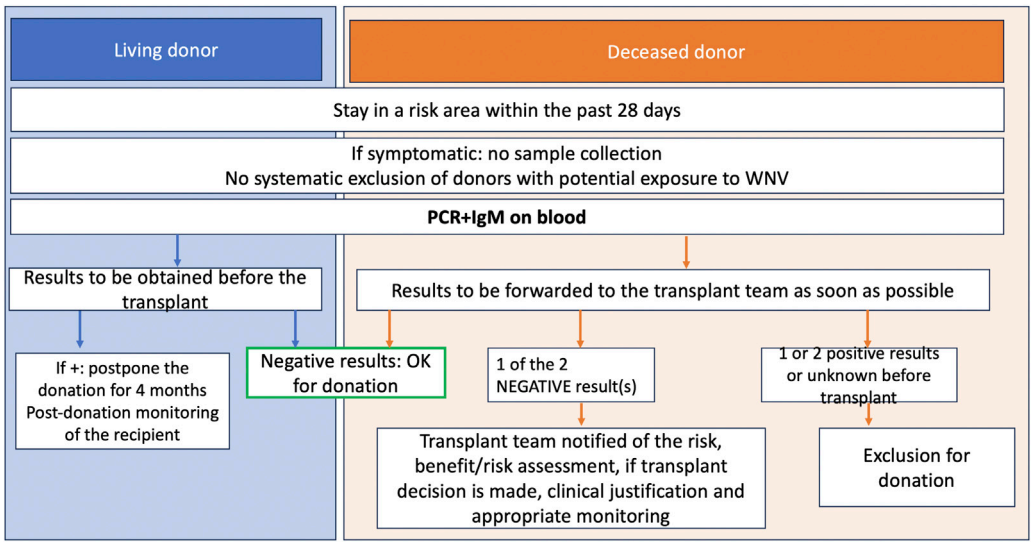


**FIGURE 3 |** Biomedecine National Agency algorithm, donor screening for West Nile Virus.

## BK POLYOMAVIRUS–FOCUS ON KIDNEY TRANSPLANTATION

Hans H. Hirsch, University of Basel, Switzerland, presented recent data and a summary of 2024 international guidelines about BK polyomavirus in kidney transplants.

The BK polyomavirus (BKPyV) is a small, environmentally resilient virus with a circular, double-stranded DNA genome. While its natural transmission route remains unclear, it likely reaches the reno-urinary tract via the bloodstream, establishing lifelong persistence [44]. Approximately 10% of BKPyV-seropositive blood donors have low levels of the virus in their urine [45] highlighting the role of donor-derived BKPyV in kidney transplantation.

BKPyV persists in the reno-urinary tract through several mechanisms, including latency, reactivation within host cell nuclei (undetectable by the immune system until cell lysis), agnoprotein expression that disrupts innate immune sensing, and viral genome variations leading to serotypes and variants [46]. There are four main BKPyV serotypes: I (most common, 71% of cases), IV (19%), II (8%), and III (2%). Genetic and immunological differences between these serotypes influence post-transplant BKPyV-related risks [47].

In immunosuppressed patients, BKPyV is linked to three major complications: BKPyV nephropathy (BKPyVAN) in kidney transplantation; BKPyV hemorrhagic cystitis in hematopoietic cell transplantation; BKPyV-associated urothelial carcinoma, often years after poorly controlled BKPyVAN [48].

For kidney transplant patients, multiple prospective studies have confirmed the continuum of presentations [49]: no/low level viruria (<100,000 copies/mL) in 60%–80%; high-level viruria (decoy cells or >10 million copies/mL) in 20%–40%; the new-onset plasma BKPyV-DNAemia in 5%–21% after 2–6 weeks, and eventually the invasive diagnosis of biopsy-proven BKPyV-nephropathy initially without and then with declining allograft function in 1%–15%.

### Sources and Risks of BKPyV

The primary sources of BKPyV in kidney transplantation include environmental exposure [50], viral reactivation in the recipient [51], and donor-derived infections (DDI) [52]. Donor-derived BKPyV plays a significant role, with genomic sequencing often matching the donor's BKPyV genotype. Recipients of kidneys from donors with urinary BKPyV shedding are at higher risk of viral replication [53]. Also, high antibody levels in the donors as a marker of significant or recent exposure, low and serotype-mismatched antibodies in the recipients have been identified as a risk factor for BKPyV-DNAemia/nephropathy [54, 55].

### New Guidelines for the Management of BKPyV in Kidney Transplantation

Pr Hirsch described the consequences on the clinical practice through summarizing [56]. The Second International Consensus Guidelines on the Management of BK Polyomavirus in Kidney Transplantation [57].

Diagnosis: Routine monitoring of plasma BKPyV-DNA levels up to 2 years post-transplant (3 years for pediatric patients). New-onset plasma BKPyV-DNAemia of >1,000 copies/mL sustained for 3 weeks was defined as probable BKPyV-nephropathy, plasma BKPyV-DNAemia of >10,000 copies/mL as presumptive BKPyV-nephropathy, and biopsy-proven BKPyV-nephropathy without and with impaired renal function, respectively. Biopsies should include systematic BKPyV-DNAemia assessment.

Management: Gradual reduction of immunosuppressants (e.g., mycophenolate, then tacrolimus) is recommended. Antiviral treatments like leflunomide and cidofovir lack strong clinical evidence. Retransplantation is viable if BKPyV-DNAemia clears, while allograft nephrectomy is deferred unless nephropathy persists.

### Future Directions

Further randomized trials are required to validate molecular diagnostics and safe antiviral strategies, including multi-virus-specific T-cell therapy, which shows promise for treating BKPyV nephropathy [58].

## THE DONOR WITH A KNOWN AND TREATABLE INFECTION: WHAT SHOULD WE DO?

J-R Zahar, Avicenne University Hospital, Paris, France did an interactive presentation about practical cases of donors with a known and treatable infection.

The shortage of transplantable organs highlights the need to correctly identify situations of potential organ availability. In case of an ongoing infection at the time of donation, there are a number of data available from different scientific societies [59, 60]. Simultaneous bacterial infections are often "unrecognized" at the time of transplantation and the diffusion of multidrug resistant micro-organisms is an additional risk for recipients. However, most case series acknowledge that bacterial infections transmitted by the donor are rare [61]. The risk of transmission depends on several factors such as the site of infection, the species involved, their antibiotic susceptibility profile, previous antibiotic treatment regimen and duration before and upon transplantation.

Four factors are to be considered (apart from immunosuppression treatment):

- The site of infection: the risk of donor-derived infection (DDI) seems to be different in the event of *in situ* infection, or at distance from the transplanted organ. The bacterial inoculum effect influences bacterial clearance, as high inoculum are associated with lower clearance, exemplified by intravascular infections in which bacterial DNA load are elevated [60].
- The microbial species: *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are known to have a higher risk of morbidity and mortality in acute severe settings. Consequently, causal microbial species must be taken into account for treatment duration.
- The mechanisms of multidrug resistance and types of antibiotic treatment received: the growing spread of multidrug resistance across communities and hospitals expose transplant recipients to a higher risk of difficult-to-treat infections. Consistently, an assessment of the prior treatments received by the candidate recipient and the donor are essentials (bactericidal activity, dose, modalities of administration and duration) to guide any empiric antibiotic strategy upon transplantation procedure.
- The transplanted organ: some infectious risks are organ-specific. It is important to take into account the risk associated with the "non-drainable" nature of the infection and the capacity of the antibiotics administered to diffuse into the infected transplanted organ.

## CONCLUSION

During the well-attended "Infection and Transplantation Group" day, the major and recent advances in the field of the current risk of donor-transmitted infections in solid organ transplantation highlighted the crucial importance of vigilance and the constant updating of knowledge in this area. This led us to keep in mind key information:

- Detailed screening of potential organ donors is essential to detect infections that could be transmitted to recipients. This includes assessing medical history, previous infections, vaccinations, and exposures.
- Various infections pose risks, such as hepatitis viruses mostly HEV, BK virus, fungal infection
- Climate change involved the increase of emerging Infections. Diseases like West Nile Virus, Dengue, are particularly important in transplantation due to their potential transmission through organs, their increase severity-risk in those immunocompromised hosts in absence of specific treatment, and have been integrated in donor's screening seasonally.
- The acceptance and outcomes of organ transplantation from HIV-positive donors to HIV-positive recipients are evolving, with successful cases.
- Globalization and travel contribute to the spread of infectious diseases, impacting donor screening protocols.

Overall, ensuring the safety of organ transplantation involves rigorous screening protocols, continuous monitoring, and adapting to emerging infectious disease risks. Each case requires careful consideration of risks versus benefits to optimize patient outcomes post-transplant, in the context of organ shortage.

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# Number of Pretransplant Therapeutic Plasma Exchange Sessions Increase the Recurrence Risk of Hepatocellular Carcinoma in ABO-Incompatible Living Donor Liver Transplantation

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Previous studies have reported comparable oncologic outcome between ABO-incompatible (ABOi) living donor liver transplantation (LDLT) and ABO-compatible (ABOc) LDLT in patients with hepatocellular carcinoma (HCC). We aimed to analyze the relationship between number of therapeutic plasma exchanges (TPE) before LDLT and HCC outcomes in ABOi LDLT. In this single-center retrospective study, 428 adult LDLT recipients with HCC were categorized into three groups according to ABO incompatibility and the number of pretransplant TPE: ABOc (n = 323), ABOi/TPE ≤5 (n = 75), and ABOi/TPE ≥6 (n = 30). The RFS and HCC recurrence rates were compared. Three groups showed similar characteristics in most demographics, pretransplant tumor markers and pathologies. The median initial isoagglutinin (IA) titer was 1:64 (range negative-1:512) in ABOi/TPE ≤5 group and 1:512 (range 1:128–1:4,096) in ABOi/TPE ≥6 group. Five-year RFS was significantly lower (75.7% vs. 72.7% vs. 50.0%, P = 0.005) and HCC recurrence was significantly higher in the ABOi/TPE ≥6 group than in the other groups(16.4% vs. 17.0% vs. 39.4%, P = 0.014). In multivariable Cox regression analysis, ABOi/TPE ≥6 was an independent risk factor for RFS (aHR 1.99, 95% CI:1.02–3.86, P = 0.042) and HCC recurrence (aHR 2.42, 95% CI:1.05–5.57, P = 0.037). More than six pretransplant TPE sessions may increase the risk of HCC recurrence after ABOi LDLT. Reducing TPE sessions to fewer than six should be considered while maintaining immunological stability through IA titer control.

**Keywords: ABO-incompatible living donor liver transplantation, hepatocellular carcinoma, plasma exchange, surgical oncology, oncologic outcome**

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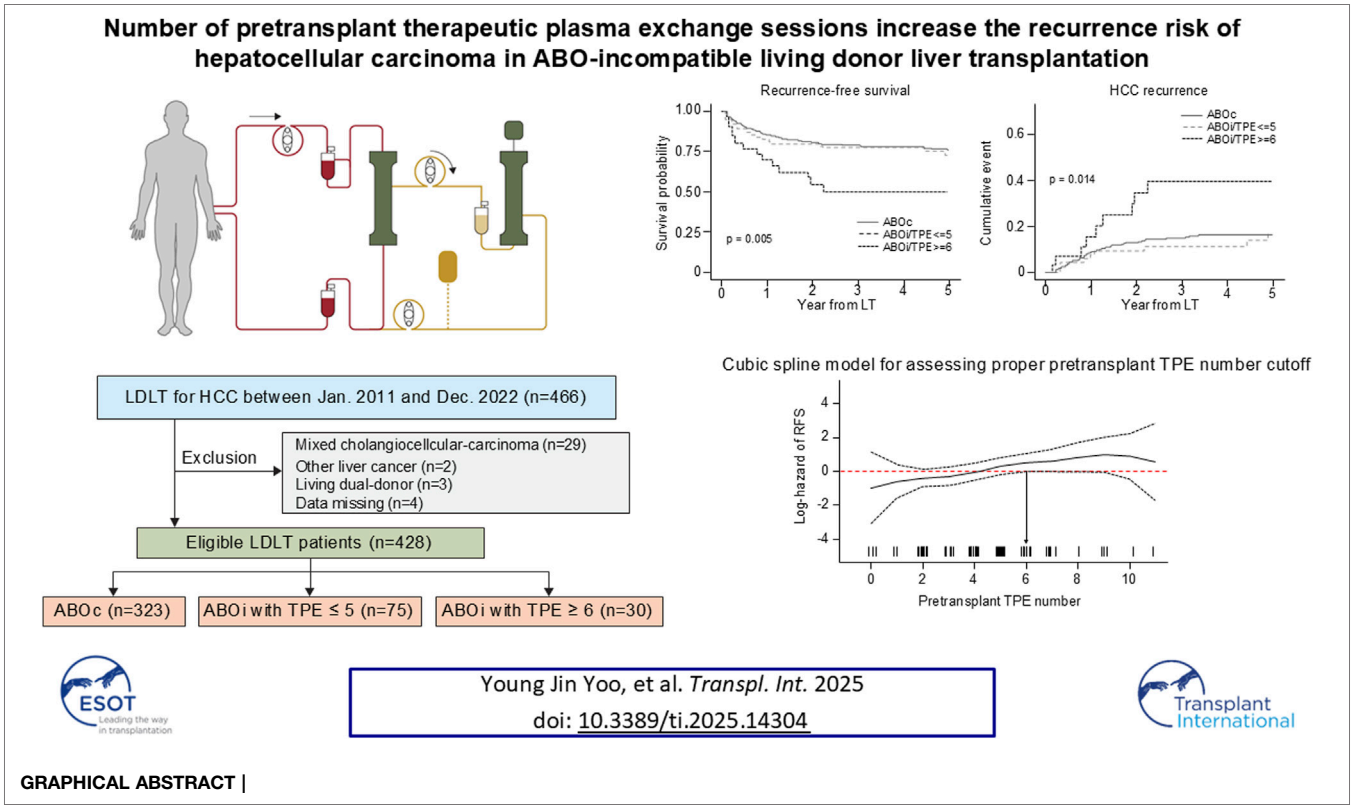
**Abbreviations:** ABOc, ABO-compatible; ABOi, ABO-incompatible; AFP, Alpha-fetoprotein; aHR, Adjusted Hazards ratio; CD, Cluster of differentiation; CI, Confidence interval; DDLT, Deceased donor liver transplantation; MELD, Model for end-stage liver disease; HCC, Hepatocellular carcinoma; HR, Hazards ratio; IA, Isoagglutinin; LDLT, Living donor liver transplantation; LT, Liver transplantation; MoRAL, Model of Recurrence After Liver transplant; PIVKA-II, protein induced by vitamin K absence or antagonist II; PVTT, portal vein tumor thrombosis; RFS, Recurrence-free survival; TPE, Therapeutic plasma exchange.




**GRAPHICAL ABSTRACT |**

## INTRODUCTION

Liver transplantation (LT) is an effective, and sometimes the only, treatment option for unresectable hepatocellular carcinoma (HCC). However, owing to organ shortages, not all patients can receive timely LT. Consequently, the demand for living donor liver transplantation (LDLT) for HCC is increasing worldwide, and numerous studies have reported comparable oncological outcomes between LDLT and deceased donor liver transplantation (DDLT) [1–7].

When an ABO-incompatible (ABOi) living donor is the only available option, ABO-incompatible LDLT (ABOi LDLT) with proper desensitization becomes a viable choice [8–15]. Despite the need for pretransplant antibody treatment and an increased risk of posttransplant infections, ABOi LDLT has been reported as a feasible treatment for patients with end-stage liver disease, offering substantial survival benefits even for those with high Model for End-Stage Liver Disease (MELD)scores [11, 12, 16, 17]. Additionally, several Korean centers have reported that ABOi LDLT has a similar impact on HCC outcomes compared to ABO-compatible (ABOc) LDLT (ABOc LDLT) [12, 18–21].

Despite these reports, ABOi LDLT necessitates more potent immunosuppression, including B-cell depleting agents, therapeutic plasma exchange (TPE), and higher maintenance immunosuppressants, which raises concerns about potentially adverse oncologic outcomes [22, 23]. Furthermore, ABOi LDLT requires additional pretransplant TPE sessions as the titer of blood group antibodies increases. However, there are no published studies examining the differences in HCC outcomes based on the degree of desensitization required.

Therefore, this study aimed to analyze the effect of the number of pretransplant TPE sessions, a critical component of pretransplant treatment, on HCC outcomes in ABOi LDLT.

## MATERIALS AND METHODS

### Study Material

In this retrospective cohort study, we analyzed single-center data from 466 patients who underwent LDLT for HCC between January 2011, when ABOi LDLT was initiated at our institution, and December 2022. The baseline characteristics and details of explant pathology were retrieved from a prospectively collected institutional database. The exclusion criteria were as follows: mixed cholangiocellular carcinoma on pathology (n = 29), liver cancer other than HCC (n = 2), LDLT from a dual living donor (n = 3), and missing data (n = 4) (**Supplementary Figure S1**, study population).

A total of 428 eligible patients were categorized according to ABO incompatibility and the number of pretransplant TPE sessions: ABO-compatible (ABOc group, n = 323, 75.5%), ABO-incompatible with fewer than 5 TPE sessions (ABOi/TPE ≤5 group, n = 75, 17.5%), and ABO-incompatible with six or more TPE sessions (ABOi/TPE ≥6 group, n = 30, 7.5%). The

cutoff for the number of TPE sessions (6 times) was determined based on the spline curve for recurrence-free survival (RFS), where the hazard began to significantly increase (**Supplementary Figure S2**, spline curve).

## Data Collection and Outcomes

All relevant information regarding recipients, donors, and LDLT surgery was retrieved from the institutional database. The underlying liver diseases associated with HCC included hepatitis B, hepatitis C, and non-B/non-C. Detailed information on explant pathology and tumor markers, such as alpha-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist-II (PIVKA-II) at the time of LDLT, was obtained. Additionally, data on pretransplant locoregional and systemic treatments, as well as previous hepatectomies, were collected for patients with HCC. RFS and HCC recurrence (time to recurrence) were the primary outcomes.

## Pretransplant Desensitization for ABO Incompatibility

Our institutional protocol for desensitization in ABOi LDLT mainly consisted of rituximab and TPE, as described previously [24, 25]. Every pretransplant TPE sessions and desensitization protocols were performed within 2 weeks prior to ABOi LDLT. A recently revised version of this protocol is provided in **Supplementary Figures S3-1–S3-2** (Desensitization protocol for ABOi LDLT). For the initial and target isoagglutinin (IA) titers, higher IgM or IgG anti-A/B titers were employed. The number of preoperative TPE sessions was determined based on the initial IA titer, the response to TPE, and the decrease in the ABO titer. Splenectomy and postoperative TPE were performed in patients at high risk of rejection, specifically those with an IA titer greater than 1:64 at the time of LT. Additional rounds of TPE were conducted postoperatively in cases of clinical rejection or IA titer rebound, defined as a resurgence to 1:64 and a minimum two-fold increase. Following TPE, intravenous immunoglobulin (IVIG) was administered at a dose of 500–800 mg/kg on an individualized basis, depending on ABO antibody levels and infection risk.

## Statistical Analysis

Depending on the type of variable, data are presented either as numbers (percentages) or as medians (interquartile range [IQR]). The Mann–Whitney U test or chi-square test was employed to compare continuous and categorical variables, respectively, when appropriate. HCC outcomes were analyzed using Kaplan–Meier curves and log-rank tests. Multivariable Cox regression was performed to evaluate HCC outcomes in the entire cohort, including covariates with significant P values <0.1 from the univariable analysis. In the risk analysis of HCC recurrence, non-HCC death was considered a competing risk, utilizing the Fine and Gray method [26] for competing risk regression. In the ABOi LDLT groups, the 5-year estimates of HCC recurrence were compared based on the number of TPE sessions (≤5 vs. ≥6) across various subgroups categorized by tumor burden, which reflects the tumor size, tumor number, and AFP and PIVKA-II levels [27–30], as well as ABO antibody strength, postoperative rebound of IA titer and TPE, and splenectomy status. Subgroup analyses were conducted in a univariate manner due to the small size of each group. All statistical analyses were performed using the R statistical package, version 4.3.0 for macOS[1], with the significance threshold set at $P < 0.05$.

## Statement of Ethics

This study was performed in accordance with the Declaration of Helsinki and the Declaration of Istanbul and was approved by the Institutional Review Board of Severance Hospital, Yonsei University Health System (IRB number 4-2024-0977). The requirement of informed consent was waived due to the retrospective nature of the study.

# RESULTS

## Baseline Characteristics

No significant difference was noted in most baseline patient characteristics (**Table 1**). The distribution of LT years was also not statistically significant (P = 0.069); however, a higher proportion of transplants in the ABOi groups occurred between 2016 and 2019. Most patients had hepatitis B as the underlying cause of HCC across all groups, with no statistical significance (76.8% in ABOc, 69.3% in ABOi/TPE ≤5, and 86.7% in ABOi/TPE ≥6, P = 0.401). Notably, the ABOi/TPE ≥6 group required a significantly higher number of red blood cell transfusions (median 4.5 packs) than the ABOc and ABOi/TPE ≤5 groups (median two packs, P = 0.014). No significant differences were noted in the pretransplant AFP and PIVKA-II levels. Additionally, history of hepatectomy, locoregional therapy (LRT), and systemic treatment were similar across the groups.

Most characteristics from explant pathology were similar across the groups, including the incidence of portal vein tumor thrombosis (PVTT), total necrosis, number of viable tumors, maximum tumor size, microvascular invasion, and poor differentiation. However, the presence of satellite nodules was significantly higher in the ABOi/TPE ≥6 group (26.7%) than in the other groups (10.8% in ABOc and 10.7% in the ABOi/TPE ≤5, P = 0.035).

## Detailed Information on Recipient of ABOi LDLT

Almost all patients who underwent ABOi LDLT received rituximab and at least one cycle of TPE for desensitization. **Table 2** presents details regarding ABO incompatibility and desensitization protocols for patients in the ABOi group, categorized by the number of pretransplant TPE sessions. A significantly higher proportion of A to O transplants was observed in the ABOi/TPE ≥6 group (56.7%) than in the ABOi/TPE ≤5 group (17.3%, P < 0.001). The median IA titer was significantly higher in the ABOi/TPE ≥6 group than in the

[1]http://cran.r-project.org/

**TABLE 1 |** Baseline characteristics of patients, according to ABO incompatibility and the number of pretransplant therapeutic plasma exchange.

| Variables | ABOc (n = 323) | ABOi/TPE ≤5 (n = 75) | ABOi/TPE ≥6 (n = 30) | *P* |
|---|---|---|---|---|
| Age, years | 56.8 ± 7.0 | 57.1 ± 6.9 | 55.6 ± 7.3 | 0.608 |
| Sex, female | 58 (18.0) | 17 (22.7) | 7 (23.3) | 0.539 |
| BMI | 23.8 (22.3–26.1) | 24.9 (23.4–26.3) | 24.0 (21.9–25.9) | 0.065 |
| LT year | | | | 0.069 |
| 2011–2015 | 112 (34.7) | 15 (20.0) | 7 (23.3) | |
| 2016–2019 | 104 (32.2) | 33 (44.0) | 14 (46.7) | |
| 2020–2022 | 107 (33.1) | 27 (36.0) | 9 (30.0) | |
| Underlying liver disease for HCC | | | | 0.401 |
| Hepatitis B | 248 (76.8) | 52 (69.3) | 26 (86.7) | |
| Hepatitis C | 23 (7.1) | 8 (10.7) | 1 (3.3) | |
| Non-B, Non-C | 52 (16.1) | 15 (20.0) | 3 (10.0) | |
| Hypertension | 74 (22.9) | 21 (28.0) | 9 (30.0) | 0.490 |
| Diabetes mellitus | 97 (30.0) | 27 (36.0) | 12 (40.0) | 0.367 |
| Pretransplant MELD | 10 (8–14) | 10 (8–13) | 11.5 (8–14) | 0.674 |
| Donor age, years | 31 (24–40) | 34 (26–40.5) | 35 (25–41) | 0.203 |
| Donor sex, female | 130 (40.2) | 26 (34.7) | 11 (36.7) | 0.647 |
| GRWR[a] <0.8 | 27 (8.4) | 5 (6.7) | 2 (6.7) | 0.856 |
| Macrovesicular steatosis ≥10% | 46 (15.2) | 9 (12.3) | 2 (6.9) | 0.422 |
| Cold ischemic time, min | 126 (106–150) | 126 (102–152.5) | 128.5 (96–180) | 0.884 |
| Transfusion RBC, packs | 2 (0–6) | 2 (0–7.5) | 4.5 (2–9) | 0.014 |
| AFP at LT, ng/mL | 6.6 (3.3–23.1) | 6.4 (3.3–14.0) | 4.3 (2.2–27.2) | 0.545 |
| PIVKA at LT, mAU/mL | 38 (22–112) | 38 (23.5–141) | 47 (20–232) | 0.666 |
| Hepatectomy history | 62 (19.2) | 13 (17.3) | 7 (23.3) | 0.779 |
| Pretransplant LRT | 246 (76.2) | 59 (78.7) | 23 (76.7) | 0.899 |
| Systemic treatment | 45 (13.9) | 9 (12.0) | 5 (16.7) | 0.812 |
| Explant pathology | | | | |
| Total necrosis | 57 (17.6) | 13 (17.3) | 4 (13.3) | 0.836 |
| Viable tumor number | 1 (1–3) | 2 (1–3) | 2 (1–3) | 0.485 |
| Maximum tumor size, cm | 1.7 (1.0–3.0) | 1.8 (0.8–3.0) | 2.4 (1.3–3.7) | 0.139 |
| Microvascular invasion | 76 (23.5) | 20 (26.7) | 9 (30.0) | 0.656 |
| Poor differentiation | 107 (33.1) | 22 (29.3) | 13 (43.3) | 0.388 |
| Satellite nodule | 35 (10.8) | 8 (10.7) | 8 (26.7) | 0.035 |
| PVTT | 5 (1.5) | 2 (2.7) | 1 (3.3) | 0.673 |

*Results presented as number (percentage) or median (interquartile range) values.*

[a]*Graft weight was directly measured during operation.*

*ABOc, ABO, compatible; ABOi, ABO incompatible; AFP, alpha-feto protein; BMI, body mass index; GRWR, graft recipient weight ratio; HCC, hepatocellular carcinoma; LRT, locoregional treatment; LT, liver transplantation; MELD, model for end-stage liver disease; PIVKA, protein induced by vitamin K antagonist-II; PVTT, portal vein tumor thrombosis; TPE, therapeutic plasma exchange.*

ABOi/TPE ≤5 group at initial assessment (1:64 vs. 1:512, P < 0.001), at the time of LT (1:8 vs. 1:32, P < 0.001), and after LT (1:16 vs. 1:32, P < 0.001).

Additionally, a significantly higher proportion of patients in the ABOi/TPE ≥6 group underwent splenectomy (5.3% vs. 23.3%, P = 0.018), pretransplant IVIG (10.7% vs. 53.3%, P < 0.001), posttransplant IVIG (8.0% vs. 30.0%, P = 0.009), and posttransplant TPE (18.7% vs. 36.7%, P = 0.049). The univariate analysis showed no significant association between recipient or donor ABO blood type and 5-year HCC recurrence, regardless of TPE sessions. Similarly, A to O donor-recipient mismatches did not show a significant impact on recurrence risk (**Supplementary Table S1**).

## HCC Outcomes

As shown in the Kaplan-Meier curves in **Figure 1**, a significant difference was observed in RFS between the ABOc group and the ABOi/TPE ≥6 group (5-year survival: 75.7% in the ABOc group vs. 50.0% in the ABOi/TPE ≥6 group, P = 0.005). Additionally, the HCC recurrence rates also differed significantly (5-year survival: 16.4% vs. 39.4%, P = 0.014).

To further evaluate the impact of TPE on oncologic outcomes, we categorized the ABO incompatibility group into subgroups based on the number of TPE sessions: ≤3 sessions (5-year RFS: 76.2%, 5-year HCC recurrence: 17.4%), 4-5 sessions (5-year RFS: 68.5%, 5-year HCC recurrence: 16.5%), and ≥6 sessions (5-year RFS: 50.0%, 5-year HCC recurrence: 39.4%). Although these results were not statistically significant, a trend related to the number of TPE sessions was observed (P = 0.056 for RFS and P = 0.051 for HCC recurrence, **Supplementary Figure S4**).

In the multivariable Cox analyses (**Table 3**), the ABOi/TPE ≥6 group was significantly associated with RFS [hazard ratio (HR) = 1.99, 95% confidence interval (CI): 1.02–3.86, P = 0.042] and HCC recurrence (HR = 2.42, 95% CI: 1.05–5.57, P = 0.037).

## Subgroup Analysis for HCC Recurrence

In the subgroup analysis (**Table 4**), the 5-year HCC recurrence rates were higher across all subgroups in the ABOi/TPE

**TABLE 2 |** Details for ABO incompatibility and desensitization of ABO incompatible group patients, according to therapeutic plasma exchange number.

| Variables | ABOi/TPE ≤5 (n = 75) | ABOi/TPE ≥6 (n = 30) | *P* |
|---|---|---|---|
| ABO type | | | <0.001 |
| *A* | 36 (48.0) | 2 (6.7) | |
| *B* | 16 (21.3) | 1 (3.3) | |
| *O* | 23 (30.7) | 27 (90.0) | |
| Donor ABO type | | | 0.008 |
| *A* | 23 (30.7) | 17 (56.7) | |
| *AB* | 25 (33.3) | 2 (6.7) | |
| *B* | 27 (36.0) | 11 (36.7) | |
| A to O | 13 (17.3) | 17 (56.7) | <0.001 |
| IA titer at initial | 1:64 (1:24–1:128) | 1:512 (1:256–1:1024) | <0.001 |
| IA titer at LT | 1:8 (1:4–1:16) | 1:32 (1:16–1:64) | <0.001 |
| Pretransplant TPE number | 3 (2–4) | 6.5 (6–7) | <0.001 |
| Pretransplant IVIG[a] | 8 (10.7) | 16 (53.3) | <0.001 |
| Rituximab | 73 (97.3) | 30 (100.0) | 0.910 |
| Rituximab conventional dose[b] | 54 (72.0) | 26 (86.7) | 0.078 |
| Pretransplant duration of MMF | 7 (4–8) | 7 (0–8) | 0.645 |
| Pretransplant MMF total dose, mg | 3,500 (3,000–4,000) | 3,500 (2000–4,000) | 0.284 |
| Splenectomy | 4 (5.3) | 7 (23.3) | 0.018 |
| Posttransplant IA titer rebound[c] | 19 (25.3) | 8 (26.7) | 0.986 |
| Posttransplant maximum IA titer | 1:16 (1:4–1:48) | 1:32 (1:16–1:128) | 0.001 |
| Posttransplant TPE[d] | 14 (18.7) | 11 (36.7) | 0.049 |
| Posttransplant IVIG[a] | 6 (8.0) | 9 (30.0) | 0.009 |

*Results presented as number (percentage) or median (interquartile range) values.*

[a]*Pretransplant IVIG total dose range was 7.5–50 g in ABOi/TPE ≤5 group, and 5.5–136 g in ABOi/TPE ≥6 group. Posttransplant IVIG total dose range was 15–127.5 g in ABOi/TPE ≤5 group, and 1–458 g in ABOi/TPE ≥6 group.*

[b]*375 ± 25 mg per body surface area (m2).*

[c]*Defined as IA titer increased to more than 1:64 after transplantation.*

[d]*Posttransplant TPE number ranges 0–10 in ABOi/TPE ≤5 group, and 0–15 in ABOi/TPE ≥6 group.*

*ABOi, ABO incompatible; IA, isoagglutinin; IVIG, intravenous immunoglobulin; LT, liver transplantation; MMF, mycophenolate mofetil; TPE, therapeutic plasma exchange.*

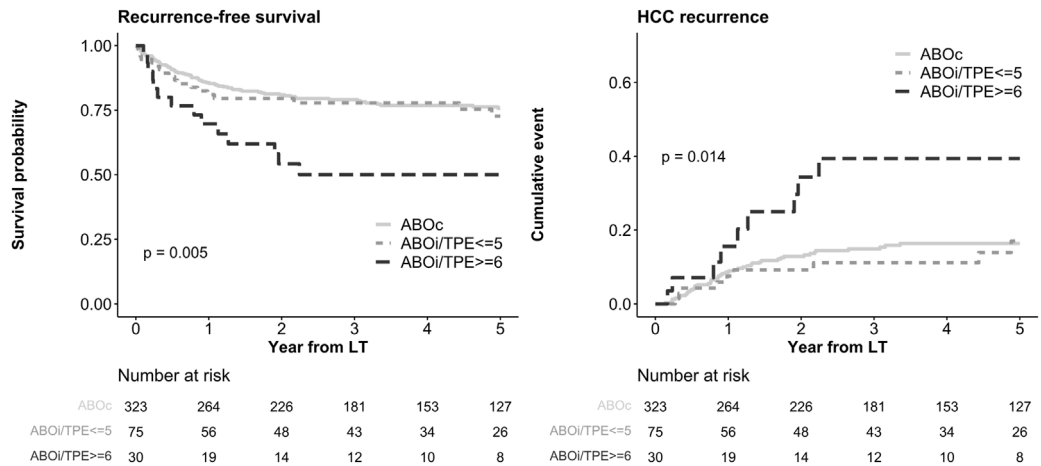



**FIGURE 1 |** Kaplan-Meier curve of RFS and HCC recurrence according to ABO incompatibility and plasma exchange numbers. RFS, recurrence free survival; HCC, hepatocellular carcinoma; ABOc, ABO compatible; ABOi, ABO incompatible; TPE, therapeutic plasma exchange; LT, liver transplantation.

≥6 group. Although this trend is numerically apparent, the small sample size limits the ability to confirm statistical significance. Interestingly, among patients with a tumor marker-based MoRAL score ≥100, the recurrence rate was significantly higher in the ABOi/TPE ≥6 group (56.7%) than in the ABOi/TPE ≤5 group with a MoRAL score ≥100 (16.1%, P = 0.017). However, patients with a MoRAL score <100 exhibited similar 5-year HCC recurrence rates between the two groups (17.6% vs. 20.5%, P = 0.75). **Supplementary Figure S5** illustrates HCC recurrence based on the number of TPE sessions and the MoRAL score. As observed, a marked difference was evident between the ABOi/TPE ≥6 group with a high MoRAL score and the other groups (P = 0.0042).

**TABLE 3 |** Multivariable Cox analysis for recurrence free survival and hepatocellular carcinoma recurrence.

| Variables | Recurrence free survival HR (95% CI) | *P* | HCC recurrence[a] HR (95% CI) | *P* |
|---|---|---|---|---|
| ABOi group | | | | |
| ABOc | Reference | | Reference | |
| ABOi/TPE ≤5 | 1.08 (0.63–1.85) | 0.777 | 0.97 (0.46–2.01) | 0.928 |
| ABOi/TPE ≥6 | 1.99 (1.02–3.86) | 0.042 | 2.42 (1.05–5.57) | 0.037 |
| Age, years | - | - | 0.96 (0.92–1.00) | 0.048 |
| BMI | 0.95 (0.89–1.01) | 0.102 | - | - |
| Pretransplant MELD | 1.06 (1.03–1.09) | <0.001 | - | - |
| Cold ischemic time, min | 1.00 (1.00–1.01) | 0.622 | - | - |
| Transfusion RBC, pack | 1.02 (1.01–1.04) | 0.002 | - | - |
| Log_AFP at LT | 1.11 (0.98–1.25) | 0.093 | 1.09 (0.94–1.25) | 0.260 |
| Log_PIVKA at LT | 1.03 (0.90–1.18) | 0.659 | 1.14 (0.98–1.34) | 0.091 |
| Pretransplant LRT, yes | 2.91 (1.45–5.84) | 0.003 | 7.00 (2.02–24.26) | 0.002 |
| Systemic treatment, yes | 2.20 (1.37–3.53) | 0.001 | 2.10 (1.16–3.82) | 0.015 |
| Viable tumor number | 1.02 (1.01–1.04) | 0.007 | 1.04 (1.01–1.07) | 0.004 |
| Maximum tumor size, cm | 0.90 (0.82–0.98) | 0.019 | 0.92 (0.82–1.03) | 0.150 |
| Microvascular invasion, yes | 1.77 (0.97–3.22) | 0.062 | 2.07 (1.01–4.24) | 0.046 |
| Poor differentiation, yes | 1.30 (0.82–2.05) | 0.268 | 1.69 (0.95–3.02) | 0.076 |
| Satellite nodule, yes | 1.67 (0.90–3.10) | 0.101 | 1.83 (0.92–3.63) | 0.085 |
| PVTT, yes | 2.83 (0.98–8.16) | 0.054 | 2.49 (0.57–10.86) | 0.226 |

*Variables which result p < 0.1 in univarible Cox analysis were included and represented at multivariable Cox analysis. Full univariate and multivariate results are represented at* ***Supplementary Tables S2, S3****.*

[a]*Multivariable analysis for HCC recurrence was performed treating non-HCC death as competing risk.*

*ABOc, ABO compatible; ABOi, ABO incompatible; AFP, alpha-feto protein; BMI, body mass index; CI, confidence interval; HCC, hepatocellular carcinoma; HR, hazard ratio; LRT, locoregional treatment; MELD, model for end-stage liver disease; PIVKA, protein induced by vitamin K antagonist-II; PVTT, portal vein tumor thrombosis; TPE, therapeutic plasma exchange.*

**TABLE 4 |** Subgroup analysis of 5-year hepatocellular carcinoma recurrence according to therapeutic plasma exchange numbers in ABO incompatible group.

| Subgroups | Patient number | | 5 years HCC recurrence | | *P* |
|---|---|---|---|---|---|
| | ABOi/TPE ≤5 (n = 75) | ABOi/TPE ≥6 (n = 30) | ABOi/TPE ≤5 (n = 75) | ABOi/TPE ≥6 (n = 30) | |
| Milan criteria | | | | | |
| Within | 44 | 15 | 9.9% | 35.2% | 0.025 |
| Above | 31 | 15 | 27.2% | 43.8% | 0.267 |
| Up-to-7 | | | | | |
| Within | 65 | 22 | 16.1% | 35.5% | 0.033 |
| Above | 10 | 8 | 27.1% | 47.5% | 0.569 |
| French risk score | | | | | |
| ≤2 | 56 | 20 | 14.8% | 31.8% | 0.082 |
| >2 | 19 | 10 | 21.3% | 55.0% | 0.117 |
| MoRAL score | | | | | |
| <100 | 49 | 16 | 17.6% | 20.5% | 0.750 |
| ≥100 | 26 | 14 | 16.1% | 56.7% | 0.017 |
| IA titer at initial | | | | | |
| ≤1:128 | 65 | 6 | 17.9% | 50.0% | 0.035 |
| ≥1:256 | 10 | 24 | 0.0% | 64.1% | 0.177 |
| IA titer at LT | | | | | |
| ≤1:16 | 61 | 11 | 19.4% | 31.8% | 0.287 |
| ≥1:32 | 14 | 19 | 0.0% | 43.5% | 0.026 |
| IA titer rebound | | | | | |
| No | 56 | 22 | 12.9% | 28.6% | 0.073 |
| Yes | 19 | 8 | 28.9% | 66.7% | 0.103 |
| Post LT TPE | | | | | |
| No | 61 | 19 | 12.1% | 24.0% | 0.203 |
| Yes | 14 | 11 | 37.3% | 59.1% | 0.246 |
| Splenectomy | | | | | |
| No | 71 | 23 | 17.9% | 36.9% | 0.075 |
| Yes | 4 | 7 | 0.0% | 46.4% | 0.137 |

*ABOi, ABO incompatible; HCC, hepatocellular carcinoma; IA, isoagglutinin; LT, liver transplantation; MoRAL, model of recurrence after liver transplant; TPE, therapeutic plasma exchange.*

Regarding the tumor burden criteria, the ABOi/TPE ≤5 group of patients within the Milan criteria exhibited a significantly lower 5-year HCC recurrence rate (9.9%) than the ABOi/TPE ≥6 group (35.2%, P = 0.025). Additionally, the 5-year HCC recurrence rate was significantly lower in the ABOi/TPE ≤5 group of patients within the Up-to-7 criteria (16.1%) than in the ABOi/TPE ≥6 group (35.5%, P = 0.033).

In the subgroup analysis based on immunological classification, patients with an initial IA titer ≤1: 128 demonstrated a significantly higher recurrence rate in the ABOi/TPE ≥6 group (50.0%) than in the ABOi/TPE ≤5 group (17.9%, P = 0.035). However, patients with an IA titer ≥1:32 at LT had a significantly higher recurrence rate in the ABOi/TPE ≥6 group (43.5%) than in the ABOi/TPE ≤5 group (0.0%, P = 0.026).

## DISCUSSION

The study evaluated the impact of pretransplant TPE sessions on HCC recurrence in patients undergoing ABOi LDLT and determined if limiting TPE sessions to fewer than six can enhance oncologic outcomes. We found that in the ABOi LDLT group, patients who underwent more than six pretransplant TPE sessions exhibited significantly worse HCC RFS and recurrence outcomes, with a similar trend observed in the subgroup analysis. Interestingly, the MoRAL score, which includes biomarkers, revealed that poorer oncologic outcomes were particularly pronounced in the high MoRAL score group. This suggests that in patients requiring a greater number of TPE sessions, biomarkers, in addition to tumor size, may play a crucial role in influencing HCC outcomes.

Moreover, the immunologic status at the time of transplantation is a critical factor influencing HCC recurrence [31, 32]. The need for multiple pretransplant TPE sessions may reflect an underlying immune dysregulation that could contribute to an increased risk of HCC recurrence. In particular, alterations in immune surveillance due to intensified desensitization protocols may affect the tumor microenvironment, potentially facilitating HCC recurrence [33, 34]. Similarly, ischemia-reperfusion injury (IRI) plays a crucial role in shaping the post-transplant microenvironment, influencing oncologic outcomes. Recent studies suggest that machine perfusion may help reduce HCC recurrence by mitigating IRI-induced inflammation and creating a more favorable post-transplant microenvironment [35–37]. Thus, assessing and managing the pretransplant immunologic status is essential for optimizing long-term oncologic outcomes in ABOi LDLT. A tailored approach that considers both desensitization requirements and immune profiling may help refine patient selection and improve posttransplant HCC prognosis. The strengths of our study include a well-organized dataset and a standardized desensitization protocol within the context of ABOi LDLT.

Globally, there has been a growing demand for LT as a definitive treatment for HCC, particularly for LDLT and ABOi LDLT due to organ shortages [15, 38]. In many countries outside East Asia, there is greater availability of deceased donors (DD), resulting in a predominant reliance on DDLT [38–40]. Consequently, these regions have limited cases and data regarding ABOi LDLT and the frequent use of TPE. In contrast, due to extreme shortages of deceased donors in Korea, LDLT is commonly performed for HCC [40, 41]. Paradoxically, this societal impact of deceased donor shortages has contributed to the accumulation of extensive data on ABOi LDLT, particularly in cases with high ABO antibody titers and a greater number of TPE sessions.

TPE is an intervention that involves the extracorporeal removal, return, or exchange of blood plasma or its components [42, 43]. The fundamental mechanism of this procedure is achieved through centrifugation or filtration using semipermeable membranes [44, 45]. In ABOi LDLT, the primary purpose of TPE is to remove IA. However, because this procedure is not selective, other immune-related factors in the blood are also removed, which presents a theoretical concern. Consequently, TPE is typically used as a primary or adjunctive treatment for conditions such as neurological diseases—including multiple sclerosis, amyotrophic lateral sclerosis, and myasthenia gravis—as well as autoimmune diseases like systemic lupus erythematosus and Kawasaki disease. Recent studies in this field have indicated that TPE promotes the differentiation and function of regulatory T cells [46–53].

Upon reviewing prior studies, it was noted that desensitization through pretransplant TPE or induction therapy in immunologically high-risk groups is associated with an increased cancer risk in certain malignancies (**Table 5**) [22, 23, 54, 55]. Although specific studies on ABOi LDLT are lacking, and the existing literature did not establish consistent protocols for TPE in kidney transplantation, direct comparisons with our study are challenging. Nevertheless, these findings underscore the relevance of desensitization and induction therapy concerning cancer risk, which was considered in our research.

Recent trends suggest that the outcomes of ABOi LDLT, including HCC outcomes and oncologic survival benefits, are comparable to those of ABOc LT [18, 19, 21]. However, these studies did not account for the cumulative and long-term effects of TPE, which prompted the initiation of our research.

In our study, the data indicated that ABOi patients requiring six or more pretransplant TPE sessions exhibited significantly poorer RFS and higher rates of HCC recurrence than ABOc patients. Additionally, our subgroup analysis shows that a higher number of pretransplant TPE sessions (≥6) was associated with a statistically significant increase in the 5-year HCC recurrence rate across several subgroups, including those within the Milan and Up-to-7 criteria, those with a high MoRAL score, and those with lower initial IA titers and higher IA titers at the time of LT.

Notably, within the size-based criteria, the ABOi/TPE ≥6 group exhibited a significantly higher recurrence rate. In contrast, regarding the tumor marker-based MoRAL score, a higher recurrence rate was observed in the ABOi/TPE ≥6 group only among patients with a score above 100. This suggests that among patients with a lower size-based tumor burden and a higher biologic-based tumor burden, those requiring more TPE sessions tended to experience poorer oncologic outcomes. Furthermore, this implies that the number of TPE sessions

**TABLE 5 |** Previous studies regarding pretransplant desensitization and cancer risk.

| Study | Yang, C.Y., et al.[a] | Motter, J.D., et al.[b] |
|---|---|---|
| Country | Taiwan | USA |
| Study period | 2007–2013 | 1997–2016 |
| Transplantation | Kidney | Kidney |
| Compared groups | DSA+ (n = 22) vs DSA – (n = 152) | ABOi LDKT (n = 858) vs ABOc LDKT (n = 12,239) |
| Plasmapheresis number | At least 4 cycles in DSA+ group | Not provided |
| Cancer type | Urothelial, endometrial, colon, and thyroid cancer | Colorectal cancer |
| *Cancer incidence* | DSA+ 19.6% vs DSA- 8.5% for 5 years (HR = 7.81, p = 0.028) | ABOi 0.6% vs ABOc 0.3% (HR = 3.27, p = 0.002) |
| *Hypothesis for higher cancer incidence* | Desensitization therapy for DSA+ including TPE might increase cancer | Desensitization therapy might increase cancer |

[a]*Yang, C.Y., et al.,* Renal transplantation across the donor-specific antibody barrier: Graft outcome and cancer risk after desensitization therapy. *J Formos Med Assoc, 2016. 115 (6): p. 426–33.*
[b]*Motter, J.D., et al.,* Cancer Risk Following HLA-Incompatible Living Donor Kidney Transplantation. *transplant direct, 2023. 9 (8): p. e1505.*
*ABOc, ABO compatible; ABOi, ABO incompatible; DSA, donor specific antibody; HR, hazard ratio; LDKT, living donor kidney transplantation; TPE, therapeutic plasma exchange.*

may be a more critical factor than the IA titer in influencing these outcomes.

Unlike previous studies, we focused on the immunomodulatory effects of T-regulatory (T-reg) cells induced by TPE and their association with HCC recurrence. As discussed earlier, while it is well established that T-reg cells are effective in treating autoimmune and neurological disorders, there are theoretical concerns that this process may reduce patient resistance to cancer [56, 57]. The literature indicates that the activation of T-reg cells can increase the risk of cancers such as HCC, with $CD4^+$CD25+FoxP3+ T cells playing a significant role in this risk [58–60]. Although the exact cytokines and mechanisms through which these cells interact with others remain unclear, their differentiation within the tumor microenvironment (TME) has been observed [33, 34, 61], suggesting a potential increase in poor long-term cancer outcomes in various malignancies, including HCC. This information is illustrated in **Supplementary Figure S6**.

In summary, our hypothesis suggests that plasmapheresis induces the activation of T-reg cells, particularly $CD4^+$ with CD25high, FoxP3+ effector T-reg cells, leading to an immunosuppressive effect within the tumor microenvironment that may facilitate tumor progression in various malignancies, including HCC. While some aspects of this pathway remain unexplained in the current foundational research, further studies are warranted to elucidate these mechanisms. Notably, the cumulative effect of TPE in the context of ABOi LT has not been extensively studied, underscoring the significance of our research.

This study has some limitations, including its retrospective and non-randomized design, the low number of ABOi/TPE ≥6 patients from a single center (n = 30), and the lack of fully established theoretical hypotheses or evidence to support our claims. Also, patients requiring more pretransplant TPE sessions may have additional unknown risk factors for HCC recurrence, highlighting the need for prospective studies to assess their impact on posttransplant outcomes [62]. However, despite these limitations, our study is significant, as it is the first to investigate the relationship between HCC outcomes and the number of preoperative TPE sessions and emphasizes the importance of comprehensive pretransplant evaluations in refining risk assessment for ABOi LDLT. In the future, we aim to address these limitations by increasing the sample size and establishing a more robust theoretical framework.

## CONCLUSION

This study demonstrated that the administration of more than six pretransplant TPE sessions in patients with HCC undergoing ABOi LDLT was associated with poorer oncologic outcomes. Based on our clinical findings and the theoretical association between TPE and HCC oncologic outcomes, we propose that limiting the number of TPE sessions to fewer than six may improve cancer outcomes in patients with HCC receiving ABOi LDLT. A strategy to reduce the number of TPE sessions to fewer than five should be implemented if possible when planning ABOi LDLT for HCC patients, ensuring adequate immunological stability through isoagglutinin titer control and maintaining comparable levels of immunological risk.

## 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 studies involving humans were approved by Institutional Review Board of Severance HospitalYonsei University Health System. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because This study

is a retrospective research model conducted using clinical data, posing minimal risk to the participants. The exemption itself does not negatively impact the welfare or rights of the participants, and the study cannot be practically conducted without the exemption. Additionally, participants will be provided with any relevant information after participation, if necessary.

## AUTHOR CONTRIBUTIONS

YY and DJ had full access to all aspects of the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. YY, D-GK, and DJ participated in the research design. YY, D-GK, E-KM, SY, MC, H-HK, MK, JL, MK, and DJ participated in the performance of the research. YY, D-GK, and DJ participated in the data acquisition. YY and D-GK participated in the statistical analysis. YY and D-GK participated in the writing of the paper. DJ supervised the study process. 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.

## 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.2025.14304/full#supplementary-material

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# Early Donor-Specific HLA Antibodies Detected by Screening in the First Month Posttransplant and Kidney Graft Outcomes

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Donor-specific HLA antibodies (DSA) are related to antibody-mediated rejection (ABMR) and graft failure. The rationale and frequency of screening for anti-HLA antibodies in stable patients are not established. The aim of our study is to evaluate the impact of early DSA appearance in the first month post-transplant on graft outcomes. All kidney transplant recipients between 1/1/2012–12/31/2022 with anti-HLA antibody screening by Luminex during the first month post-transplant were included. Patients with preformed or historical DSA and those with DSA detection after graft loss were excluded. The mean fluorescence intensity cut-off was 1,500. Three hundred fifty-three patients were included and the median time from transplant to first antibody sample was 30.0 days. During 3.8 years of follow-up, graft loss occurred in 9.1% and 19.5% had ABMR. A total of 8.5% developed early-DSA in the first month. Patients with early-DSA detection had more HLA sensitization at the time of transplant ($p < 0.001$). Multivariable analysis showed that the presence of early-DSA was an independent risk factor for ABMR. In conclusion, sensitized patients at the time of transplant have a higher risk of DSA formation in the first month, probably reflecting alloimmune memory, therefore early HLA antibody screening should be performed in this high-risk population.

**Keywords: kidney transplant, antibody-mediated rejection, donor-specific antibodies, graft outcomes, HLA screening**



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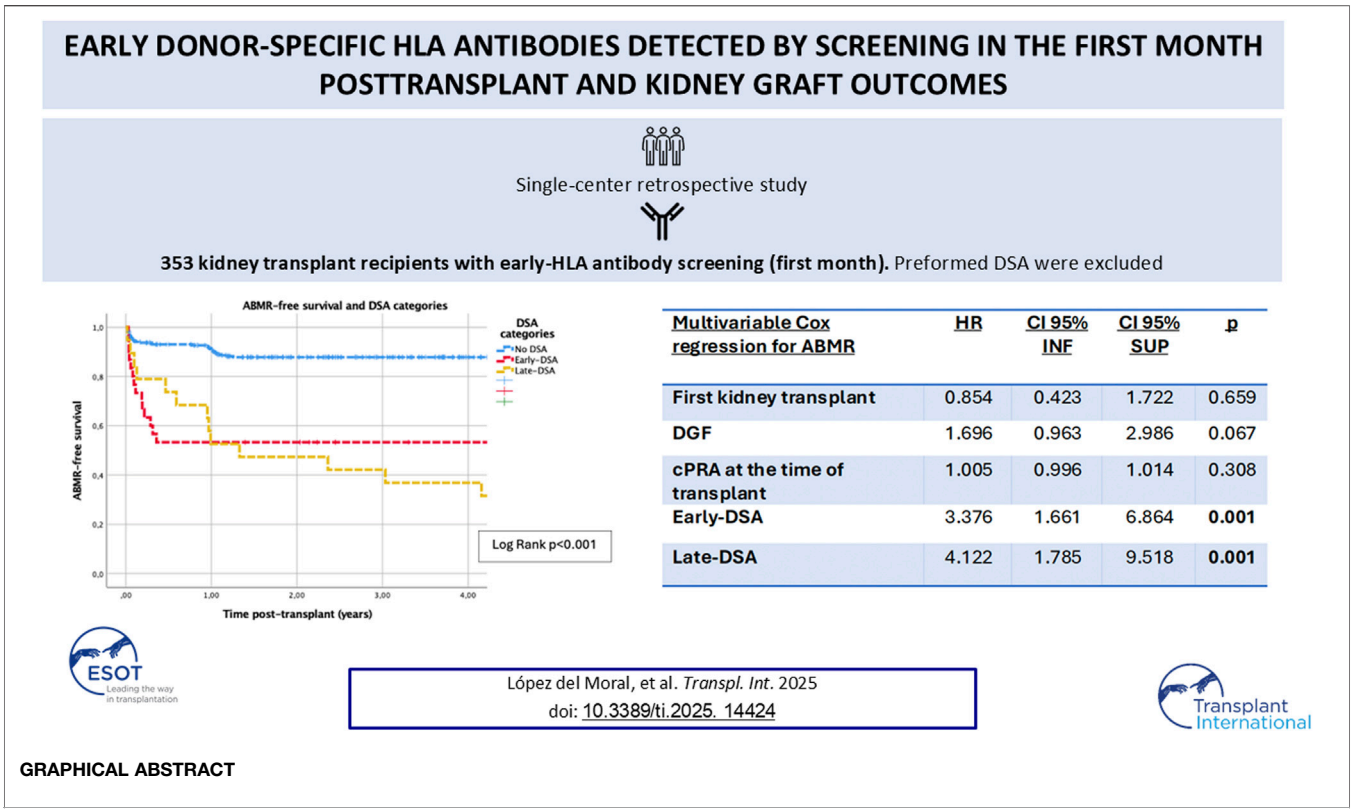
## INTRODUCTION

Donor-specific HLA antibodies (DSA) are a key factor for the diagnosis of antibody-mediated rejection (ABMR) and are associated with poor outcomes after kidney transplantation [1–6]. Immunological risk assessment before and after transplant has improved with solid-phase immunoassays in the Luminex system that provide sensitive and specific information on HLA antibodies with screening and single-antigen bead (SAB) assays, but their results must be interpreted appropriately [7–14]. In particular, the semiquantitative value of mean fluorescence intensity (MFI) and the problem of establishing a fixed and universal MFI positivity threshold hinder the correlation of DSA with clinical outcomes and the unification of results [15, 16].




**GRAPHICAL ABSTRACT**

Preformed and *de novo* DSA (dnDSA) are related to alloimmune injury [17–19]. The risk factors for dnDSA development are, among others, under-immunosuppression, graft inflammation and high HLA mismatch [17, 20–24]. Although dnDSA may appear at any time after transplantation, the STAR Working Group notes that the development of DSA between 2 weeks and 3 months post-transplant may represent a memory response [15, 25]. A history of HLA sensitizing events such as previous transplants, pregnancies or blood transfusions, and the presence of non-DSA HLA antibodies prior to transplant are risk factors for latent alloimmune memory [15].

Despite the impact of DSA on graft outcomes, there is still no consensus on the indication for DSA screening after transplantation in stable patients. Recently, the OuTSMART trial demonstrated that optimization of baseline immunosuppression after DSA detection had no impact on graft survival [26], showing that universal screening may be controversial, and the cost-effectiveness of this strategy is not determined [27, 28]. Furthermore, the frequency of routine surveillance for HLA antibodies is not established [15], and a recent ESOT Working Group proposed a monitoring scheme with screening in the first 3–6 months after transplant and annually thereafter (2C recommendation) [29]. Although an earlier assessment of HLA antibodies has been suggested in patients at potential risk of latent memory [15, 30], the exact timing of the first post-transplant HLA determination is not currently settled. Moreover, most series have described early DSA as those detected in the first year posttransplant [31–35], and there are few data on the specific evaluation of HLA monitoring in the first month.

The aim of our study is to evaluate the impact of early HLA antibody screening in the first month posttransplant on kidney graft outcomes and identify patients at risk of early DSA formation.

## MATERIALS AND METHODS

### Study Population

For this retrospective analysis we included all kidney transplant recipients from 1/1/2012–12/31/2022 at Marqués de Valdecilla University Hospital with HLA antibody screening during the first month post-transplant (range 10–60 days). Those patients with preformed or historical DSA described in pre-transplant sera and with positive flow cytometry crossmatch (FCXM) were excluded. Patients with graft failure in the first 60 days and those who developed DSA after graft loss were also excluded. The primary outcome variable was time to antibody-mediated rejection. The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the regional Ethics Committee of our institution (2024.196).

Demographic and clinical data, including recipient and donor data (type, age), induction immunosuppression, cold ischemia time (CIT), delayed graft function (DGF), HLA mismatch, early blood transfusions - within the first 30 days after transplant, and

biopsy data were collected from the prospectively maintained database of renal transplant patients at our center. All rejections were categorized according to Banff 2019 classification [36]. Allograft biopsies were performed by clinical indication and by protocol 1-year after transplantation.

Our induction protocol consisted of anti-thymocyte globulin (ATG) in highly sensitized patients and in some patients with a previous transplant lost due to rejection. Anti-IL2R was administered in patients at high risk of post-transplant acute tubular necrosis, primarily due to advanced donor age, prolonged expected CIT, or donation after circulatory death. The corticosteroid treatment protocol at our center was an intravenous pulse of 500 milligrams (mg) of methylprednisolone for induction. Oral prednisone was continued at 20 mg for the first 2 weeks after transplant, 15 mg of prednisone 2 weeks later until the first month and 10 mg at 1 month after transplant, with a subsequent reduction to 7.5 mg after 2 months and 5 mg after 3 months. Discontinuation or maintenance of baseline prednisone after 3 months was performed individually and according to clinical indication.

## HLA Antibodies

Regular monitoring of HLA antibodies was performed in the first- and sixth-month post-transplant and annually thereafter as routine clinical practice in our center, and in case of signs of impaired allograft function or clinical request. Patients had pre- and post-transplant sera screened using a mixed panel beads (LABScreen Mixed Class I and II, One Lambda, Canoga Park, CA) and if a positive result was detected, further LABScreen® SAB assay class-I and class-II (One Lambda, Canoga Park, CA) was performed by Luminex® technology. Pre-transplant sera before 2012 were assessed by enzyme-linked immunosorbent assay (ELISA) as this was the available technique, but all patients had at least one pre-transplant serum screened by Luminex®. According to the policy of our center, anti-HLA antibody testing was performed every 3 months in patients on the transplant waiting list. An additional anti-HLA antibody sample was collected on day 0 if sensitizing events, such as blood transfusions, occurred between the day of transplant and the last serum sample. The last pretransplant anti-HLA antibody sample was used to calculate pretransplant cPRA, but all pretransplant sera were reviewed to exclude patients with preformed or historical DSA.

The general positivity threshold in our laboratory was set at 1,500 MFI, and the presence of DSA was defined by the Histocompatibility laboratory considering the MFI positivity cut-off and other factors such as the evolution of HLA antibodies posttransplant or epitope sharing phenomena [15, 16]. In laboratory routine, we included dilution sera in highly sensitized patients and in those with suspected prozone, as described [37].The most probable 2-field HLA typing of the donor [38] and haplotype frequencies [39, 40] for missing information on specific HLA loci were considered to assign DSA. Calculated panel-reactive antibody (cPRA) was obtained through the Virtual PRA Calculator of the Eurotransplant Reference Laboratory [41], and delta cPRA >0% was recorded (difference between cPRA in the first serum at 1-month post-transplant and cPRA in the last pre-transplant serum).

## Patient Groups

Patients with early DSA detection in the first month (10–60 days) posttransplant were categorized as "early-DSA," and those patients with first DSA detection >60 days - and without DSA in the first month - were categorized as "late-DSA." Patients without DSA detection during the follow-up period were classified as "no-DSA." "Transient" DSA was defined as disappearance of DSA at 3 and/or 6 months after first detection (if >1 DSA per patient, disappearance of at least one DSA).

## Statistical Analysis

Continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR) according to their distribution. Categorical variables were described as relative frequencies. Continuous variables with non-normal distribution were compared using non-parametric tests (Mann-Whitney U test to compare 2 groups and Kruskal-Wallis test to compare 3 groups). A chi-square test was used to compare the average values of categorical variables. Univariable and multivariable Cox regression were performed to determine which variables were associated with ABMR, and hazard ratios (HR) were reported with 95% confidence intervals. To evaluate the predictive capacity of DSA by Cox regression, patients with ABMR before DSA appearance were eliminated from this analysis. Time-to-event outcome data were assessed by Kaplan–Meier plots and log-rank tests. A p-value <0.05 was defined as statistical significance. Statistical analysis was conducted using the SPSS statistical software package (Version 25.0. Armonk, NY: IBM Corp.).

# RESULTS

## Baseline Characteristics

In total, we included 353 patients with early HLA antibody screening in the first month post-transplant (**Figure 1**). The time between transplant and last pre-transplant antibody sample was 42.0 days (IQR 16.0–73.0), and the median time from transplant to first anti-HLA antibody test was 30.0 days (IQR 26.0–37.0). Most patients (297, 84.1%) had systematic early HLA screening by protocol, and 56/353 (15.9%) underwent early HLA screening in the first month also for clinical indication (rise in creatinine and/or proteinuria). No significant differences were observed between DSA groups regarding early HLA antibody testing solely by protocol or by clinical indication (p = 0.178). At the time of early HLA antibody screening, the median creatinine (Cr) value was 1.49 mg/dL (IQR 1.13–1.97), the estimated glomerular filtration rate (eGFR) by CKD-EPI was 49.3 mL/min/1.73 $m^2$ (IQR 34.4–67.1), and the median urine albumin-to-creatinine ratio was 69.6 mg/g (IQR 27.5–176.9). The time of initial hospital admission for transplant was 17.0 days (IQR 11.0–25.0), and most early HLA determinations in the first

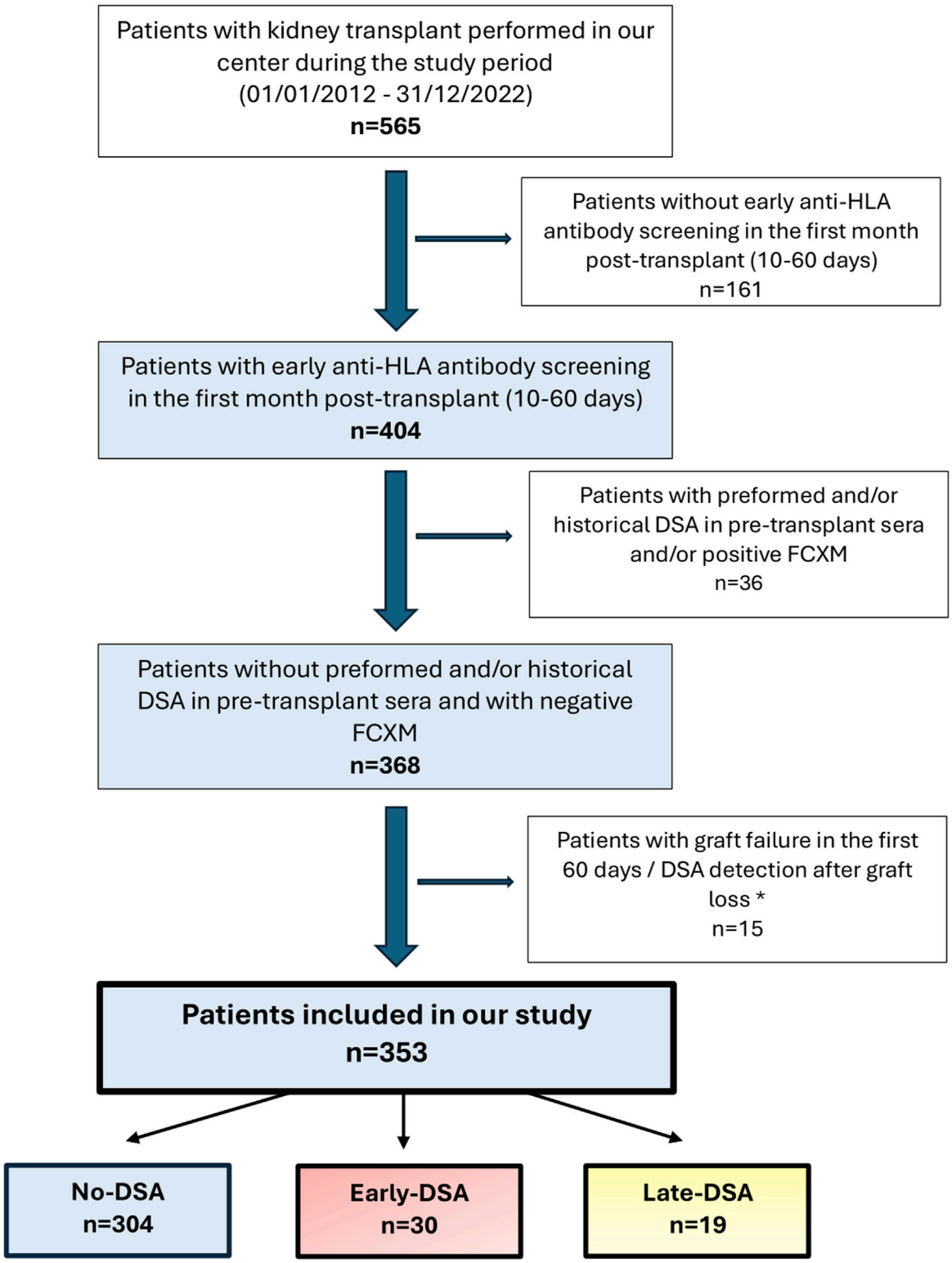



**FIGURE 1 |** Flowchart of the patients included in our study. DSA: donor-specific antibodies. FCXM: flow cytometry crossmatch. *Causes of graft loss: graft bleeding requiring nephrectomy (6.7%, 1/15), death with functioning graft (13.3%, 2/15), hyperacute/acute rejection (20.0%, 3/15), arterial thrombosis (33.3%, 5/15) and venous thrombosis (26.7%, 4/15).

**TABLE 1 |** Baseline characteristics of patients included in our study.

| Baseline characteristics | All patients (n = 353) | No-DSA (n = 304) | Early-DSA (n = 30) | Late-DSA (n = 19) | p |
|---|---|---|---|---|---|
| Recipient age (years)[a] | 57.0 (45.0–65.0) | 58.0 (46.0–65.0) | 49.5 (41.0–64.2) | 45.0 (32.0–63.0) | 0.235 |
| Recipient sex (male) (n, %)[a] | 245 (69.4%) | 217 (71.4%) | 17 (56.7%) | 11 (57.9%) | 0.133 |
| Recipient sex (female) (n, %) | 108 (30.6%) | 87 (28.6%) | 13 (43.3%) | 8 (42.1%) | 0.133 |
| • Pregnancy before transplant | 65 (60.2%) | 53 (60.9%) | 8 (61.5%) | 4 (50.0%) | 0.829 |
| Death-censored graft failure (n, %) | 32 (9.1%) | 19 (6.3%) | 6 (20.0%) | 7 (36.8%) | **<0.001** |
| Death (n, %) | 37 (10.5%) | 31 (10.2%) | 5 (16.7%) | 1 (5.3%) | 0.406 |
| Donor age (years)[a] | 55.0 (45.0–63.0) | 55.0 (45.0–63.0) | 55.0 (44.7–63.2) | 52.0 (41.0–69.0) | 0.982 |
| Donor sex (male) (n, %) | 210 (59.5%) | 180 (59.2%) | 18 (60.0%) | 12 (63.2%) | 0.942 |
| Donor type (deceased) (n, %) | 337 (95.5%) | 293 (96.4%) | 27 (90.0%) | 17 (89.5%) | 0.120 |
| • DBD (n, %) | 213 (60.3%) | 183 (60.2%) | 18 (60.0%) | 12 (63.2%) | 0.542 |
| • DCD (n, %) | 124 (35.1%) | 110 (36.2%) | 9 (30.0%) | 5 (26.4%) | |
| Induction immunosuppression (n, %) | 253 (71.7%) | 216 (71.1%) | 23 (76.7%) | 14 (73.7%) | 0.793 |
| • ATG (n, %) | 120 (47.4%) | 92 (42.6%) | 18 (78.3%) | 10 (71.4%) | **0.001** |
| • Anti-IL2R (n, %) | 133 (52.6%) | 124 (57.4%) | 5 (21.7%) | 4 (28.6%) | |
| First kidney transplant (n, %) | 257 (72.8%) | 228 (75.0%) | 18 (60.0%) | 11 (57.9%) | 0.069 |
| Retransplant (n, %) | 96 (27.2%) | 76 (25.0%) | 12 (40.0%) | 8 (42.1%) | 0.069 |
| • Repeated HLA mismatch with previous donors (n, %) | 30 (31.3%) | 23 (30.3%) | 5 (41.7%) | 2 (25.0%) | 0.675 |
| Combined transplant (n, %) | 23 (6.5%) | 20 (6.6%) | 2 (6.7%) | 1 (5.3%) | 0.974 |
| • Pancreas-kidney (n, %) | 20 (87.0%) | 17 (85.0%) | 2 (100.0%) | 1 (100.0%) | 0.772 |
| • Liver-kidney (n, %) | 3 (13.0%) | 3 (15.0%) | 0 (0.0%) | 0 (0.0%) | |
| CIT (hours)[a] | 19.0 (10.0–23.0) | 19.0 (10.0–23.0) | 20.0 (11.2–22.2) | 12.0 (5.0–22.0) | 0.506 |
| DGF (n, %) | 91 (25.8%) | 82 (27.0%) | 6 (20.0%) | 3 (15.8%) | 0.746 |
| Early blood transfusion (n, %)[b] | 199 (56.4%) | 169 (55.6%) | 19 (63.3%) | 11 (57.9%) | 0.710 |
| cPRA at the time of transplant (%)[a] | 0.0 (0.0–0.0) | 0.0 (0.0–0.0) | 40.4 (0.0–87.8) | 0.0 (0.0–36.3) | **<0.001** |
| cPRA ≥5% at the time of transplant (n, %) | 79 (22.4%) | 56 (18.4%) | 17 (56.7%) | 6 (31.6%) | **<0.001** |
| cPRA ≥85% at the time of transplant (n, %) | 33 (9.3%) | 24 (7.9%) | 8 (26.7%) | 1 (5.3%) | **0.003** |
| HLA-A mismatch >0 (n, %) | 312 (88.4%) | 266 (87.5%) | 29 (96.7%) | 17 (89.5%) | 0.323 |
| HLA-B mismatch >0 (n, %) | 322 (91.2%) | 277 (91.1%) | 27 (90.0%) | 18 (94.7%) | 0.838 |
| HLA-DRB1 mismatch >0 (n, %) | 297 (84.1%) | 257 (84.5%) | 24 (80.0%) | 16 (84.2%) | 0.810 |
| IS regimen at the time of early HLA screening (n, %) | 344 (97.5%) | 296 (97.4%) | 29 (96.7%) | 19 (100.0%) | 0.748 |
| • Tacrolimus, MMF/MPA, prednisone | 7 (2.0%) | 7 (2.3%) | 0 (0.0%) | 0 (0.0%) | 0.562 |
| • Tacrolimus, mTORi, prednisone | 2 (0.6%) | 1 (0.3%) | 1 (3.3%) | 0 (0.0%) | 0.106 |
| • Others | | | | | |
| Early HLA screening only by protocol (n, %) | 297 (84.1%) | 260 (85.5%) | 22 (73.3%) | 15 (78.9%) | 0.178 |
| Plasmapheresis before early HLA screening (n, %)[c] | 35 (9.9%) | 26 (8.6%) | 7 (23.3%) | 2 (10.5%) | **0.035** |
| IVIG before early HLA screening (n, %)[c] | 30 (8.5%) | 21 (6.9%) | 7 (23.3%) | 2 (10.5%) | **0.008** |
| ABMR (Banff 2019 Classification) (n, %)[d] | 69 (19.5%) | 38 (12.5%) | 16 (53.3%) | 15 (78.9%) | **<0.001** |
| • Time from transplant to ABMR (months)[a] | 3.3 (0.6–12.1) | 2.7 (0.5–12.0) | 1.7 (0.5–3.6) | 11.4 (1.5–35.7) | **0.027** |
| Biopsy with ABMR diagnosis by clincal indication or 1-year protocol | 54 (78.3%) | 26 (68.4%) | 16 (100.0%) | 12 (80.0%) | **0.036** |
| • Clinical indication (graft dysfunction and/or DSA appearance) (n, %) | 15 (21.7%) | 12 (31.6%) | 5 (31.3%) | 2 (16.7%) | |
| - Only for DSA (n, %) | | | 11 (68.8%) | 10 (83.3%) | |
| - Graft dysfunction (n, %) | | | 0 (0.0%) | 3 (20.0%) | |
| • 1-year-Protocol (n, %) | | | | | |
| TCMR (Banff 2019 Classification) (n, %)[d] | 96 (27.2%) | 75 (24.7%) | 11 (36.7%) | 10 (52.6%) | **0.014** |

[a]*Median and interquartile range. For continuous variables with non-normal distribution, the Kruskal-Wallis test was used to compare the 3 groups.*
[b]*Early blood transfusion: at least one blood transfusion within the first 30 days after transplant.*
[c]*Treatment with plasmapheresis and/or IVIG, before the first early HLA, determination was performed as rejection prophylaxis in high-risk patients, suspected rejection and inability to perform a biopsy, or biopsy-proven ABMR.*
[d]*Rejection episodes were categorized according to Banff 2019 Classification. Borderline rejection was included in the category of T-cell mediated rejection (TCMR).*
*DBD: donor brain death. DCD: donor circulatory death. ATG, antithymocyte globulin; Anti-IL2R, anti-interleukin-2, receptor. CIT: cold ischemia time. DGF: delayed graft-function. cPRA: calculated panel-reactive antibody (Eurotransplant). IS: immunosuppression. MMF/MPA: Mycophenolate mofetil/Mycophenolic acid. mTORi: mTOR, inhibitor. IVIG: Intravenous immunoglobulin. ABMR: antibody-mediated rejection. TCMR: T-cell mediated rejection.*

month (279/353, 79.0%) were performed on an outpatient basis, after first hospital admission.

The study cohort comprised mainly patients with a first single-kidney transplant from a deceased donor (**Table 1**) with a median follow-up of 3.8 years (IQR 2.1–6.4). By design of the study, none of the patients had pretransplant DSA and most patients were not sensitized (median cPRA 0.0%). Specifically analyzing patients with a first kidney transplant (n = 257, 72.8%), 20.6% had a pregnancy before transplantation. In this group of first transplants, pregnancy before transplant was significantly higher in sensitized patients with cPRA ≥5% (32.1% vs. 4.9%, $p < 0.001$) and cPRA ≥85% (7.5% vs. 0.5%, $p = 0.001$).

Graft failure occurred in 9.1% of patients and 19.5% had ABMR. Delta cPRA >0% developed in 54 patients (15.3%) whereas 30 out of 353 (8.5%) had early-DSA detection in the first month, and the total number of patients with DSA

appearance during the complete follow-up period was 49/353 (13.9%). In our cohort, the median time from transplant to first DSA detection was 1.5 months (IQR 1.0–11.4) (**Supplementary Figure S1**).

When evaluating the characteristics of patient groups (**Table 1**), they were comparable in terms of recipient age, recipient sex, donor age and donor type. 71.7% of patients received induction immunosuppression, with more induction with anti-thymocyte globulin (ATG) in the early-DSA group ($p = 0.001$). Patients in the early-DSA group had more HLA sensitization at the time of transplant compared to patients with late-DSA and no-DSA (cPRA 40.4% vs. 0.0% vs. 0.0%, $p < 0.001$). There was a higher proportion of patients receiving plasmapheresis and intravenous immunoglobulin (IVIG) treatment before early screening in the early-DSA group ($p = 0.035$ and $p = 0.008$, respectively). There were differences between groups in T-cell mediated rejection (TCMR) episodes, with a higher incidence in patients with late-DSA ($p = 0.014$). Patients with early-DSA and late-DSA had more proportion of ABMR compared to patients without DSA (53.3% vs. 78.9% vs. 12.5%, $p < 0.001$), and the median time from transplant to first ABMR episode was shorter in the early-DSA group ($p = 0.027$). A higher proportion of biopsies with ABMR diagnosis were performed for clinical indication in the early and late-DSA groups ($p = 0.036$). Histological data are shown in **Supplementary Tables S1–S3**.

## Patients With Early-DSA

Most patients with early-DSA had class-I (56.7%), 33.3% had class-II and 10.0% had class-I and class-II. Regardless of the antibody class, 33.3% had >1 DSA in the same first serum after transplant. The median MFI level at first early-DSA detection was 4,912.0 (IQR 2,505.7–7,235.2). "Transient" early-DSA were detected in 20/30 patients (66.7%), and 60.0% (12/20) had early-DSA negativity after rejection and specific active treatment whereas 40.0% (8/20) had "spontaneous" disappearance of early-DSA without treatment for rejection.

The median values of estimated glomerular filtration rate and urine albumin-creatinine ratio in the early-DSA group at the time of early-DSA appearance were 37.5 mL/min/1.73 $m^2$ (IQR 28.7–60.0) and 91.0 mg/g (IQR 43.8–332.7), respectively. At least one allograft biopsy was performed in 83.3% of patients with early-DSA. Technical difficulty, high-risk due to anticoagulation, patient refusal or "transient" early-DSA without evidence of graft dysfunction were the reasons for not performing a biopsy in 5/30 patients. ABMR was present in 53.3% of patients with early-DSA, and 46.7% had "subclinical" early-DSA without evidence of ABMR. In patients with ABMR, 31.3% (5/16) had rejection before early-DSA appearance in the first month and 68.8% (11/16) presented ABMR at the time or after early-DSA detection. Patients with early-DSA and ABMR were associated with lower allograft survival compared to those patients with "subclinical" early-DSA (log rank $p = 0.012$) (**Supplementary Figure S2**). 36.7% of patients with early-DSA (11/30) presented TCMR, and 36.4% of these patients (4/11) had TCMR before first early-DSA appearance. The characteristics of DSA in patients with early-DSA and late-DSA are illustrated in **Table 2**. The evolution of eGFR in patients with DSA is shown in **Supplementary Table S4** and **Supplementary Figures S3, S4**.

## DSA Status, Rejection and Graft Survival

The presence of early-DSA and late-DSA was associated with lower death-censored allograft survival (log rank $p = 0.001$), as shown in **Figure 2**. Independently of DSA status, patients with ABMR were associated with lower allograft survival compared to patients without ABMR (log rank $p = 0.001$). Similarly, the presence of TCMR was associated with lower graft survival (log rank $p = 0.006$). These results are shown in **Supplementary Figures S5, S6**.

Analyzing the relationship between DSA and ABMR, the presence of DSA was associated with lower ABMR-free survival (log rank $p < 0.001$) (**Figure 3**). Different patient characteristics were associated with ABMR in univariable Cox regression analyses (**Table 3**). Specifically analyzing factors that were associated with ABMR posttransplant by multivariable Cox regression (**Table 4**), early-DSA and late-DSA were independent risk factors for ABMR (HR 3.3, CI 95% 1.6–6.8, $p = 0.001$ and HR 4.1, CI 95% 1.7–9.5, $p = 0.001$, respectively). Conversely, first kidney transplant, DGF or HLA sensitization at the time of transplant were not contributors in the multivariable model.

# DISCUSSION

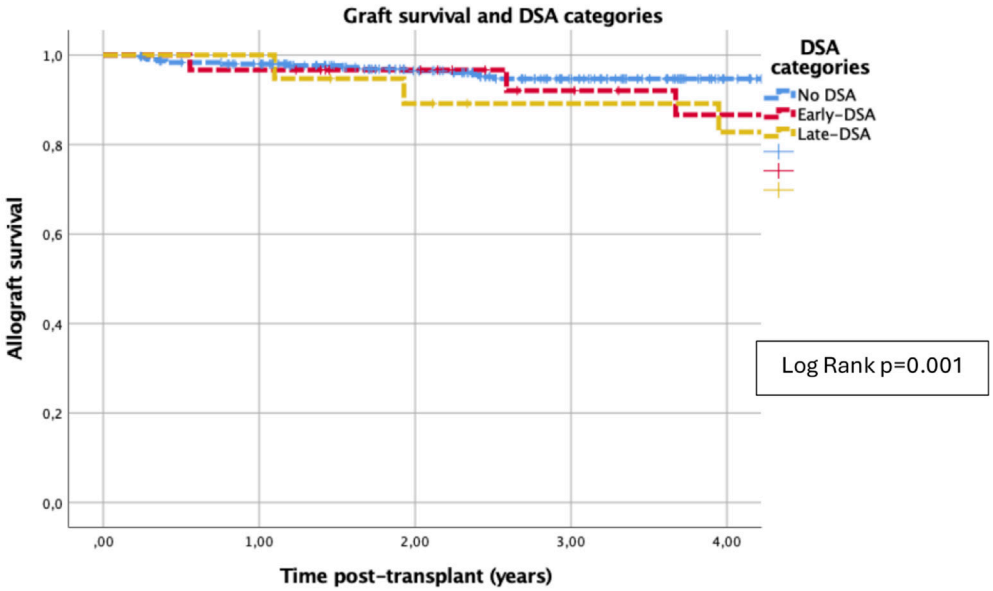
Despite the widely described impact of DSA on graft outcomes in kidney transplantation [1–6], the indication of universal post-transplant HLA antibody screening remains unclear [26, 29]. Data on the cost-effectiveness of DSA monitoring are scarce, and different strategies have been proposed to select high-risk patients [27, 28]. The frequency of HLA screening is also not determined and current recommendations have a low level of evidence [15, 29, 30]. In our center we established regular DSA screening in the first month posttransplant more than 10 years ago and in this study, we describe a large cohort of mostly non-sensitized patients without preformed or historical DSA and with early HLA antibody screening by Luminex in the first month. After 3.8 years of follow-up, DSA develop in 13.9% of patients with a median time to first DSA appearance of 1.5 months post-transplantation. Early-DSA are predominant (8.5%) and are an independent risk factor for ABMR. Patients with early-DSA had more HLA sensitization at the time of transplant, presumably reflecting alloimmune memory even in the absence of preformed DSA, therefore we suggest that early-DSA screening should be performed in this high-risk population. Consequently, with our data we highlight that assessing the HLA sensitization status and immunological risk of patients may be the best tool to generate a post-transplant DSA monitoring scheme.

The timing of DSA appearance after transplantation is variable and most series have described a higher incidence in the first year, ranging from 3% to 20%, with a lower annual rate thereafter [17, 29, 33]. A recent study showed that 77% of DSA detected by screening appeared in the first 100 days post-transplant [32]. However, some series have shown that dnDSA can appear up to 10 years after transplant [16, 29], demonstrating that the risk of

**TABLE 2 |** DSA characteristics in patients with early-DSA and late-DSA.

| DSA characteristics | Early-DSA (n = 30) | Late-DSA (n = 19) | p |
|---|---|---|---|
| Class of DSA (n, %) | | | 0.230 |
| • Class-I | 17 (56.7%) | 6 (31.6%) | |
| • Class-II[a] | 10 (33.3%) | 10 (52.6%) | |
| • Both class-I and class-II[a] | 3 (10.0%) | 3 (15.8%) | |
| Number of DSA per patient: >1 DSA in the first sample (n, %) | 10 (33.3%) | 7 (36.8%) | 0.801 |
| "Transient" DSA (n, %) | 20 (66.7%) | 7 (36.8%) | **0.041** |
| MFI at DSA first occurrence[b] | 4912.0 (2505.7–7235.2) | 2428.5 (1432.5–11,109.0) | 0.365 |
| IS at the time of first DSA detection | | | 0.739 |
| • Tacrolimus (n, %) | 29 (96.7%) | 18 (94.7%) | 0.739 |
| • MMF/MPA (n, %) | 29 (96.7%) | 18 (94.7%) | 0.306 |
| • Prednisone (n, %) | 29 (96.7%) | 17 (89.5%) | 0.070 |
| • mTORi (n, %) | 0 (0.0%) | 2 (10.5%) | |
| ABMR (Banff 2019 Classification) (n, %) | 16 (53.3%) | 15 (78.9%) | 0.070 |
| • ABMR before DSA detection | 5 (31.3%) | 7 (46.7%) | 0.370 |
| • ABMR at the time/after DSA detection | 11 (68.8%) | 8 (53.3%) | |
| Time from DSA to ABMR diagnosis (months)[b] | 1.4 (0.3–3.1) | 3.2 (2.0–5.2) | |

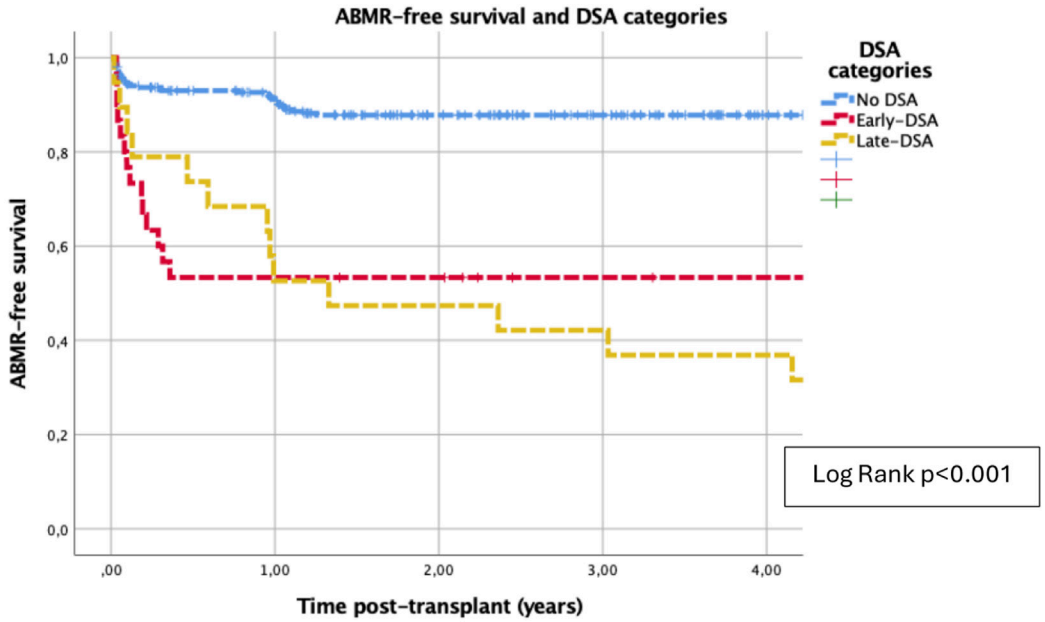
*[a]Of patients with class-II DSA, in both groups (alone or together with class-I DSA), 18/26 (69.2%) had anti-DQB, 5/26 (19.2%) had anti-DQA, and 1/16 (3.8%) had anti-DP DSA.*
*[b]Median and interquartile range. For continuous variables with non-normal distribution, the Mann-Whitney U test was used to compare the 2 groups.*
*DSA: donor-specific HLA, antibody. MFI: mean fluorescence intensity. IS: immunosuppression. MMF/MPA: Mycophenolate mofetil/Mycophenolic acid. mTORi: mTOR, inhibitor.*




| N at risk | 0 years | 1 years | 2 years | 3 years | 4 years |
|---|---|---|---|---|---|
| **No DSA** | 304 | 287 | 232 | 188 | 139 |
| **Early-DSA** | 30 | 29 | 25 | 19 | 16 |
| **Late-DSA** | 19 | 19 | 16 | 14 | 13 |

**FIGURE 2 |** Kaplan-Meier survival analysis of death-censored graft failure for DSA categories. Patients with early-DSA and late-DSA were associated with lower allograft survival compared to patients without DSA (log rank p = 0.001). Graft survival at 3 years posttransplant was 94.7% (± 1.4%) in no-DSA patients, 92.1% (5.5%) in patients with early-DSA in the first month and 89.2% (± 7.2%) in patients with late-DSA.

developing DSA should be considered at any time during functional graft life. In our cohort, the incidence of DSA is almost 14% (49/353) over about 4 years of follow-up, and although the number of patients with DSA is not very high, our incidence is in line with previous observations [5, 34, 42]. Surprisingly, most of them (30/49) are detected early in the first




| N at risk | 0 years | 1 years | 2 years | 3 years | 4 years |
|---|---|---|---|---|---|
| **No DSA** | 304 | 250 | 189 | 154 | 115 |
| **Early-DSA** | 30 | 16 | 15 | 11 | 10 |
| **Late-DSA** | 19 | 10 | 9 | 8 | 7 |

**FIGURE 3 |** Kaplan-Meier survival analysis of antibody-mediated rejection (ABMR) for DSA categories. Patients with early-DSA and late-DSA were associated with lower ABMR-free survival compared to patients without DSA (log rank p < 0.001). At 3 years posttransplant, ABMR-free survival was 87.8% (± 1.9%) in no-DSA patients, 53.3% (9.1%) in patients with early-DSA, and 42.1% (11.3%) in patients with late-DSA.

**TABLE 3 |** Univariable Cox regression analysis for antibody-mediated rejection (ABMR).

| ***Univariable cox regression for ABMR***[a] | **HR** | **CI 95% INF** | **CI 95% SUP** | **p** |
|---|---|---|---|---|
| Recipient age | 0.985 | 0.966 | 1.006 | 0.158 |
| Donor age | 0.982 | 0.964 | 1.001 | 0.061 |
| Deceased donor | 2.000 | 0.798 | 5.015 | 0.139 |
| DCD | 0.910 | 0.519 | 1.519 | 0.743 |
| Induction immunosuppression | 1.478 | 0.795 | 2.747 | 0.216 |
| First kidney transplant | 0.523 | 0.308 | 0.888 | **0.016** |
| Combined transplant | 0.645 | 0.201 | 2.068 | 0.461 |
| CIT | 1.022 | 0.986 | 1.059 | 0.235 |
| DGF | 1.779 | 1.026 | 3.083 | **0.040** |
| cPRA at the time of transplant | 1.009 | 1.003 | 1.016 | **0.007** |
| HLA-A mismatch >0 | 1.506 | 0.601 | 3.773 | 0.382 |
| HLA-B mismatch >0 | 1.237 | 0.448 | 3.418 | 0.682 |
| HLA-DRB1 mismatch >0 | 2.059 | 0.822 | 5.157 | 0.123 |
| HLA mismatch – sum of A, B and DRB1 mismatch | 1.069 | 0.884 | 1.293 | 0.490 |
| Early-DSA | 3.279 | 1.696 | 6.339 | **<0.001** |
| Late-DSA | 3.981 | 1.882 | 8.421 | **<0.001** |

*ABMR: antibody-mediated rejection. DCD: donor circulatory death. CIT: cold ischemia time. DGF: delayed graft-function. cPRA: calculated panel-reactive antibody (Eurotransplant). DSA: donor-specific HLA, antibody.*

[a]*To evaluate the predictive capacity of DSA, by Cox regression, patients with ABMR before DSA appearance were eliminated from this analysis.*

month. These results are probably explained by the more intensive monitoring in our center with a first HLA determination in the first month, allowing rapid detection of DSA. A relevant proportion of patients with early-DSA (8/30) present "spontaneous" negativity at 3 or 6 months after first appearance, and these "transient" early-DSA cannot be detected if first early HLA determination is omitted, which could explain our high incidence. Also, our study describes two exclusive categories

**TABLE 4 |** Multivariable Cox regression analysis for antibody-mediated rejection (ABMR).

| *Multivariable cox regression for ABMR*[a] | HR | CI 95% INF | CI 95% SUP | p |
|---|---|---|---|---|
| First kidney transplant | 0.854 | 0.423 | 1.722 | 0.659 |
| DGF | 1.696 | 0.963 | 2.986 | 0.067 |
| cPRA at the time of transplant | 1.005 | 0.996 | 1.014 | 0.308 |
| Early-DSA | 3.376 | 1.661 | 6.864 | **0.001** |
| Late-DSA | 4.122 | 1.785 | 9.518 | **0.001** |

*ABMR: antibody-mediated rejection. DGF: delayed graft-function. cPRA: calculated panel-reactive antibody (Eurotransplant). DSA: donor-specific HLA, antibody.*
[a]*To evaluate the predictive capacity of DSA, by Cox regression, patients with ABMR before DSA appearance were eliminated from this analysis.*

of patients based on first detection of DSA, and patients of the early-DSA group may develop new DSA later after transplantation. Despite these considerations, our study shows a relevant proportion of patients with DSA appearance in the first month, emphasizing that early HLA screening allows prompt detection of patients at potential risk of poor outcomes.

The STAR Working Group noted that DSA up to the third month after transplant are likely preformed and reflect alloimmune memory [15, 25]. Previous transplants are a risk factor for memory responses [15] and in patients undergoing retransplantation it has been described that re-exposure to mismatched HLA antigens may be associated with increased immunological risk [43]. In our cohort there are no differences between groups in the proportion of first transplants. 31.3% of our retransplanted patients present a repeated HLA mismatch with previous donors, without differences between patient groups, being in line with previous observations showing that a repeated HLA mismatch within negative FCXM and without described preformed DSA is not associated with DSA detection after transplant [44]. The presence of non-DSA HLA antibodies at the time or before transplantation may also be a risk factor for latent alloimmune memory responses [15]. In our study, patients with HLA sensitization present a higher risk of early-DSA detection in the first month, probably reflecting a memory response even with a negative FCXM and in the absence of preformed DSA. ATG induction is higher in the early-DSA group, possibly explained by the greater number of highly sensitized patients. Because of this high risk, patients with early-DSA also more frequently received plasmapheresis and/or IVIG prior to early HLA screening in the first month and early-DSA detection, showing that current treatments are not fully effective in suppressing DSA. Despite this, there is an important proportion of non-sensitized patients (cPRA = 0% in 12/30) who develop DSA rapidly in the first month. These data likely show that the percentage of cPRA in an isolated serum is not completely informative about the HLA sensitization status or the risk of memory responses, and a cPRA of 0% does not necessarily represent an immunologically naïve patient [15]. Patients with prior exposure to alloantigens through previous transplants, pregnancies or transfusions may not have detectable anti-HLA antibodies, and preexisting DSA below the positivity threshold could be present but not detected by initial screening [13] and triggered by transplantation. In fact, to study alloimmune memory beyond the presence or absence of anti-HLA antibodies, other immune assays that detect donor-specific B and T cell memory, such as the interferon-γ enzyme-linked immunospot (ELISpot), have been described as a monitoring tool to assess cellular immune risk [45–48]. Therefore, with our data we highlight the complexity of the clinical measurement of immune memory and the importance of stratifying the immunological risk of patients to predict the risk of developing DSA.

The development of ABMR regardless of DSA status is significantly associated with lower graft survival in our cohort (log rank p = 0.001), emphasizing ABMR as a fundamental cause of allograft failure, as widely described [1–6]. In our study, more than half of the patients with ABMR (55.0%) did not have detectable DSA, highlighting that the presence of C4d staining in the biopsy is a sufficient factor that allows ABMR diagnosis without serological evidence of DSA [36, 49]. The presence of DSA is associated with ABMR and graft failure, however the clinical evolution of patients with DSA is variable [50]. The MFI level may have predictive capacity, but the relationship of a single MFI value with clinical outcomes is not established, and some characteristics of DSA such as class, specificity, IgG subclass or complement binding capacity may be prognostic factors [16, 17, 51–53]. In our cohort, 19.5% of patients develop ABMR, with a higher incidence in patients with DSA. Despite this, almost half of patients with early-DSA have "subclinical" DSA without evidence of rejection and 26.6% present "transient" early-DSA with "spontaneous" disappearance, underscoring the different clinical course of patients with DSA and the urgent need to improve our knowledge of DSA characteristics and prognostic factors to determine patients at highest risk of rejection after DSA detection [50, 53].

Our study shows early-DSA as a determining factor of poor outcomes, since the presence of early-DSA is associated with a 3.3-fold higher risk of ABMR in multivariable analysis independently of other variables such as DGF, first kidney transplant or HLA sensitization. Furthermore, the presence of late-DSA is significantly associated with lower allograft survival (log rank p = 0.001) and ABMR-free survival (log rank p < 0.001), and remains a strong, independent risk factor for ABMR in multivariable analysis (HR 4.1). Altogether, our data support that the presence of DSA at any time after transplant is a consistent risk factor for ABMR, with worse outcomes in the group of patients with late-DSA. It has been described that class-II DSA usually appear later after transplant and are the most common dnDSA, being more harmful and resistant to treatment [16, 54]. Our group of patients with late-DSA presents a higher

percentage of class-II, the proportion of "transient" DSA is lower as well as the number of "subclinical" DSA, and they have more chronic ABMR changes, showing a different profile of patients with DSA that could explain our findings and determine the worse outcomes compared to the early-DSA group. It has been shown that patients with dnDSA present lower allograft survival compared to preexisting DSA [19]. Moreover, it has been demonstrated that patients with preformed DSA that persist after transplant have a higher incidence of ABMR than patients without DSA, but with a significantly lower risk of ABMR compared to those with dnDSA [55]. Accordingly, these results support our hypothesis that early-DSA could represent a memory response and have a better outcome than late-DSA.

The indication and frequency of universal HLA screening are not established, and it has been proposed a monitoring scheme with a first determination at 3–6 months post-transplant [29]. Although it seems that monitoring of preformed DSA may be useful [29, 56], high-quality data are lacking. It has been described that an early determination in the first month may be necessary in intermediate-risk patients with historical DSA or HLA sensitization [30], but there are currently no clear guidelines and the STAR 2017 Working Group suggests an early post-transplant HLA assessment in patients at risk for latent memory responses, without making specific recommendations on frequency and duration due to the absence of robust evidence [15]. In our cohort, early-DSA are consistently associated with ABMR and appear more frequently in sensitized patients. Most patients with early-DSA (68.8%) present ABMR at the time or after DSA detection. The time to ABMR diagnosis is shorter in patients with early-DSA compared to those with late-DSA (1.7 vs. 11.4 months), demonstrating that early screening potentially identifies high-risk patients and reduces the time to ABMR diagnosis, allowing prompt therapy initiation. Hence, until the clinical measurement of immune memory is better known and implemented in clinical routine to assess immunological risk, we suggest that early-DSA monitoring in the first month should be performed at least in patients with HLA sensitization, without waiting for the third or sixth month.

It should be noted that there is a time between the initial follow-up in our cohort and the determination of anti-HLA antibodies in which the appearance of DSA is not possible, mainly for analytical reasons, since there is a period without measurement of anti-HLA antibodies. Of note, all patients included in our study had a recent anti-HLA antibody sample prior to transplantation (median 42 days), and all patients underwent anti-HLA antibody testing at a specific time point or "landmark," the first month (median 30 days). Therefore, we closely monitor pre- and post-transplant HLA antibodies, and we perform a very early first HLA determination after transplant, which allows us to correctly assess the clinical impact of DSA on graft outcomes.

Nevertheless, our study has several limitations. This is a single-center, retrospective analysis that includes patients with HLA monitoring by Luminex, with a limited sample size and a low number of patients with DSA during the follow-up period. Our study presents a cohort of patients without preformed DSA and with negative FCXM, but with a variable risk of memory responses due to the inclusion of women with previous pregnancies, retransplants, and different degrees of HLA sensitization; despite this, the fact that our cohort is heterogeneous emphasizes our fundamental finding that early-DSA appearance represents memory and allows us to identify patients at risk. Although it is the best technique available, Luminex has multiple limitations and in our center SAB assay is only performed if initial screening is positive. The MFI positivity threshold is set at 1,500 as widely accepted in the literature, however the MFI cut-off value is controversial and can be affected by several parameters. The MFI level at the time of DSA detection is recorded but not the evolution of MFI in subsequent samples. Some characteristics of DSA that may be related to poor outcomes, such as complement binding capacity, are not analyzed as they are not performed routinely. Adherence to treatment and levels of immunosuppressive drugs are also not evaluated at the time of DSA detection. Pretransplant history of transfusions to assess immune memory is not registered. Classical antigen HLA mismatch is considered, without analyzing epitope mismatch. The lack of complete donor typing, especially in DP and DQ, does not allow us to rule out previous sensitization and limits the definition of post-transplant DSA at these HLA loci. To calculate repeated HLA mismatch in retransplants, it must be noted that most previous donor typings are low resolution, and repeated mismatches are not assessed at a molecular level. Finally, the presence of non-HLA antibodies is not analyzed. The fundamental strength of our study is having a large and well-described cohort of patients with early HLA determination in the first month posttransplant. Furthermore, we have clinical and histological data available that allow us to evaluate the impact of early-DSA on graft outcomes and the clinical course of patients after routine early HLA monitoring.

While more accurate knowledge of immunological risk and clinical measurement of immune memory is needed, our study describes a relevant proportion of patients with DSA detection in the first month (8.5%), probably showing alloimmune memory even in the absence of preformed DSA described in pretransplant sera and in the context of negative FCXM. Although almost half of these early-DSA are 'subclinical' without evidence of humoral injury, the presence of early-DSA remains a consistent risk factor for ABMR. The risk of developing early-DSA is significantly higher in patients with HLA sensitization, therefore routine early HLA screening in the first month may be reasonably performed in these high-risk patients. In conclusion, we provide granular evidence on the impact of early-DSA detected by screening on clinical events, being strongly related to ABMR and inferior outcomes. High-quality data on the clinical course of patients with DSA detected by universal screening and cost-effectiveness studies are essential to improve results and provide an appropriate post-transplant DSA monitoring strategy.

## DATA AVAILABILITY STATEMENT

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

## ETHICS STATEMENT

The studies involving humans were approved by Regional Ethics Committee of our institution: Comité de Ética de la Investigación con medicamentos de Cantabria (code: 2024.196). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because The clinical variables and sera of kidney transplant recipients included in the study have already been analyzed and were routinely collected as clinical practice after transplant, without requiring written informed consent from the patients.

## AUTHOR CONTRIBUTIONS

CL and JR conceived and designed the study. CL wrote the article. MO and MM-B provided technical support and acquired data. CL analyzed and interpreted the data. DS and ML-H performed HLA antibody testing. RV, LB, MV, and ER advised on the preparation of the article and provided conceptual advice. JR supervised the research. All authors contributed to the article and approved the submitted version.

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## CONFLICT OF INTEREST

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

## 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.2025.14424/full#supplementary-material

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# One-Year HbA1c Predicts Long-Term Pancreas Graft Survival Following SPK Transplantation: A US Population Cohort Study

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Understanding which factors shape long-term pancreas graft outcomes after the critical first year post-transplantation is an ongoing challenge. This study assesses one-year HbA1c as a predictor of subsequent pancreas graft survival. A retrospective cohort study was conducted using the UNOS registry on all simultaneous pancreas-kidney (SPK) transplants between 2017 and 2023. Regression models with multiple imputations for missing data were used to evaluate predictors of long-term function. Non-linear relationships were modelled with restricted cubic splines (RCS). Among 2,917 SPK recipients (median follow-up 44 months, IQR: 25–60), one-year HbA1c was the strongest independent predictor of long-term graft survival. An HbA1c of 6.8% versus 4.4% (95th vs. 5th percentile) was associated with significantly worse graft survival (aHR = 2.48, 95% CI: 1.72–3.58). Simulated trial sample size calculations found that detecting a statistically and clinically significant reduction in one-year HbA1c from 7% to 6.5% would require 65 patients per group, whereas detecting a reduction in one-year graft loss from 12% to 9% would require 1,631 patients per group. HbA1c at 1 year is a robust, continuous marker of long-term graft function and may serve as a feasible, objective surrogate endpoint in future clinical trials, enabling smaller, more efficient study designs to evaluate interventions.

**Keywords: pancreas transplantation, simultaneous pancreas-kidney transplantation, glycosylated hemoglobin (HbA1c), graft survival, long-term outcomes**

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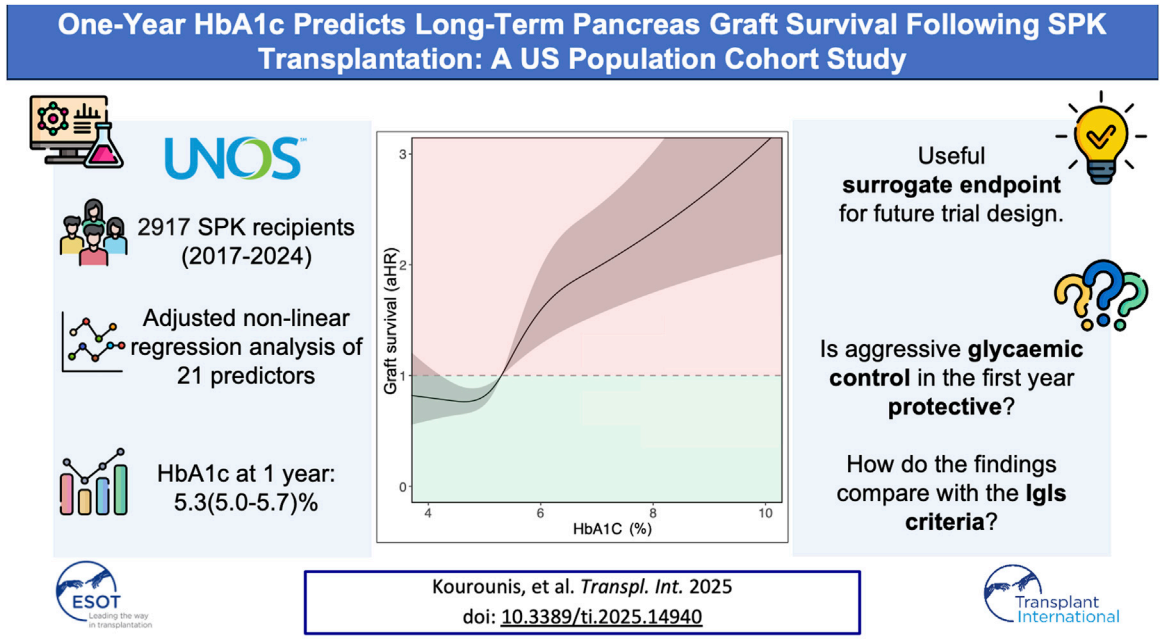
**Abbreviations:** aHR, adjusted hazard ratio; CI, confidence interval; DBD, donation after brain death; DCCT, Diabetes Control and Complications Trial; DCD, donation after circulatory death; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated hemoglobin; IFCC, International Federation of Clinical Chemistry; IQR, interquartile range; OPTN, Organ Procurement and Transplantation Network; PA, pancreas alone; PAK, pancreas after kidney; RCS, restricted cubic splines; SPK, simultaneous pancreas-kidney; UNOS, United Network for Organ Sharing.




**GRAPHICAL ABSTRACT |**

## INTRODUCTION

Diabetes has been described as a non-infectious pandemic disease of the modern era and is a leading cause of chronic kidney disease, non-traumatic lower limb amputations, and eye disease across the world [1–3]. Medical therapy in individuals with labile diabetes, even when optimized with hybrid closed loop insulin pump and continuous glucose monitoring systems, cannot restore optimal glycemic control with unavoidable continued exposure to low glucose levels and unremitting daily self-management burden, making β-replacement therapies the treatment of choice to restore long-term normoglycemia [4, 5]. In selected patients with diabetes-related kidney disease, simultaneous pancreas-kidney (SPK) transplantation offers optimal metabolic control, long-term insulin independence, fewer secondary complications, improved quality of life, and increased survival [2, 3, 6, 7].

While SPK transplantation has been shown to offer significant advantages, factors that can help predict long-term graft survival remain unclear. Previous registry analyses exploring predictors of graft survival have predominantly focused on donor and recipient factors at the time of transplantation. Factors linked with long-term graft survival have included donor age, donor cause of death, recipient and donor BMI, and cold ischemic times [3, 6]. These registry analyses have also shown that the first year post-transplant is critical, with graft loss occurring most frequently within the first year after transplant [2, 6, 8].

Among patients whose grafts continue to function beyond the first year, our understanding of the determinants of long-term outcomes remains limited. Moreover, little is known about modifiable factors that could guide post-transplant management and improve prognosis. With recipient HbA1c now routinely recorded in the UNOS dataset, there is an opportunity to investigate whether HbA1c at 1 year can serve as an independent predictor of subsequent graft survival in this cohort of patients. HbA1c is an intuitive candidate for this role. Given its routine use and potential to reflect ongoing pancreas function it has already been incorporated into post-transplantation scoring systems, such as the BETA-2 score [9, 10]. However, the independent association of HbA1c with long-term pancreas graft outcomes has not yet been clearly established. If proven to be predictive, HbA1c could serve as a clinically meaningful surrogate marker, informing patient management and guiding updates to clinical practice and transplant policy.

This study aimed to investigate whether one-year HbA1c serves as an independent predictor of long-term pancreas graft survival in SPK recipients whose grafts survived beyond the first year post-transplantation. The secondary objective was to evaluate the utility of one-year HbA1c as a surrogate marker of transplant outcomes, similar to the established role of one-year eGFR in kidney transplantation [11–13].

## MATERIALS AND METHODS

### Study Design and Population

This population cohort study was conducted using data from the United Network for Organ Sharing (UNOS) Registry. Ethical review, approval, or informed consent specific to

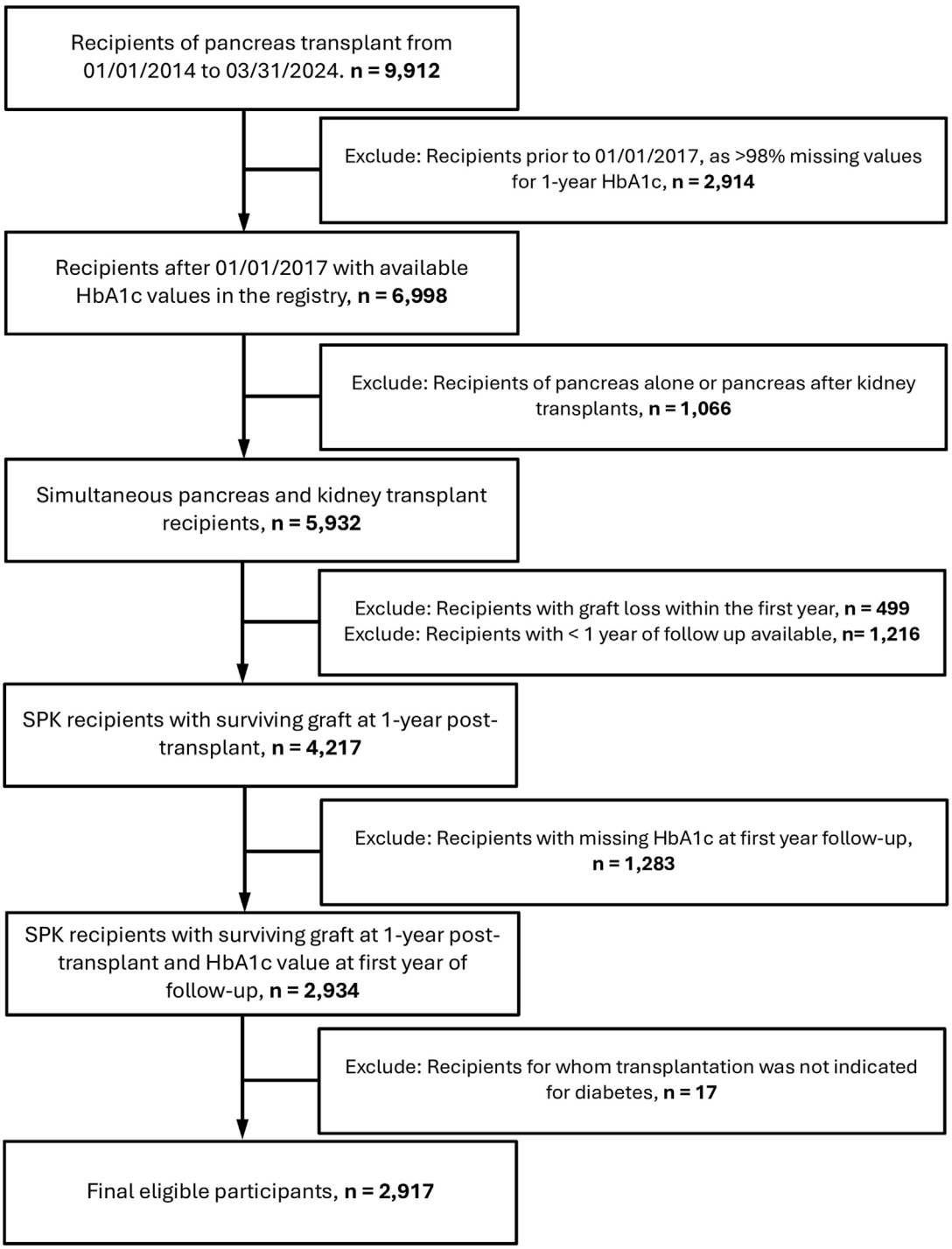



**FIGURE 1 |** Flowchart of recipients included in the study.

this study was not required as this was a secondary analysis of de-identified registry data. We included all recipients of SPK transplants between 1 January 2017, and 31 March 2023, with follow-up data available up to 31 March 2024. Exclusion criteria included recipients with graft loss within the first year post-transplant, patients with missing one-year HbA1c values, and those whose transplant indication was not diabetes. Although UNOS began recording follow-up HbA1c values in 2014, more than 98% of values were missing between 2014 and 2016, so data from those years were excluded. The study

**TABLE 1 |** Donor and recipient demographic characteristics.

| Variable | Levels | Values |
|---|---|---|
| Donor Age (years) | Median (IQR) | 23.0 (18.0–30.0) |
| Donor Sex | Female | 895 (30.7) |
| | Male | 2022 (69.3) |
| Donor Ethnicity | White, Non-Hispanic | 1714 (58.8) |
| | Black, Non-Hispanic | 577 (19.7) |
| | Hispanic/Latino | 512 (17.6) |
| | Asian, Non-Hispanic | 67 (2.3) |
| | Other | 47 (1.6) |
| Donor BMI (kg/m$^2$) | Median (IQR) | 23.6 (21.1–26.2) |
| Cause of Death | Head Trauma | 1,535 (52.6) |
| | Drug overdose | 437 (15.0) |
| | Other | 945 (32.4) |
| Donor Type | DBD | 2,827 (96.9) |
| | DCD | 90 (3.1) |
| Recipient Age (years) | Median (IQR) | 42.0 (35.0–49.0) |
| Recipient Sex | Female | 1,123 (38.5) |
| | Male | 1794 (61.5) |
| Recipient Ethnicity | White, Non-Hispanic | 1,388 (47.6) |
| | Black, Non-Hispanic | 830 (28.4) |
| | Hispanic/Latino | 520 (17.8) |
| | Asian, Non-Hispanic | 130 (4.5) |
| | Other | 49 (1.7) |
| Recipient BMI (kg/m$^2$) | Median (IQR) | 25.7 (23.1–28.6) |
| Recipient Diabetes Type | Type 1 | 2,232 (76.5) |
| | Type 2 | 680 (23.3) |
| Waiting Time (days) | Median (IQR) | 161.0 (50.0–421.0) |
| cPRA | ≤20 | 2,361 (80.9) |
| | >20 | 553 (19.0) |
| Previous Pancreas Transplant | No | 2,890 (99.1) |
| | Yes | 27 (0.9) |
| Recipient Dialysis Status | No | 659 (22.6) |
| | Yes | 2,256 (77.4) |
| HLA Mismatch | ≤2 | 102 (3.5) |
| | 3 | 322 (11.0) |
| | 4 | 755 (25.9) |
| | 5 | 1,084 (37.2) |
| | 6 | 654 (22.4) |
| CMV Match | P/N = Yes | 748 (25.6) |
| | P/N = No | 2,137 (73.3) |
| Duct Management | ED | 2,723 (93.4) |
| | BD | 86 (3.0) |
| | Other | 108 (3.7) |
| Steroid Maintenance | 0 | 805 (27.6) |
| | 1 | 2007 (68.8) |
| Tacrolimus and MMF Maintenance | No | 91 (3.1) |
| | Yes | 2,795 (95.8) |
| HbA1c at 1 Year (%) | Median (IQR) | 5.3 (5.0–5.7) |
| Treatment for Pancreas Rejection in 1st Year | No | 2,230 (76.5) |
| | Yes | 199 (6.8) |
| eGFR at 1 Year | Median (IQR) | 71.2 (57.8–87.3) |
| Treatment for Kidney Rejection in 1st Year | No | 2,251 (77.2) |
| | Yes | 170 (5.8) |

*BD, Bladder drainage; BMI, Body mass index; cPRA, Calculated panel reactive antibody; CMV, Cytomegalovirus; DBD, Donation after brain death; DCD, Donation after circulatory death; ED, Enteric drainage; eGFR, Glomerular filtration rate; HbA1c, Glycosylated hemoglobin; HLA, Human leukocyte antigen; HTK, Histidine-Tryptophan-Ketoglutarate; IQR, Interquartile range; MMF, Mycophenolate mofetil; P/N, Donor positive, recipient negative; SKP, Simultaneous kidney-pancreas transplantation; UW, University of Wisconsin solution.*

followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines [14].

## Outcomes and Definitions

The primary outcome was recipient long-term graft survival. UNOS defines graft loss in pancreas transplantation as removal of the transplanted pancreas, recipient re-registered for pancreas or islet transplant, recipient returned to ≥0.5 units per kilogram per day of insulin for a duration of >90 days, or recipient death. At the time of data sharing UNOS confirmed that this definition had been in place since June 2015 and for the duration of this study.

UNOS records HbA1c values using the % Diabetes Control and Complications Trial (DCCT) units, where a value of 5.7% corresponds to 38.8 mmol/mol International Federation of

Clinical Chemistry (IFCC) units. UNOS does not directly record center volume or follow-up eGFR. Instead, center volume was derived from the anonymized center identifiers in the registry by summing all SPK transplants performed at each center over the study period. The one-year follow-up eGFR values were calculated using the 2021 Chronic Kidney Disease-Epidemiology Collaborative (CKD-EPI) formula without race adjustment [15]. Median follow-up was estimated using the reverse Kaplan-Meier method with graft survival [16].

## Statistical Analysis

The approach to statistical analysis was similar to that described previously by our group [17]. Cox proportional hazards regression models were employed for the graft survival analyses. Multiple imputation was used to address missing data. The method used preserved non-linear relationships [18]. This was important as the outcome models employed non-linear modelling. For graft survival outcomes, we incorporated both the event indicator variable and the cumulative hazard of the event in the model to maintain the relationships between the outcome and the missing covariates [19].

Adjustment for a wide range of confounders was performed. Potential confounders were selected *a priori* based on prior research and clinical expertise [2, 3, 6, 20]. Statistical variable selection techniques (e.g., stepwise selection) were avoided [21]. A full list of covariates included along with justification for covariate selection, is provided in **Supplementary Table S1**. To account for potential non-linear relationships, restricted cubic splines were applied to continuous variables associated with the outcome. Three knots (10th, 50th, and 90th percentiles) were used for 1 year C-peptide, GFR, and recipient age, while four knots (5th, 35th, 65th, and 95th percentiles) were used for 1 year HbA1c to capture potential non-linearity while avoiding overfitting. An *a priori* decision was made to use splines for these variables. As transplanting center could impact post-transplant outcomes, we employed hierarchical modelling to adjust for this factor. This was done with a frailty Cox model, incorporating transplanting center as a random effect.

Acknowledging that donor and transplant factors might interact in ways that affect graft survival differently than when considered separately, we built models with interaction terms to account for these combined effects [22]. We also carried out sensitivity analyses to adjust for potential confounders excluded from the main models because of missing data or multicollinearity concerns [23].

Kaplan-Meier plots were generated to show crude graft survival, stratified by HbA1c levels <5.7% or ≥5.7%. This cutoff was selected based on previous literature [24] and the American Diabetes Association's definition of a normal range (<5.7%) [25]. HbA1c was maintained as a continuous variable and was not stratified in the multivariable analyses.

To demonstrate the differing sample size requirements when using continuous outcomes versus binary outcomes, we simulated sample size calculations in R [26] with the 'pwr' [27] package. Assuming $\alpha = 0.05$ and power = 0.80, our simulation varied effect sizes at 1 year post-transplant across a range of differences in graft loss incidence (binary outcome) and HbA1c values (continuous outcome). This range of effect sizes was selected to ensure that we captured all feasible minimally important differences. This approach enabled visualization and comparison of the sample sizes required.

We also conducted exploratory analyses on pancreas transplant alone (PA) and pancreas-after-kidney (PAK) recipients. Identical inclusion criteria, outcome definitions, and variable derivations were applied to these cohorts. Due to smaller sample sizes, only unadjusted Kaplan-Meier analyses were performed for PTA and PAK recipients.

Continuous variables were summarized using median and interquartile ranges. Outputs of models have been given as effect estimates with 95% confidence intervals. All analyses were performed in R (R Foundation for Statistical Computing, Vienna, Austria, version 4.4.1) [26], using the following packages; "tidyverse," "finalfit," "rms," "Hmisc," "survminer," and "pwr" [27–32]. Plots were also generated in R using "ggplot2," and "cowplot" [33, 34].

# RESULTS

## Patient Demographics

From 2017 to 2023, a total of 5,153 SPK transplant recipients were identified, of which 2,917 met the inclusion criteria and had a functioning graft 1 year post-transplant. The process of exclusion and final cohort selection is outlined in the study flow diagram (**Figure 1**). Key cohort demographics are summarized in **Table 1**. Full demographic information, including missing data, is summarized in **Supplementary Table S2**.

The median 1-year HbA1c was 5.3% (IQR: 5.0%–5.7%), with its distribution shown in **Figure 2A**. The median follow-up time from transplantation, determined using the reverse Kaplan-Meier method, was 44 months (IQR: 25–60 months). Kaplan-Meier analysis (**Figure 2B**) illustrates crude univariable 5-year graft survival stratified by HbA1c levels (<5.7% vs. ≥5.7%).

## HbA1c as a Predictor of Long-Term Graft Survival in SPK

Multivariable Cox regression model analysis was used to assess the association of recipients' HbA1c value at 1 year with long-term graft survival, adjusting for a wide range of factors (**Table 2**). Of these variables, one-year HbA1c was the strongest predictor of graft survival after 1 year (**Figure 3**), with its non-linear relationship confirmed in RCS analysis ($p < 0.001$, **Figure 4A**). One-year eGFR was the second strongest predictor of graft survival (**Figure 3**), with a non-linear relationship also confirmed in RCS analysis (<0.001, **Figure 4B**). Recipient age and one-year C-Peptide restricted cubic spline terms were visualized in **Supplementary Figure S1**.

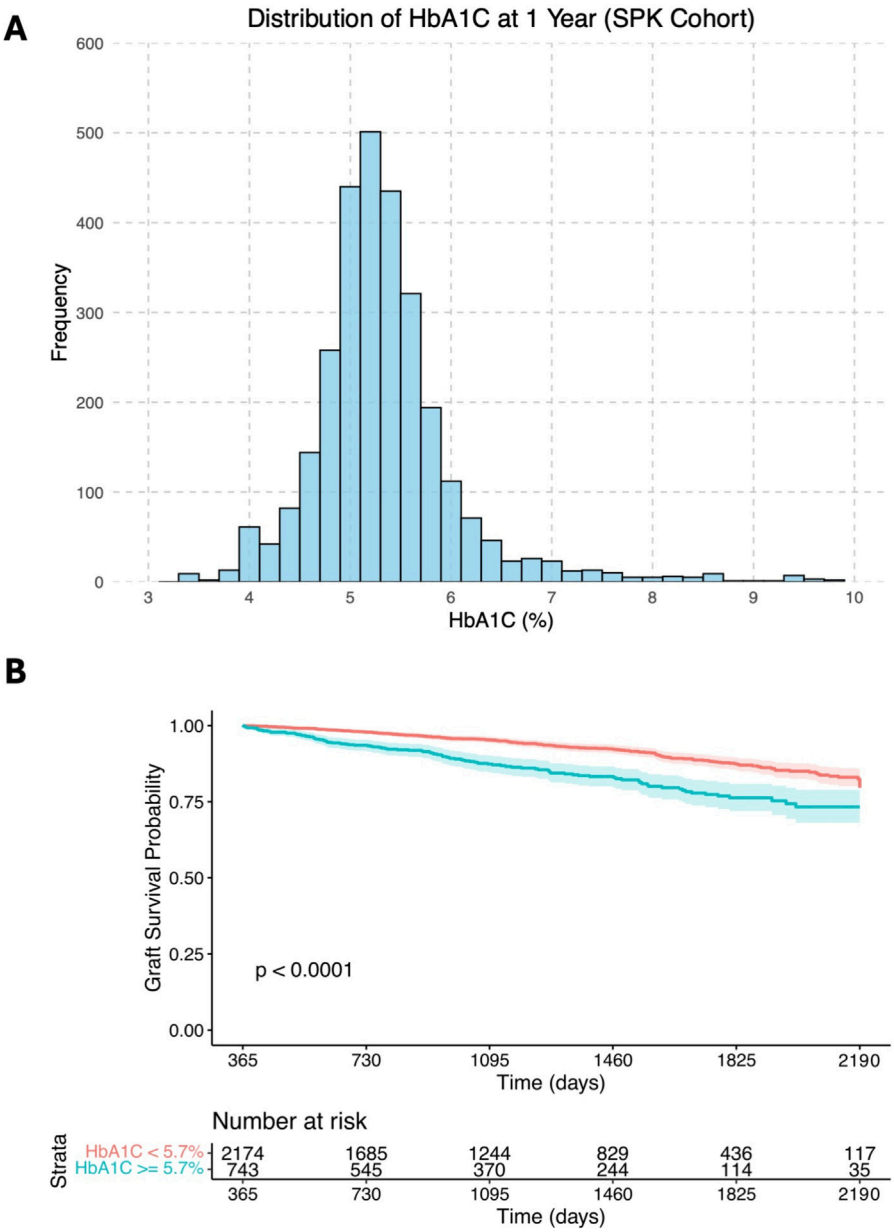



**FIGURE 2 | (A)** Histogram showing the distribution of 1-year HbA1c levels in the study cohort; **(B)** Kaplan-Meier survival curves illustrating crude univariable long-term graft survival over days post- transplantation (starting from 1 year after transplantation), stratified by HbA1c levels of <5.7% and ≥5.7%.

In this cohort of patients who had survived with a functioning graft for at least 1 year, a one-year HbA1c of 6.8% (50.8 mmol/mol) at the 95th percentile compared to 4.4% (24.6 mmol/mol) at the 5th percentile was linked to poorer graft survival after 1 year (aHR = 2.48, 95% CI 1.72–3.58). Adjusting for Cox model covariates, an HbA1c of 5.0% (31.1 mmol/mol), which was the median in this cohort, resulted in a five-year post-transplant graft loss probability of 6.4% (95% CI 2.8%–9.9%). This increased to 10.5% (4.7%–15.9%) for an HbA1c of 5.7% (38.8 mmol/mol), the third quartile for this cohort. Among the Igls criteria thresholds, an HbA1c of 6.5% (47.5 mmol/mol) yielded a predicted graft loss of 13.8% (6.0%–20.9%), and an HbA1c of 7.0% (53.0 mmol/mol) resulted in a predicted graft loss of 14.8% (6.5%–22.4%). A full summary of predicted graft loss by HbA1c level at 2–5 years post-transplant, adjusted for Cox model covariates, is provided in **Table 3**.

To adjust for potential individual transplant center effects, a Cox frailty model with random effects for transplant center was performed, revealing no significant variation between transplant centers (P = 0.324, $\chi^2$ = 1.949, df = 1.756). When adjusting for this between-center variation, the RCS estimates for the impact of HbA1c on graft survival did not meaningfully change.

**TABLE 2 |** Multivariable Cox regression model for long-term graft survival, pooled from 20 imputed datasets. For restricted cubic splines see **Figure 4** and **Supplementary Figure S1**.

| Variable | Hazard ratio (95% CI) | P value |
|---|---|---|
| Donor age | 1.011 (0.996–1.026) | 0.164 |
| Donor BMI | 0.991 (0.960–1.024) | 0.607 |
| Donor cause of death | | |
| Head trauma | Ref | |
| Drug overdose | 0.860 (0.601–1.231) | 0.411 |
| Other | 0.763 (0.577–1.008) | 0.057 |
| Donor type | | |
| DBD | Ref | |
| DCD | 1.218 (0.613–2.418) | 0.573 |
| Pancreas preservation time | 0.986 (0.956–1.016) | 0.359 |
| HLA Mismatch | 0.950 (0.848–1.065) | 0.380 |
| CMV Match | | |
| P/N = Yes | Ref | |
| P/N = No | 0.812 (0.620–1.062) | 0.129 |
| Duct management | | |
| Enteric Drainage | Ref | |
| Bladder Drainage | 1.608 (0.920–2.809) | 0.095 |
| Other | 1.171 (0.688–1.995) | 0.561 |
| Steroids maintenance | | |
| Yes | Ref | |
| No | 0.966 (0.732–1.276) | 0.810 |
| Donor ethnicity | | |
| Black | Ref | |
| White | 0.996 (0.734–1.351) | 0.979 |
| Other | 0.946 (0.664–1.350) | 0.761 |
| Recipient BMI | 1.004 (0.972–1.038) | 0.809 |
| cPRA | | |
| <20 | Ref | |
| ≥20 | 1.069 (0.790–1.447) | 0.664 |
| Recipient diabetes type | | |
| Type 1 | Ref | |
| Type 2 | 1.170 (0.849–1.612) | 0.338 |
| Previous pancreas transplant recipient | | |
| No | Ref | |
| Yes | 0.706 (0.165–3.016) | 0.639 |
| Recipient on dialysis | | |
| No | Ref | |
| Yes | 1.545 (1.123–2.126) | 0.008 |
| Treated for pancreas rejection in first year | | |
| No | Ref | |
| Yes | 1.744 (1.201–2.532) | 0.003 |
| Treated for kidney rejection in first year | | |
| No | Ref | |
| Yes | 1.118 (0.736–1.697) | 0.601 |
| RCS: Recipient age | RCS terms | 0.010 |
| RCS: HbA1c at first year follow-up | RCS terms | <0.001 |
| RCS: C-Peptide at first year follow-up | RCS terms | 0.034 |
| RCS: eGFR at first year follow-up | RCS terms | <0.001 |

*BMI, Body mass index; cPRA, Calculated panel reactive antibody; CMV, Cytomegalovirus; DBD, Donation after brain death; DCD, Donation after circulatory death; eGFR, Glomerular filtration rate; HbA1c, Glycosylated hemoglobin; HLA, Human leukocyte antigen; P/N, Donor positive, recipient negative; RCS, Restricted cubic splines.*

Sensitivity analyses were carried out using additional Cox proportional hazards models that incorporated factors such as donor age, ethnicity, smoking history, terminal lipase, time from admission to death, recipient waiting time, induction medications, center volume, and preservation fluid. An additional analysis with a death-censored graft failure endpoint was also carried out (**Supplementary Table S3**; **Supplementary Figure S2**). None of these analyses altered the conclusions derived from the main model findings.

Formal interaction assessments were conducted to check if the impact of HbA1c on graft survival was different along different levels of eGFR, or whether the recipient had received any insulin during the first year post-transplant. There were no associations between HbA1c and eGFR ($p = 0.408$), or HbA1c and insulin use ($p = 0.636$). Overall, this further supports that HbA1c is an independent predictor of long-term graft loss across all levels of these factors.

## Utility of HbA1c as a Surrogate Marker of Outcome

As HbA1c at 1 year was confirmed to be the strongest predictor of long-term graft survival, we sought to assess its utility as a surrogate marker for transplant outcomes in the context of clinical trial design. Our simulations revealed that a clinical trial using incidence of graft loss as its primary outcome, powered to detect a relative risk reduction of at least 25%—i.e. an absolute risk reduction from 12% to 9%—would require a total sample size of 3,262. In contrast, if the continuous HbA1c was used as a primary outcome measure, detecting a reduction of at least 0.5% (for example, from 7% to 6.5%) would require a total sample size of 130.

**Figure 5** illustrates the required sample sizes across a range of possible minimum effect sizes for both the continuous HbA1c, and the binary relative risk of graft loss at 1 year. Due to the considerable difference in sample sizes between these outcomes, a log-transformed y-axis was necessary to effectively visualize these trends.

## Exploratory Analyses

Because of the limited number of PA and PAK recipient outcomes available in the UNOS registry since HbA1c data collection began, a comparable multivariable analysis was not possible. We have summarized the donor and recipient characteristics for these cohorts in **Supplementary Table S4**. Exploratory univariable analyses using Kaplan–Meier plots yielded similar results for the PA cohort, while no significant differences in long-term graft survival were observed in the PAK cohort (**Supplementary Figures S3 and S4**).

In the primary analysis, no significant association was observed between C-Peptide levels below 5 ng/mL and graft loss. However, the hazard of graft loss increased at C-Peptide levels above 5 ng/mL (**Supplementary Figure S1**). To further explore this relationship, we conducted an interaction analysis to assess whether the relationship between C-Peptide and graft loss differed by insulin use (**Supplementary Figure S5**). In this assessment, among recipients receiving exogenous insulin, the risk of graft loss was high at lower C-Peptide levels (<3 ng/mL). In contrast, for those not receiving exogenous insulin, the risk of graft loss was high at higher C-Peptide levels (>5 ng/mL). Associations outside these ranges were not significant.

We also applied our multivariable Cox-regression methodology to evaluate predictors of kidney-graft survival in

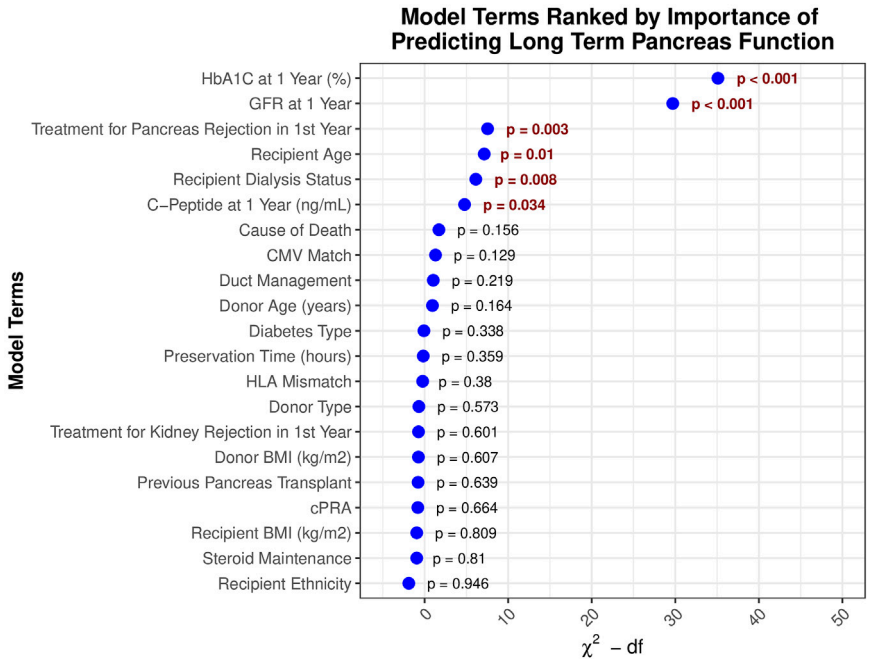



**FIGURE 3 |** Model terms ranked by significance in predicting long-term pancreas graft survival.

the same cohort. As expected, eGFR emerged as the strongest predictor in this analysis, while HbA1c showed no association with graft outcome (**Supplementary Figure S6**).

## DISCUSSION

Our findings demonstrate that one-year HbA1c was the strongest independent predictor of long-term graft survival in recipients of SPK transplants whose pancreas continued to function beyond the first year. The findings also highlight the potential utility of HbA1c as a primary endpoint in clinical trials, as it is a good surrogate for long-term graft survival. As a continuous measure, HbA1c could enable a substantial reduction in required sample sizes compared to binary measures of outcome.

This is also the first study to evaluate the association between one-year eGFR and subsequent pancreas graft survival in this population. One-year eGFR emerged as the second strongest independent predictor of pancreas graft outcomes, with lower eGFR values associated with an increased risk of pancreas graft loss. As this study includes only SPK recipients, reduced kidney function at 1 year likely reflects early rejection episodes affecting both organs, leading to subsequent pancreas graft loss.

These findings align with previous cohort studies that have explored the link between early metabolic assessments in pancreas transplant recipients and graft failure [9, 35–37]. Chetbourn et al. found that BETA-2 scores calculated 3 months post-transplantation in 209 pancreas transplant recipients were a marker of long term insulin independence [9]. Similarly, in an adjusted multivariable analysis of 210 pancreas transplant recipients, Mittal et al. found that oral glucose tolerance tests performed within 2 weeks of transplantation were the strongest independent predictor of graft failure [35]. Although we used different measures, our results similarly indicate that post-transplant glycemic control is the strongest predictor of long-term graft survival, even when adjusting for a range of other clinically relevant variables.

A significant strength of this study is the cohort size and methodology employed, including the use of a Cox frailty model to adjust for center-specific effects. This is particularly important given longstanding concerns among clinicians that pancreas graft loss may be reported inconsistently across centers. Although a standardized UNOS definition has been in place since June 2015, center-level variation in how outcomes are recorded or interpreted can still occur. These differences—whether due to follow-up protocols, thresholds for insulin restart, or documentation practices—can introduce variability in reported outcomes. By using a Cox frailty model, the analysis accounts for these unmeasured center-level differences. Importantly, the association between HbA1c and long-term graft survival remained even after this adjustment, strengthening the evidence that the finding is not affected by any potential inter-center variability.

In addition, multivariable analyses with interaction terms enabled us to control for potential confounders and assess whether associations varied across different variable levels. Kidney function was of particular interest, as renal impairment makes HbA1c interpretation more challenging and might synergistically influence graft survival with HbA1c. Our interaction analyses confirmed that the effect of HbA1c on graft outcomes remained consistent regardless of differences in

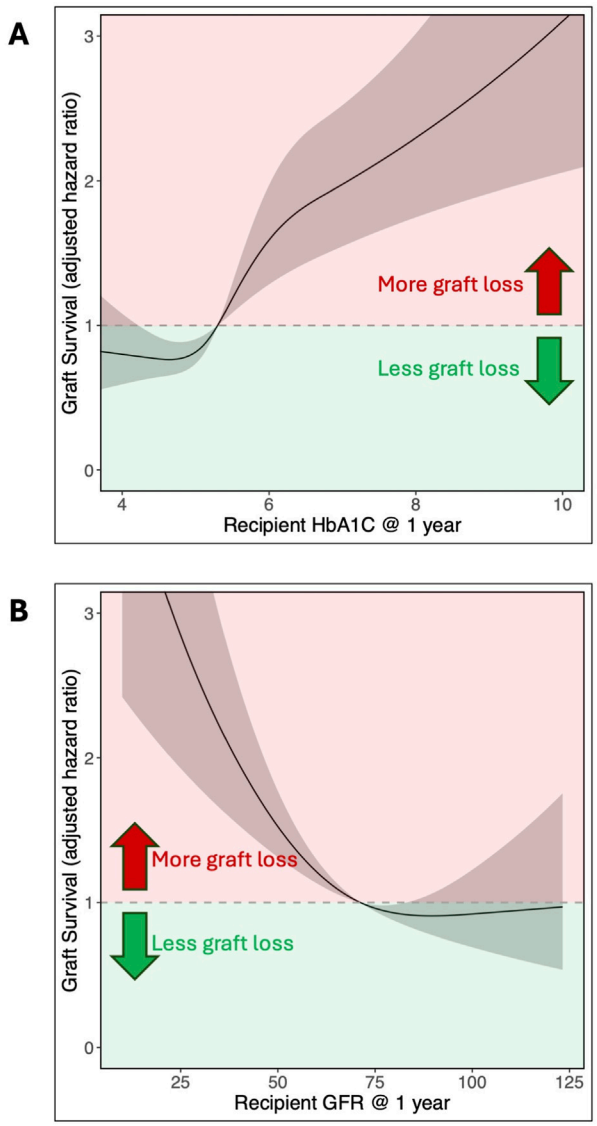



**FIGURE 4 |** Adjusted hazard ratio for pancreas graft survival based on recipient **(A)** HbA1c (% DCCT) and **(B)** eGFR (mL/min/1.73 $m^2$) at 1 year. The non-linear relationship is shown with restricted cubic splines analysis. HbA1c was modeled with 4 knots, while eGFR used 3 knots.

eGFR levels–HbA1c was predictive of outcome even in those with low eGFR.

The results could significantly impact future pancreas transplantation research. Graft loss is becoming increasingly less prevalent and is a binary measure of outcome [2, 6, 8]. This combination poses significant challenges for powering clinical trials, as it necessitates large numbers of patients per group. In kidney transplantation, one-year eGFR has been adopted as an effective surrogate endpoint [11, 12]. HbA1c, a similarly continuous measure, also has the potential to serve as a surrogate marker in pancreas transplantation. Our findings demonstrate that HbA1c not only predicts long-term graft survival but also reduces the required sample size for adequately powered trials. Adopting HbA1c as a trial outcome could lower trial costs, speed up research timelines, and help bring effective interventions to clinical practice sooner.

In terms of identifying predictors of long-term pancreas survival, this is the first registry analysis to demonstrate how these predictors change over time. Previous registry analyses have only explored predictors of graft survival at the time of transplantation, reporting factors such as younger donor age, donor body mass index, and cause of death as important predictors of long-term graft survival [3, 6]. However, in recipients whose grafts survived up to the first year these factors became less important, with HbA1c and eGFR emerging as the strongest predictors. In other words, events during the first year post-transplant carry greater prognostic value than the donor and recipient characteristics present at transplantation.

This study is also the first to quantify the adjusted hazard ratio (aHR) of graft loss along a continuum of HbA1c values, made possible by the restricted cubic spline analysis. In our cohort of SPK recipients, the aHR for graft loss begins to increase at an HbA1c of 5.7%, which aligns with the American Diabetes Association's cut-off for a normal range [25]. This contrasts to the Igls criteria for β-cell replacement therapy that defines optimal and good glycemic control at ≤6.5% and <7% HbA1c respectively [7, 38]. Our data suggest that these thresholds may be too high for SPK recipients. Three-quarters of recipients in our analysis had HbA1c values at or below 5.7% (the third quartile), with few recipients exceeding 6.5%. In addition, both the 6.5% and 7% thresholds fell within the range associated with significantly increased aHRs for graft loss (**Figure 4**).

In addition to evaluating the predictive value of one-year HbA1c for long-term graft survival, we also sought to explore whether its impact varied according to the recipients' use of diabetes medications. Unfortunately, the registry data had significant gaps on recipients' dosing and duration of oral hypoglycemic and insulin therapy post-transplant. This limited our ability to determine whether individuals with low HbA1c had better outcomes independently of medication use. Future studies should aim to capture more detailed information on medication use so as to evaluate whether targeted interventions to optimize glycemic control in the first year may lead to improved one-year and long-term pancreas outcomes. Supported by data showing the intrinsic impact of islet graft function on HbA1c regardless of exogenous insulin use, we propose that HbA1c is likely to be an important predictor even in those using glucose lowering medications [39].

The observed association between elevated C-peptide levels and graft loss was noteworthy. Although C-peptide concentrations below 5 ng/mL were not significantly associated with outcomes, levels exceeding this threshold were independently associated with an increased risk of graft failure. Interaction analysis further distinguished this to be the case only in those not receiving any exogenous insulin. This finding is not novel and aligns with prior reports linking elevated C-peptide with adverse graft

**TABLE 3 |** Predicted graft loss by HbA1c levels at 2–5 years post-transplant, adjusted for Cox model covariates.

| HbA1c level (% DCCT/mmol/mol IFCC) | Predicted graft loss (% & 95% Confidence interval) | | | |
|---|---|---|---|---|
| | 2 years | 3 years | 4 years | 5 years |
| 4.4%/24.6 mmol/mol | 1.1% (0.4%–1.8%) | 2.5% (1%–3.9%) | 3.8% (1.5%–6%) | 6.1% (2.5%–9.5%) |
| 5.0%/31.1 mmol/mol | 1.2% (0.5%–1.9%) | 2.6% (1.1%–4.1%) | 4% (1.7%–6.2%) | 6.4% (2.8%–9.9%) |
| 5.3%/34.4 mmol/mol | 1.5% (0.6%–2.3%) | 3.2% (1.4%–4.9%) | 4.9% (2.2%–7.5%) | 7.8% (3.5%–11.8%) |
| 5.7%/38.8 mmol/mol | 2% (0.8%–3.2%) | 4.3% (1.9%–6.7%) | 6.6% (2.9%–10.1%) | 10.5% (4.7%–15.9%) |
| 6.5%/47.5 mmol/mol | 2.7% (1.1%–4.3%) | 5.7% (2.4%–9%) | 8.7% (3.7%–13.4%) | 13.8% (6%–20.9%) |
| 7.0%/53.0 mmol/mol | 2.9% (1.1%–4.6%) | 6.2% (2.6%–9.6%) | 9.4% (4%–14.5%) | 14.8% (6.5%–22.4%) |
| 8.0%/63.9 mmol/mol | 3.4% (1.3%–5.4%) | 7.1% (2.9%–11.2%) | 10.8% (4.5%–16.7%) | 17% (7.3%–25.7%) |

*Cox model adjusted for: donor age, donor BMI, donor cause of death, donor type, pancreas preservation time, HLA mismatch, CMV match, duct management strategy, steroid maintenance, donor ethnicity, recipient age, recipient BMI, cPRA, recipient diabetes type, prior pancreas transplant, dialysis at transplant, treatment for pancreas rejection in the first year, treatment for kidney rejection in the first year, C-peptide at one-year follow-up, HbA1c at one-year follow-up, and eGFR at one-year follow-up.*

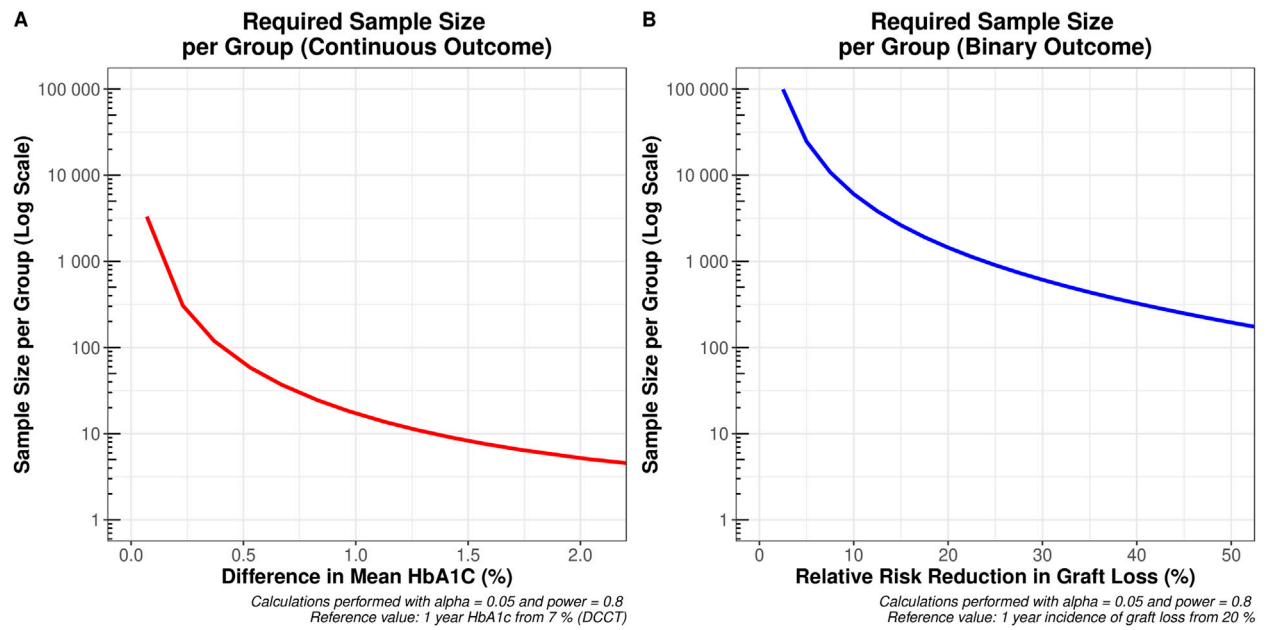



**FIGURE 5 |** Sample sizes required to detect the desired minimum clinically important difference across the range of values on the x-axis **(A)** a change in one-year mean HbA1c from 7% (e.g., a reduction from 7% to 6.5% would require 65 patients per group) and **(B)** a relative risk reduction in graft loss from 20% at 1 year (e.g., a reduction from 20% to 16% would require 1,444 patients per group).

outcomes [40, 41]. High C-Peptide in this cohort could reflect more insulin resistance or be an early marker of rejection. As the UNOS registry does not specify whether C-peptide measurements were taken in the fasting or stimulated state, these findings should be interpreted with caution. The absence of standardized collection highlights the need for more rigorous and consistent data capture for this key variable in future studies.

Another limitation of the UNOS registry is a lack of information on hemoglobin values at 1 year post transplantation. Hemoglobin is a potentially important confounder as anemia can make the interpretation of HbA1c more challenging. Analyzing the effect of anemia and adjusting for this was not possible with the current dataset.

Overall, these limitations also prevented us from comparing the Igls criteria for β-cell graft function to the UNOS graft failure definition. Key Igls components such as severe hypoglycemic episodes, insulin dosing, and reliably recorded fasting or stimulated C-peptide levels were not available in the UNOS registry. Future prospective studies should aim to capture these parameters to evaluate how different graft failure definitions impact observed event rates and trial design considerations.

Finally, we recognize that the results may not readily generalize to recipients of pancreas alone (PA) and pancreas after kidney (PAK) transplants. A robust multivariable analysis of this group was not possible due to the limited sample size. These

exploratory findings are a call to action for further detailed studies across centers and improved data capture within the UNOS registry.

## CONCLUSION

One-year HbA1c was the strongest independent predictor of long-term graft survival in SPK recipients whose grafts survived beyond 1 year. This supports the potential utility of HbA1c as a surrogate endpoint for clinical trials, enabling more efficient study designs with smaller sample sizes. Moving forward, more granular and nuanced data are needed—whether through improved adherence to UNOS registry protocols or through collaborative efforts among individual centers—to determine whether targeted interventions during the first year can improve both one-year and long-term pancreas outcomes.

## DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The data that support the findings of this study are available from UNOS upon reasonable request. Requests to access these datasets should be directed to https://optn.transplant.hrsa.gov/data/view-data-reports/request-data/.

## ETHICS STATEMENT

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

## AUTHOR CONTRIBUTIONS

Concept and design: CoW, GK, and ST. Data acquisition, cleaning and statistical analysis: GK and ST. Data interpretation: All authors. Drafting of the article: GK and ST. Revision and approval of the article: All authors. All authors contributed to the article and approved the submitted version.

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## AUTHOR DISCLAIMER

The views expressed are those of the author(s) and not necessarily those of the NIHR, NHS Blood and Transplant or the Department of Health and Social Care.

## 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.2025.14940/full#supplementary-material

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LETTER TO THE EDITOR
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# The Safety and Efficacy of Daprodustat for Recipients in Peritransplant Period: a Single-Center Retrospective Study on Post-Transplant Anemia Management in Kidney Transplantation

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**Keywords: HIF-PH inhibitor, erythropoiesis-stimulating agent, renal transplantation, anemia, chronic kidney disease (CKD)**

Dear Editors,

Post-transplant anemia (PTA) is a common complication after kidney transplantation, with a prevalence ranging from 38.6% to 45.6% [1]. PTA is associated with poor graft function and graft survival [1, 2]. While erythropoiesis-stimulating agents (ESAs) are widely used for PTA [3], hypoxia-inducible factor prolyl hydroxylase (HIF-PH) inhibitors have recently emerged as alternative oral therapies [4, 5]. Daprodustat is a once-daily oral HIF-PH inhibitor that stabilizes HIF-α subunits, including HIF-1α and HIF-2α, maintains their activity, and activates the downstream genes of HIF signaling [6]. HIF-2 induces renal and hepatic EPO synthesis in response to daprodustat, which stimulates erythropoiesis. Although HIF-PH inhibitors, including daprodustat, are widely used for anemia of chronic kidney disease, evidence for their use in the immediate post-transplant period remains limited.

We conducted a single-center retrospective study to assess the safety and efficacy of daprodustat, a HIF-PH inhibitor, in managing PTA during the peritransplant period. We included adult recipients of living-donor kidney transplants from June 2019 to March 2023 who received treatment for PTA within 1 week after transplantation. Patients who received daprodustat (n = 59) or ESAs (n = 74) were identified, and 42 patients from each group were matched using propensity scores calculated using preemptive transplantation, ABO incompatibility, and prior ESA use. The target hemoglobin was 10–12 g/dL. Both groups received iron supplementation as needed.

Hemoglobin levels declined initially in both groups, reaching a nadir in the first postoperative week, and subsequently increased over 12 weeks, with no significant difference between the groups (**Figure 1A**). There were also no significant differences in progression of renal function within 12 weeks after transplantation (**Figure 1B**). As hemoglobin increased, more than half of patients in the daprodustat group discontinued treatment within 12 weeks (**Figure 1C**). Three patients in the ESA group transitioned to HIF-PH inhibitors after 12 weeks. At 1 year, both hemoglobin levels and eGFR were comparable between groups; hemoglobin was 12.5 ± 1.6 g/dL in the daprodustat group

**Abbreviations:** eGFR, Estimated Glomerular Filtration Rate; ESA, Erythropoiesis-Stimulating Agent; HIF-PH, Hydroxylation-Inducible Factor Prolyl Hydroxylase; MACE, Major adverse cardiovascular event; PTA, Post-Transplant Anemia.

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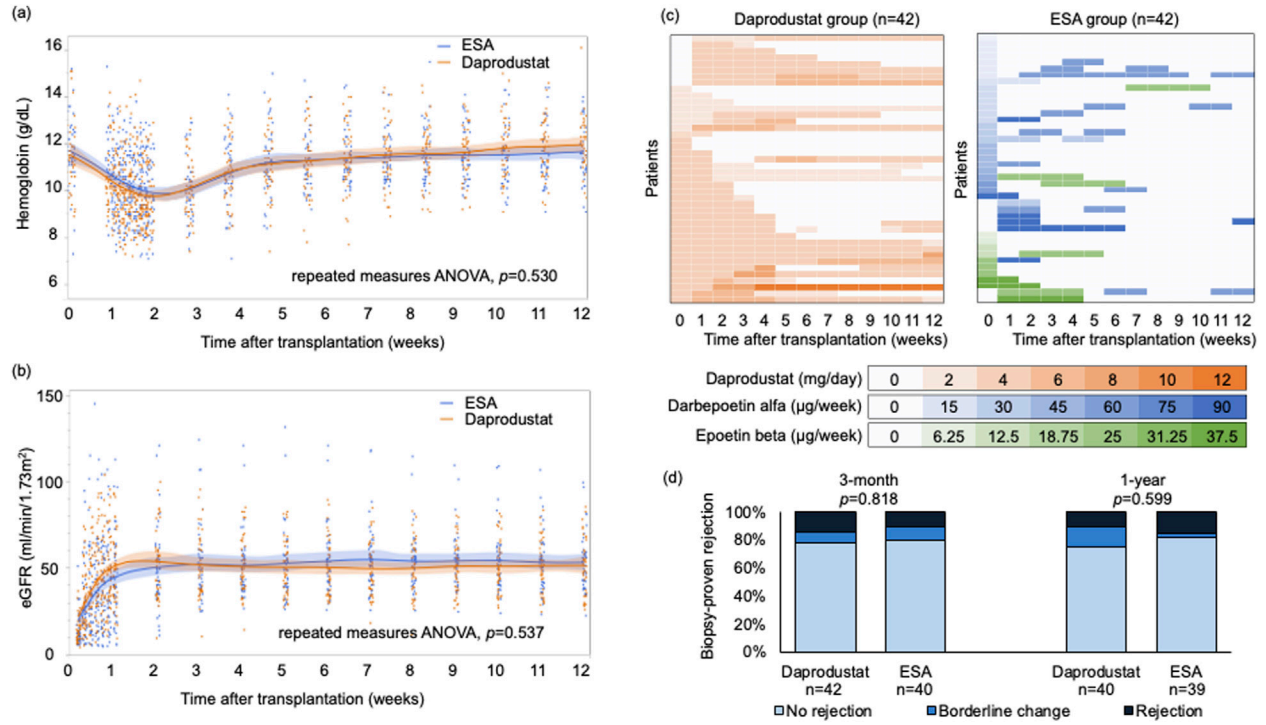


**FIGURE 1 | (A)** Hemoglobin levels and **(B)** eGFR of the groups within 12 weeks after transplantation. Data are presented as mean values with 95% confidence intervals. Repeated measures analysis of variance showed no significant differences between the groups in either hemoglobin levels or eGFR. **(C)** Dose of daprodustat or ESAs of each patient. Vertical axis represents each patient, and color intensities indicate the dosage levels at each time point. Darker colors correspond to higher doses of daprodustat or ESAs. **(D)** Biopsy-proven rejection rates were compared between the groups. The biopsy results were classified in the order of no rejection, borderline change, and rejection, and compared with two-sided Mann-Whitney U test. ANOVA, analysis of variance; eGFR, estimated glomerular filtration rate; ESA, erythropoietin-stimulating agent.

versus 13.0 ± 1.7 g/dL in the ESA group (p = 0.205), and eGFR was 49.3 ± 15.5 mL/min/1.73 $m^2$ versus 50.9 ± 14.0 mL/min/1.73 $m^2$, respectively (p = 0.620).

Safety outcomes were also similar. One thromboembolic event occurred in the daprodustat group (pulmonary embolism), and one major adverse cardiovascular event (MACE) was observed in each group. No deaths or graft losses occurred. Rates of biopsy-proven rejection at 3 months and 1 year did not differ significantly (**Figure 1D**).

In our study, the effectiveness of daprodustat in maintaining hemoglobin and renal function was similar to ESAs during the early post-transplant period, suggesting that daprodustat can be safely initiated in kidney transplant recipients during peritransplant period. Because it is suggested that rapid hemoglobin elevation and iron deficiency induced by HIF-PH inhibitors may increase the risk of thrombotic events, monitoring hemoglobin and serum iron levels could be important to avoid hemoglobin overshoot and iron deficiency. In our institution, HIF-PH inhibitors were initiated during hospitalization, and patients underwent weekly monitoring for the first 3 months after transplantation. This allowed fine adjustment of HIF-PH inhibitor dosing to avoid hemoglobin overshoot. In addition, especially when using HIF-PH inhibitors, serum iron and ferritin are monitored, and iron supplementation is performed if needed. However, because a meta-analysis has shown that long-term use of HIF-PH inhibitors increases the risk of thrombotic events compared with ESAs [7], we should pay attention to patients requiring long-term use of daprodustat.

It is known that PTA is associated with impaired allograft function and increased mortality following kidney transplantation [1, 8]. Moreover, correcting PTA with ESAs has been shown to improve allograft function and prolong survival in prospective interventional trials [9, 10]. Hypoxia in the tubulointerstitium due to anemia may contribute to kidney allograft damage and the development of CKD in transplant recipients [9]. Therefore, early correction of PTA after transplantation may be crucial for the long-term prognosis of kidney allografts. Our study demonstrated that daprodustat treats PTA as effectively as ESAs, suggesting that correcting PTA with daprodustat could also improve kidney allograft outcomes similar to ESAs.

This study has several limitations. First, as this was a retrospective study, it was susceptible to selection bias. There were significant differences in background characteristics between the groups; therefore, we conducted a propensity score matching analysis to create comparable cohorts. Additionally, the retrospective design resulted in some missing data, including iron metabolism markers such as ferritin.

Monitoring of iron markers is crucial for assessing and managing anemia; therefore, further investigation is needed after accumulating sufficient data. Second, we could only evaluate short-term outcomes within 1 year after transplantation. Given that nearly half of the patients in the daprodustat group discontinued daprodustat and a certain number of patients in the ESA group started taking daprodustat or other HIF-PH inhibitors even beyond 3 months after transplantation, we could not accurately assess the long-term effects of daprodustat on transplant outcomes. Despite these limitations, this study provides valuable insights into the safety and efficacy of perioperative daprodustat administration in kidney transplant recipients.

In conclusion, daprodustat can be safely used to manage anemia in kidney transplant recipients in the early post-transplantation period.

## 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 studies involving humans were approved by Ethical Committee of Kyushu University. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because of the retrospective nature of the study.

## AUTHOR CONTRIBUTIONS

YS contributed to study design, data collection, data analysis, and drafting of the manuscript. HN was involved in study design and critically revised the manuscript. SK, YH, and KK acquired and analyzed the data and revised the manuscript. ST interpreted the pharmacological data. YO and MN were responsible for study supervision and final approval of the manuscript. All authors contributed to the article and approved the submitted version.

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## CONFLICT OF INTEREST

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

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LETTER TO THE EDITOR
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# Positive Impact of ERAS Programme on Living and Deceased Donor Renal Transplant Recipients During COVID-19 Pandemic

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**Keywords: enhanced recovery after surgery (ERAS), kidney, COVID-19, length of stay, perioperative care**

Dear Editors,

We watch with interest as the transplant community adopts Enhanced Recovery After Surgery (ERAS) protocols. There was a significant reduction in recipient length of stay after introduction of an ERAS protocol in our unit when compared with an earlier, matched population with no evidence of adverse events and high acceptability to patients and staff. We believe that the ERAS protocol contributed to this difference and helped change staff mindset.

ERAS protocols are multi-modal perioperative care pathways designed to improve recovery after major surgery by maintaining preoperative body composition and physiological organ function and modifying the surgical stress response. ERAS is now standard of care in many surgical specialties as it reduces post-operative complications, pain, and length of stay (LoS) with increased patient satisfaction. ERAS in solid organ transplantation is less well established but there is increasing evidence that ERAS can positively impact on living donor, recipient and organ outcomes with additional financial benefits [1–7]. However, in a survey of all UK renal transplant units, only three had established ERAS programmes [3]. The most cited barrier was embedded peri-transplant management culture within the unit along with limited evidence and lack of existing guidelines in the heterogeneous UK transplant environment [1].

Our centre proposed an ERAS quality improvement programme before the COVID-19 pandemic, which accelerated momentum due to an increasing awareness of nosocomial transmission risk aiming to reduce hospital exposure for vulnerable transplant recipients. The aim of the ERAS quality improvement project was to create a straight-forward, acceptable, protocol that would result in a reduced in-patient stay aiming for day five discharge (as seen safely in other units [2, 4]) without an increase in readmissions or morbidity. A successful ERAS programme requires staff buy-in, managerial support and compliance to an agreed plan, so a multidisciplinary team meeting was convened where available literature, feedback from other UK units and patients were considered and a renal transplant recipient protocol agreed (**Figure 1**).

The main differences included earlier catheter removal (day 3 as supported by Cole et al [7]), encouragement of early mobilisation goals through a new physiotherapy referral pathway, dietary advice and analgesia guidance to reduce opioid use. To further encourage mobilisation and reduce analgesia requirements, we recommended reduction of auxiliary lines including surgical drains, central lines (given that CVP is a poor marker for vascular filling in most cases [8]) and early discontinuation of IV fluids. No specific fluid overload scoring system has been

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**ERAS bedside checklist**

Please note that this is a suggested plan and some patients may require a modified plan.

| Plan | | Check as complete |
|---|---|---|
| Pre-op | Check patient aware of enhanced recovery aims and anticipated progress (patient information booklet and physio advice sheet) | |
| | Based on confirmed theatre time, encourage diet until 6 hrs pre-op and clear carbohydrate drink 8 hrs and 2 hrs pre-op (check with anaesthetist) | |
| Post op day 0 | Encourage oral intake as soon as able with aim to stop IVF early | |
| | Fluid balance as per team plan | |
| | Offer oral analgesia, laxatives and anti-emetics | |
| Day 1 | Out of bed for meals with aim for 4hrs chair and circulatory exercises | |
| | Encourage normal diet as tolerated with access to independent snack trolley | |
| | Oxycodone MR by 0800 to allow PCA to come down 1000<br>Paracetamol QID<br>Oxycodone IR as required (with laxatives) | |
| | walk supervised (aim for 20m) | |
| | walk supervised (aim for 40m) | |
| | Encouragement of deep breathing (described in physio sheet)<br>Refer physio if on-going O2 requirements or high risk | |
| | Weight | |
| | Start self-medication discussion | |
| Day 2 | Out of bed for meals with aim for 6 hours in chair | |
| | Encourage normal diet as tolerated with access to independent snack trolley | |
| | PCA down and regular oral analgesia | |
| | 60m walk | |
| | 60m walk | |
| | Encouragement of deep breathing | |
| | Weight | |
| | Self medication | |
| | Discharge discussion (clothes, family, medication, travel, follow-up) | |
| Day 3 | Change into home clothes | |
| | Catheter out if no concerns about bladder function | |
| | 6 hours in chair | |
| | 60m walk | |
| | 60m walk | |
| | 60m walk | |
| | Weight | |
| | Routine physio review | |
| | Self medication | |
| Day 4 | Aim for discharge with advice sheet, medication, information on phone numbers if concerns and anticipated follow-up calls | |
| | Weight | |
| | Complete self medication | |
| | Catheter out if anuria pre-op or high risk TWOC | |
| | Independent mobilisation +/- stair assessment | |

All suggestions for improvement welcome to Rachel.thomas@nhslothian.scot.nhs.net

Edinburgh Transplant Centre Recipient checklist v5 19th November 2020

**FIGURE 1 |** ERAS checklist.

validated and therefore we continued with individualised goal-directed fluid regimes to limit weight gain and reduce fluid overload that has negative impact on gut and graft function [1, 8].

Patients on the renal transplant waiting list and scheduled live donor transplant recipients were informed, and the updated recovery pathway was included in the pre-listing information briefing. Staff were updated through unit meetings and a local ERAS nurse delivered educational events to help with a smooth transition.

The outcomes from the first 35 consecutive renal transplants after introduction of ERAS (ERAS cohort, December 2020- February 2021) were compared to 35 consecutive recipients before the introduction (pre-ERAS cohort, June- November 2020). It is acknowledged that this comparison might be biased as ERAS had been discussed widely prior to implementation and our unit was already aiming for expedited discharge due to the pandemic. Therefore, an additional analysis of 35 consecutive pre-COVID-19 recipients were used for further LoS analysis (pre-COVID-19 December 2019-March 2020).

The median LoS for the 35 consecutive recipients on the ERAS pathway was 5 days, compared to 8 days (pre-COVID-19, $p = 0.008$) and 7 days (pre-ERAS or standard cohort $p = 0.050$) (Mann Whitney U test). The aim for day five discharge was met for 54% (19 patients) of the transplants compared to 17% pre-ERAS ($X^2$, $p = <0.001$) and only two pre-COVID-19 (5%, $X^2$, $p = <0.001$).

There was no evidence that the reduced LoS resulted in more readmissions within 30 days compared to either group (2 ERAS vs. 2 pre-ERAS vs. 7 pre-COVID-19) or more adverse events such as recatheterisation (1 vs. 4 vs. 3) or significant complications such as return to theatre (1 vs. 2 vs. 3).

There was no evidence that recipients in the ERAS group were less complex; they were older (median age 58 vs. 52 pre-ERAS vs. 53 pre-COVID-19, p = NS), had fewer live donor transplants (9 vs. 13 vs. 12, p = NS), less pre-emptive transplantation (5 vs. 9 vs. 7, p = NS) and were more obese (19 vs. 11 vs. 8 [BMI >30], $p = 0.02$). However, likely in keeping with the change in donor acceptance during COVID, there were equivalent DCD donors compared to pre-ERAS but more in pre-COVID-19 group (23% deceased donors vs. 21% vs. 39% but did not reach significance) and this may have impacted on LoS.

80% of ERAS patients met the goal of mobilisation on day one and were more likely to be discharged earlier from physio (median discharge day 3.6 ERAS vs. day 5.8 pre-ERAS). There was no significant difference in opiate use although a trend to less use (238 mg equivalent oral morphine dose vs. 266mg pre-ERAS). Unfortunately, two potential local analgesic methods (wound catheters and transversus abdominis plane (TAP) blocks), which can reduce opioid use and improve bowel and pulmonary function were not consistently available during this period [9].

Further analysis of those who did not meet the discharge target found that while some patients had graft issues that required longer inpatient stay for additional investigations or treatment, some (17%) could have been discharged earlier due to preventable issues such as medication issuing delay, earlier specialty referral and transport issues. This provided a focus for future improvements and recognises the need for system-wide buy-in to address all aspects of care. Of note, the lead author (and ERAS contact surgeon) left the unit and LoS was noted to have increased again to pre-ERAS levels (November 2022- February 23, median 8.5 days). While this increase is likely multifactorial, when reviewed, the ERAS checklist (**Figure 1**) was neither physically in the casenotes nor mentioned in electronic record for 70% (14/20) recipients, potentially underscoring the acknowledged benefit of a dedicated ERAS lead to drive the process, which is a challenge in an over-stretched health system.

Patients and staff satisfaction with ERAS was sought. All 35 ERAS patients were phoned for feedback and 56% responded. All who answered thought that their discharge was safe, had adequate analgesia and found physiotherapy input was helpful. 25/28 members of the MDT completed the written questionnaire with positive comments on analgesia, physio input and safety. 92% of staff and patients who completed the feedback recommended a continuation with the protocol in its current form.

We should also acknowledge that prehabilitation complements ERAS care and we aim to develop a prehabilitation programme, which may be funded by the savings associated with reduced LoS.

In conclusion, our implementation of ERAS for renal transplant was safe, feasible and acceptable, in line with results from other centres. We acknowledge that the reduction in length of stay may have been a multimodal response to practice change but the results from this retrospective, quality improvement project has encouraged development of pancreas and liver transplant recipient protocols and supported funding for a dedicated ERAS transplant nurse. ERAS guidelines for renal transplant are underway from the ERAS Society and NHS Blood and Transplant [10].

## 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

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

## AUTHOR CONTRIBUTIONS

RT, HU, HC, and GO devised and led this quality improvement project. SW, OP, ES-D, and MW collected supplementary data. HG performed statistical analysis. RT wrote the first draft of 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.

## GENERATIVE AI STATEMENT

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# Outscoring "Fire and Forget"? Current Practice of Lipid Management in Kidney Transplant Recipients

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**Keywords: LDL-C, hyperlipidaemia, mortality, kideny transplantation, cardiometabolic disease**

Dear Editors,

Kidney transplant recipients (KTR) have a very high cardiovascular risk and cardiovascular disease (CVD) is the major cause of death contributing to decreased life expectancy in this population [1]. Dyslipidemia is a major modifiable cardiovascular risk factor, and effective management can significantly improve patient outcomes [2]. The current 2013 KDIGO guideline advocates for statin therapy and recommends a "fire and forget" strategy without specification of low-density lipoprotein cholesterol (LDL-C) target levels [3]. In contrast, the European Society of Cardiology (ESC) guideline recommends a "treat to target" approach with LDL-C treatment goals based on risk stratification [4]. So far, evidence-based recommendations for optimal lipid management in the vulnerable KTR population are missing. In order to get a clear view on current practice in KTR lipid management, we conducted a survey among all transplant centers within the Eurotransplant (ET) network. Of 107 centers in total (n = 81 DACH region and n = 26 international), 18 centers responded (response rate 17%, with 13 German and 5 international ET-Centers). 61% (n = 11) of the responders reported having a standardized protocol for lipid management. The treatment goals for LDL-C varied significantly. In total, 77% reported a defined target range. 44% referenced the ESC guideline high risk treatment goals (**Figure 1**). The most common LDL-C target ranges were <55 mg/dL (1.4 mmol/L) and 70–100 mg/dL (1.4–1.8 mmol/L). Only 17% (n = 3) of centers had no specific treatment goal, as according to the KDIGO guidelines. A single center reported using individualized treatment goals that did not follow any guidelines or recommendations.

Therefore, our survey reveals striking heterogeneity in the management of dyslipidemia in KTRs. Based on the ambiguous recommendations of KDIGO and ESC and the lack of randomized controlled trials evaluating LDL-C target values in KTR, this is not surprising. Interestingly, our results suggest that many centers are moving towards targeted approach, congruent with the ESC guidelines. As a notable constraint, 39% (n = 7) of centers report to not have a standard protocol, indicating a substantial proportion of patients receive treatment dependent on physician's personal preferences.

We argue that KTRs deserve an optimal standardized therapy of their CV risk factors: Most of these patients have a years-long history of CKD and dialysis, which are associated with accelerated atherosclerosis and CVD remains the leading cause of death in KTR.

In the ALERT Trial, Fluvastatin showed effective risk reduction for cardiac death and non-fatal myocardial infarction while adverse events to statin therapy were not reported [5]. The results suggest that beneficial results of statin therapy from non-transplant cohorts are projectable to the KTR population. Our data underlines that most practitioners share this view, albeit studies comparing a targeted vs. non-targeted approach are missing.

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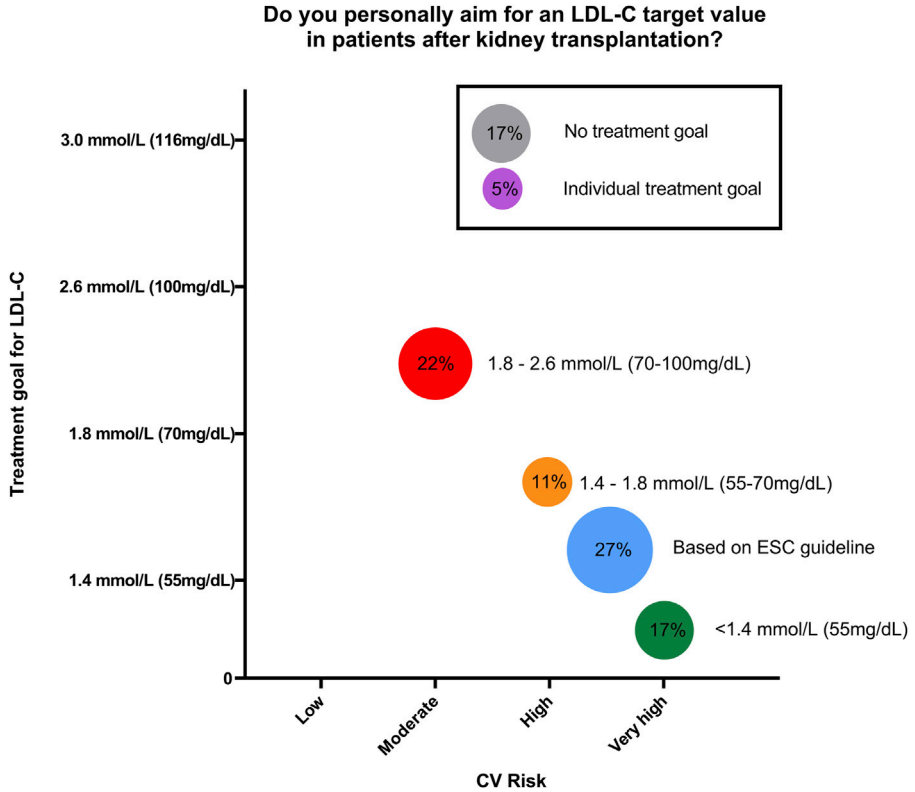


**FIGURE 1 |** Schematic representation of answers from Eurotransplant Kidney transplant centers to the question "Do you personally aim for an LDL-C target value in patients after kidney transplantation" (n = 18; circle size correlates with percentage).

Importantly, targeted therapy may require higher statin doses or combination therapy with ezetimibe, bempedoic acid and/or PCSK9-inhibitors, raising the question for cost-benefit analysis in this setting.

Taken together, there is significant variability in lipid management practices among transplant centers regarding LDL-C target values. Our survey did not capture patient outcomes nor detailed information on the lipid-lowering therapies used.

The majority of transplant centers does not stick to the "fire-and-forget" strategy advocated by the KDIGO but KTR lipid management is oriented at the ESC guidelines. Apparently, real-world KTR lipid management has outpaced the current KDIGO recommendations, underscoring the need for further research and updated nephrological guidelines for lipid management in KTR. Standardized protocols could improve patient outcomes across centers as the management of CVD remains the essential challenge in this high-risk population.

## DATA AVAILABILITY STATEMENT

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

## AUTHOR CONTRIBUTIONS

RS and LS drafted the survey, EA performed the survey and evaluated the results, EA and LS drafted the manuscript and the figure, RS, LS, and EA rewrote and finalized 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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# Impact of Era on Acute Cellular Rejection After Lung Transplantation

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**Keywords: lung, transplantation, acute cellular rejection, era, immunosuppression**

Dear Editors,

According to the International Society for Heart and Lung Transplantation (ISHLT) registry, survival after lung transplantation has improved significantly over time [1]. In addition, several randomized controlled trials have shown that improvements in maintenance immunosuppression are associated with a lower incidence of acute cellular rejection (ACR) [2, 3]. The ISHLT registry has also shown a modest decrease in the incidence of ACR between 2014 and 2018 (29.0% in 2014, and 27.3% in 2018) [4]. Clearly, ACR remains common despite advances in our understanding of mechanisms of rejection and immunosuppressive therapy. We conducted this study to assess the incidence of ACR and its risk factors over time.

This was a single-center, retrospective cohort study. Between 2009 and 2021, a total of 937 consecutive adult lung transplants were performed at Barnes-Jewish Hospital in St. Louis, Missouri. Among these, 773 patients were included in this study; re-transplants, multi-organ transplants, single lung transplants, and donation after circulatory death-donor transplants were excluded. These patients were stratified into 3 eras based on when they underwent transplantation: Era 1 (2009–2013), Era 2 (2014–2017), and Era 3 (2018–2021). The study protocol was approved by our center's Institutional Review Board (#202008193). All patients were treated with a triple drug maintenance immunosuppressive regimen after lung transplantation, including a calcineurin inhibitor (tacrolimus or cyclosporine), an antiproliferative agent (mycophenolate mofetil), and corticosteroids. Corticosteroids were initiated on postoperative day 0 at 1 g of methylprednisone daily for 3 days followed by 1 mg/kg prednisone (maximum of 40 mg) with a predetermined taper down to 5 mg by 3 months. Lung transplant recipients undergo surveillance bronchoscopy with transbronchial lung biopsies and bronchoalveolar lavage at 1, 2, 3, 6, and 12 months after transplantation. ACR was scored according to the standard ISHLT criteria [5] and defined as the occurrence of ACR grade ≥ A2 detected at any point during surveillance bronchoscopy within the first year after lung transplant. Cox proportional hazards modeling was used for univariate and multivariate analyses of risk factors for ACR, and all variables with $p < 0.05$ in univariate analyses were included in multivariate models.

There were increases of anoxia as a donor cause of death from 16.3% in Era 1–27.6% in Era 3 ($p = 0.02$) and distant donors from 43.3% in Era 1–74.5% in Era 3 (**Supplementary Table S1**, $p < 0.001$). In recent eras, patients have been older (median age: 56.0 in Era 1, 59.0 in Era 2, and 61.0 in Era 3, $p < 0.001$). The use of intraoperative cardiopulmonary bypass (CPB) in recent eras (Era 2 and 3) has

**Abbreviations:** ACR, acute cellular rejection; CI, confidence interval; CPB, cardiopulmonary bypass; HR, hazard ratio; ISHLT, International Society for Heart and Lung Transplantation.



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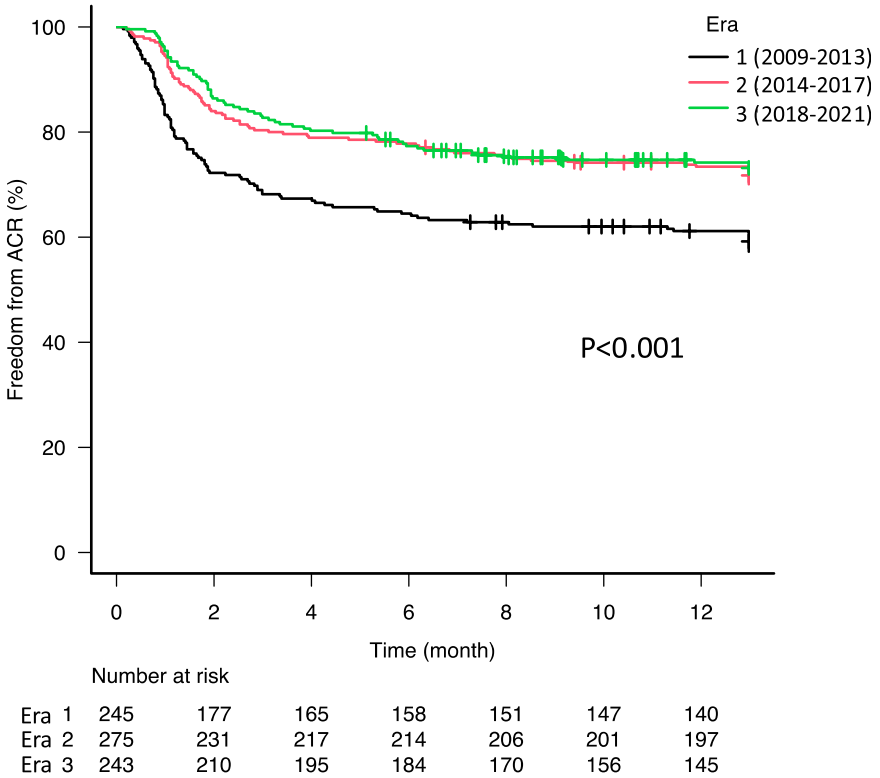


**FIGURE 1 |** Kaplan-Meier freedom from acute cellular rejection (ACR) curves stratified by era. The 1-year freedom from ACR was significantly higher in era 2 (70.1%) and 3 (72.2%) compared to era 1 (57.2%, $p < 0.001$).

decreased in comparison with the early era (Era 1), while the use of intraoperative extracorporeal membrane oxygenation and nitric oxide have increased over time from 0.4% to 36.6% ($p < 0.001$) and from 65.2% to 92.6% ($p < 0.001$), respectively. Of note, the proportion of patients who developed primary graft dysfunction grade 3 after lung transplantation has gradually decreased from 34.1% in Era 1–24.0% in Era 2, and 19.8% in Era 3 ($p < 0.001$). The use of basiliximab for induction immunosuppression increased over time from 73.9% in Era 1–93.0% in Era 3 ($p < 0.001$). The combination of tacrolimus and mycophenolate mofetil was the most commonly used maintenance immunosuppression regimen at discharge and increased in recent eras ($p < 0.001$). The incidence of ACR has decreased in recent eras (2014–2017 and 2018–2021) compared to the early era (2009–2013). Freedom from ACR at 1 year was 57.2% in Era 1, 70.1% in Era 2, and 72.2% in Era 3 (**Figure 1**, $p < 0.001$).

Multivariate Cox proportional hazards analysis showed that Eras 2 and 3 were associated with a decreased risk of ACR compared to Era 1 (**Supplementary Table S2**, hazard ratio [HR]: 0.602, 95% confidence interval [CI]: 0.449–0.808, $p = 0.002$, HR: 0.666, 95% CI: 0.477–0.924, $p < 0.001$, respectively). Although the number of lung transplants performed in patients with cystic fibrosis has drastically decreased over time (**Supplementary Table S1**), multivariate analysis revealed that cystic fibrosis was not a significant factor associated with the recent reduction in the incidence of ACR.

Previous studies have shown younger patients have been reported to be at increased risk in multiples studies [6]. Although over 70% of lung recipients are treated with basiliximab and its use has become more commonplace internationally according to the ISHLT Registry [1] the use of basiliximab was not associated with a lower risk of ACR in our study. Indeed, the superiority of basiliximab in comparison with thymoglobulin has not been demonstrated in other studies [7]. In contrast, a number of randomized controlled trials have demonstrated a lower risk of ACR in patients treated with tacrolimus compared to those treated with cyclosporine A [2, 8]. Small case series suggested that mycophenolate mofetil was superior to azathioprine in preventing ACR, but this finding has not been consistent in other studies [9]. Taken together, these data and our results suggest that recipient age and the more frequent use of tacrolimus may contribute to the decreased incidence of ACR in the more recent era.

This study has important limitations inherent to its design. This was a retrospective single-center study. However, the results are consistent with the ISHLT Registry. Nonetheless, it is difficult to make firm conclusions about the impact of the immunosuppressive regimen on ACR because of the retrospective design and categorizing patients based on their maintenance immunosuppression at the time of discharge from the index hospitalization and at 6 months after transplantation. Finally, it is possible that we did not account

for other potential factors that may influence the risk of ACR in this analysis, such as human leukocyte antigen mismatches, donor-specific antibodies, antibody-mediated rejection, and respiratory infections following lung transplantation.

Our findings demonstrate an improvement in the incidence of ACR after lung transplantation over time. Recent eras (2014–2017 and 2018–2021) were associated with a significantly lower risk of ACR. There were no significantly independent factors leading to the recent improvement in the incidence of ACR over time in this study. This is likely multifactorial, but we suspect that increasing recipient age, decreasing use of CPB, and the use of tacrolimus and mycophenolate mofetil in recent eras contribute to this lower risk of ACR.

## 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 studies involving humans were approved by Washington University School of Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because of the retrospective nature of the study.

## AUTHOR CONTRIBUTIONS

YT and TT contributed to the study design, data analysis, and manuscript writing. All authors contributed to the article and approved the submitted version.

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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.2025.14534/full#supplementary-material

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