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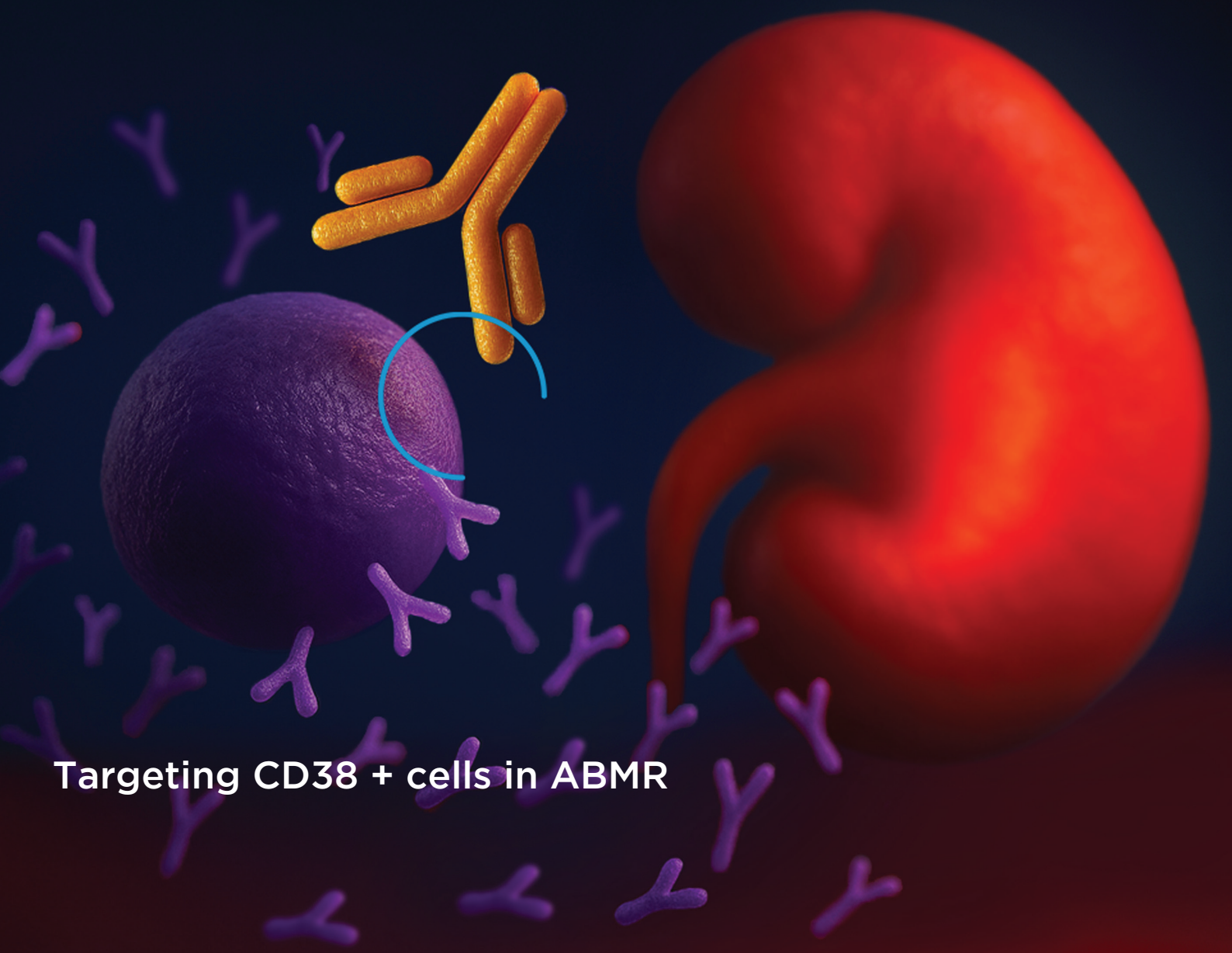

Targeting CD38 + cells in ABMR

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TRANSPLANT TRIAL WATCH
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Transplant Trial Watch

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

[1]Centre for Evidence in Transplantation, Nuffield Department of Surgical Sciences, University of Oxford, Oxford, United Kingdom, [2]University Hospitals Coventry and Warwickshire, Coventry, United Kingdom, [3]Oxford Transplant Centre, Churchill Hospital, Oxford, United Kingdom

Keywords: randomised controlled trial, liver transplantation (LT), kidney transplantation (KT), hypothermic oxygenated machine perfusion, immunosupression

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

RANDOMISED CONTROLLED TRIAL 1

Real-Time Biomarkers of Liver Graft Quality in Hypothermic Oxygenated Machine Perfusion.

by Zhylko, A., et al. Journal of clinical medicine 2025; 14(2): 13.

Aims

To determine whether lactate concentration measured in real time during hypothermic oxygenated machine perfusion (HOPE) of liver grafts can serve as a biomarker to predict post-transplant graft function and early clinical outcomes.

Interventions

Intervention Group (dHOPE): Liver grafts underwent dual hypothermic oxygenated machine perfusion for ≥120 min (portal vein + hepatic artery perfusion). Control (SCS): A separate arm of patients received conventional static cold storage. The paper's focus is on 26 grafts in the dHOPE arm. During perfusion, perfusate was sampled every 30 min for lactate, oxygen, and flavin mononucleotide (FMN) measurements-up.

Participants

Total Randomized Trial: 102 patients (26 allocated to dHOPE, 76 to standard cold storage), all receiving donor-after-brain-death (DBD) liver grafts. dHOPE Cohort Analyzed: 26 adult recipients meeting inclusion criteria (≥18 years old, informed consent). Median donor age was 53 years, and median recipient MELD was 12.

Outcomes

Primary Outcome: Predictive value of perfusate lactate (and FMN) for Early Allograft Dysfunction (EAD) – a standard measure of post-transplant graft function. Secondary Outcomes: Correlation of perfusate biomarkers with post-transplant hospital and ICU stay, peak liver enzymes, post-transplant complications (e.g., Clavien-Dindo grade ≥3 events), and other composite clinical scores (MEAF, L-GrAFT7)

Follow-Up

1 year posttransplantation.

Transplant Trial Watch

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***Correspondence**
John O'Callaghan,
ocallaghan.john@gmail.com
John Fallon,
john.fallon@nds.ox.ac.uk
Simon Knight,
simon.knight@nds.ox.ac.uk

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CET Conclusion

by John Fallon

The authors present analysis of a cohort of 26 patients within a single-centre RCT, the 26 patients received dHOPE and the control arm had standard care of SCS. The perfusate lactate during hypothermic preservation is utilised as a possible biomarker for transplant outcomes. Lactate assessment is feasible given it can be measured on a standard blood gas analyser, making it a quick and cheap biomarker compared with FMN, which requires a spectrofluorometer. Within the 26 patients they found lactate concentration in the perfusate after 120 min of dHOPE (≥3.45 mmol/L) predicted a significantly higher rate of EAD (67% vs. 6% below that threshold) and that elevated perfusate lactate correlated with longer hospital stays and higher peak transaminases, aligning with more severe graft dysfunction. Comparing lactate utility with FMN, which measured at 30 min also had predictive utility (AUC ~0.83). It seems lactate best discriminated EAD after a longer perfusion (2 h), presumably reflecting time-dependent metabolic changes.

The study is limited by small sample size (n = 26 in dHOPE) and primarily DBD donors with relatively low-risk characteristics, which for a UK recipient cohort is less translatable. In the centres which use end-ischemic HOPE, it typically starts when the transplant is already in progress, so perfusate-based decisions to accept/reject might be limited. The absolute lactate level could be confounded by large graft weight, making delta-lactate (accumulation over 2 h) potentially more informative. Finally, methodologically there was no formal blinding described, but objective biomarker endpoints reduce detection bias.

Overall, while a secondary analysis of a relatively small single-centre randomised study, it was prospective with clearly defined endpoints and a structured HOPE protocol. Their findings reinforce that real-time lactate during HOPE could help gauge graft quality, complementing or substituting more complex measurements (e.g., FMN). This concept should be taken forward into a future multicentre validation, especially in broader donor populations (e.g., DCD, more steatotic grafts) and with extended HOPE. In a device to donor setting this may provide timing predictive information to influence clinical decision making.

Trial Registration

ClinicalTrials.gov - NCT04812054.

Funding Source

Non-industry funded.

RANDOMISED CONTROLLED TRIAL 2

Induction of Immune Tolerance in Living Related HLA-Matched Kidney Transplantation: A Phase 3 Randomized Clinical Trial.

by Kaufman, D. B., et al. American Journal of Transplantation 2025 [record in progress].

Aims

This study aimed to investigate whether the MDR-101, a donor-derived cellular product, was able to induce immune tolerance compared to standard treatment in renal transplant patients.

Interventions

Participants were randomised to either receive MDR-101 or standard immunosuppression.

Participants

30 adult kidney transplant recipients from 2-haplotype human leukocyte antigen (HLA) –matched living siblings.

Outcomes

The primary efficacy endpoint was the proportion of patients that achieved functional immune tolerance. Other outcomes measured were quality of life, adverse events, and renal and metabolic function.

Follow-Up

36 months.

CET Conclusion

by Simon Knight

This interesting multicentre randomised study investigated the ability of a donor stem-cell derived cell therapy product (MDR-101) to induce clinical tolerance in recipients of HLA-matched sibling renal transplants. Recipients received rATG induction and low-dose lymphoid irradiation post-transplant, followed by MDR-101 cell therapy on day 11. During the first-year post-transplant, immunosuppression was gradually withdrawn until tacrolimus was stopped completely at 1 year in patients with evidence of mixed chimerism. 75% of patients (15/20) remained IS-free for 2 years with an acceptable safety profile. Although small (just 30 patients), this is a challenging study to undertake, and the results are impressive. Application is currently limited to 2-haplotype matched siblings, but the ability to deliver therapy post-transplant provides scope to extend to previously transplanted eligible recipients.

Jadad Score

3.

Data Analysis

Modified intention-to-treat analysis.

Allocation Concealment

Yes.

Trial Registration

ClinicalTrials.gov - NCT03363945.

Funding Source

Non-industry funded.

CLINICAL IMPACT SUMMARY

by John O'Callaghan

This is a well-written report of a very interesting clinical trial in renal transplantation. The results in terms of the successful withdrawal of immune suppression, and continued freedom from immune suppression, are very exciting.

The trial was conducted in a randomised, multicentre study, without blinding. The allogeneic cellular product "MDR-101" was tested in the induction of mixed chimerism and functional immune tolerance. Potential recipients were limited to those receiving their first transplant from a living related donor between 18 and 70 years. Donors were healthy adults with 2-haplotype HLA match with the recipient. Functional immune tolerance was defined as remaining off all immune suppressing drugs for at least 24 months, with no episodes of biopsy proven acute rejection, development of *de novo* DSA, GVHD, transplant loss of patient death. The sample size was small (20 patients in the study group and 10 patients in the control group) but this should not be taken as a criticism in this study.

A significant number of patients in the study group (19/20) established mixed chimerism for at least 6 months, this reduced to 56% by day 1095. Patients remained off immune suppression even if mixed chimerism was lost. At 24 months 15/20 patients in the study group were off immune suppression and 4/20 resumed immune suppression after complete withdrawal. The overall rates of adverse events were similar and there was no graft versus host disease.

The study significantly surpassed the FDA threshold for success, which was set at 48% functional tolerance for 2 years after withdrawal of immune suppression. There are limitations in terms of the selected patient population for this particular trial. However, with further testing and development, this study will mark a key point on the road towards transplantation without long-term immune suppression.

Clinical Impact

4/5.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

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

GENERATIVE AI STATEMENT

The authors declare that no Generative AI was used in the creation of this manuscript.

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REVIEW
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Targeting CD38 in Antibody-Mediated Rejection

Katharina A. Mayer[1], *Klemens Budde*[2], *Matthias Diebold*[1,3], *Philip F. Halloran*[4] *and Georg A. Böhmig*[1]*

[1]Division of Nephrology and Dialysis, Department of Medicine III, Medical University of Vienna, Vienna, Austria, [2]Department of Nephrology, Charité Universitätsmedizin Berlin, Berlin, Germany, [3]Clinic for Transplantation Immunology and Nephrology, University Hospital Basel, University of Basel, Basel, Switzerland, [4]Alberta Transplant Applied Genomics Centre, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada

Antibody-mediated rejection (AMR) remains a major challenge in clinical transplantation. Current therapies have yielded inconsistent outcomes, highlighting the need for innovative approaches. CD38, a multifunctional glycoprotein, is highly expressed on plasma cells and natural killer (NK) cells, potentially offering a dual mechanism of action that could intervene in the pathophysiologic course of AMR: depleting alloantibody-producing plasma cells and NK cells. This review focuses on recent results from CD38-targeted therapies, with felzartamab emerging as a promising option. Previous case reports and series suggested that off-label daratumumab treatment could effectively reverse AMR. Felzartamab has now demonstrated safety and efficacy in a phase 2 trial for late AMR. Reductions in microvascular inflammation, downregulation of rejection-associated transcripts, and decreases in donor-derived cell-free DNA paralleled a substantial decrease in NK cell counts. However, felzartamab did not significantly affect donor-specific antibodies, which may reflect its distinct mechanism of action, primarily involving antibody-dependent cellular cytotoxicity and phagocytosis. The effects on rejection activity may have a rapid onset, but are transient. The potential benefits of prolonged therapy are currently being investigated in a recently launched phase III trial. Future studies may expand the applications of CD38 targeting to early AMR or broader indications, such as DSA-negative microvascular inflammation.

Keywords: antibody-mediated rejection, CD38, natural killer cells, kidney transplantation, treatment

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***Correspondence**
Georg A. Böhmig,
georg.boehmig@meduniwien.ac.at

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INTRODUCTION

Antibody-mediated rejection (AMR) after kidney transplantation is a major clinical challenge [1, 2], often leading to chronic injury and declining graft function, which contributes to poor graft survival [3–5]. Despite advances in immunosuppression, AMR remains a frequent cause of allograft failure [6, 7], and there are currently no approved therapies, highlighting the urgent need for effective treatments [8, 9].

During the pathophysiologic course of AMR, donor-specific antibodies (DSA) arise from alloantigen-specific T cell-dependent B cell activation. This process generates a reservoir of donor-specific memory B cells and/or plasma cells [10, 11]. DSA produced by plasma cells may bind to HLA molecules on the surface of the allograft endothelium, inducing microvascular inflammation (MVI), the histologic hallmark lesion of AMR [12]. Beyond complement activation, DSA can also mediate graft damage through direct signaling and Fc-mediated

effector mechanisms, including the binding of the Fc portion of DSA to Fc receptors (FcR) on innate immune cells, such as $CD16^+$ natural killer (NK) cells and monocytes/macrophages [10, 11, 13, 14].

Over recent decades, research into novel therapies for AMR has focused on identifying targets to reduce DSA levels and impair plasma cell function. However, controlled studies investigating treatments that target various aspects of B cell and plasma cell immunity have failed to demonstrate clear therapeutic benefits [8, 9]. This is particularly evident for late (active and chronic active) AMR, where interventions aimed at plasma cell generation and/or integrity (e.g., interleukin-6 blockade [15] or proteasome inhibition [16]), DSA removal (e.g., immunoglobulin G degradation using imlifidase [17]), complement inhibition [18], or depletion of early B cell populations (e.g., rituximab in combination with intravenous immunoglobulin [19]) have not produced convincing outcomes. According to the Transplantation Society Working Group, optimizing baseline immunosuppression is the primary recommendation for late AMR, while apheresis combined with intravenous immunoglobulin remains the standard for early active AMR, despite limited evidence [8].

Recently, novel treatment strategies have emerged, with the most promising being the targeting of CD38 via monoclonal antibodies, as supported by the positive results from a recent phase 2 trial of the CD38 antibody felzartamab [20, 21]. The application of CD38 antibodies has broadened therapeutic options beyond plasma cell-directed therapies to a multifaceted strategy that includes simultaneous depletion of plasma cells and innate effector cells [20–24].

In this review, we summarize the current evidence supporting monoclonal CD38 antibodies as a novel therapeutic option for AMR and discuss their potential future applications, including HLA desensitization.

CD38 – A MULTIFUNCTIONAL MOLECULE

The complexity of CD38 is detailed in recent reviews, emphasizing its diverse and often poorly understood biological roles, such as in infection defense, chronic inflammation, and autoimmunity [25]. Immunologically, CD38 regulates cell differentiation, proliferation, cytokine release, apoptosis, phagocytosis, chemotaxis, and transmigration, the latter potentially involving selectin-like binding of hematopoietic cells to endothelial cells via CD31 [25]. CD38 is a non-lineage-restricted, single-chain transmembrane glycoprotein comprising 300 amino acids with a molecular weight of 45 kDa. It lacks an internal signaling domain and is encoded in humans on chromosome 4. First identified in the early 1980s, CD38 was initially described as a surface protein on T cells capable of inducing cell activation [26]. CD38 is constitutively expressed and upregulated upon activation in various immune and hematopoietic cells (T cells, B cells, NK cells, dendritic cells, etc.) and precursors. CD38 is also found in tissues like the prostate, pancreas, smooth muscle, kidney, gut, and brain [25].

Over recent years, CD38 has gained attention as a marker and therapeutic target in hematopoietic malignancies, particularly multiple myeloma [27]. In organ transplantation, its high expression on plasma cells and NK cells suggests a dual mechanism for CD38-targeting antibodies in immune cell depletion [28]. However, this view may oversimplify the complex physiology of CD38. Such antibodies might also modulate enzymatic activity or affect the function and activation of other immune cell subsets, including regulatory cells.

Interestingly, CD38 shares striking molecular similarity with an enzyme from the mollusk *Aplysia californica*. This resemblance has led to its identification as a NAD-depleting ectoenzyme with ADP-ribosyl cyclase and hydrolase activities. CD38 converts nicotinamide adenine dinucleotide (NAD^+) into nicotinamide and cyclic ADP-ribose (cADPR), which is then hydrolyzed to ADP-ribose (ADPR), but its enzymatic function has turned out to extend much farther [29, 30]. Expressed on cell surfaces and in intracellular compartments, CD38 has two orientations: type II and type III, the latter positioning the catalytic domain toward the cytosol and implicating CD38 in intracellular NAD^+ regulation, vital for mitochondrial function and metabolism. Its enzymatic activity also links CD38 to intracellular signaling, as ADPR and cADPR serve as second messengers that regulate Ca^{2+} levels. This enzymatic versatility highlights potential roles in health and disease, with NAD homeostasis changes contributing to various pathologies [25, 31, 32].

Of particular relevance to AMR, where NK cells have recently garnered interest [10, 11, 14], is the role of CD38 as a receptor that regulates NK cell cytokine release and cytotoxicity [33, 34]. Experiments with interleukin-2-activated NK cells revealed that ligation of CD38 with agonistic monoclonal antibodies significantly increased intracellular Ca^{2+} levels and induced tyrosine phosphorylation of cytoplasmic substrates, resembling activation via FcγRIIIA (CD16) [34]. CD38 engagement also elevated HLA class II and CD25 expression, promoted IFN-γ and granulocyte-macrophage colony-stimulating factor release, and enhanced cytolytic effector functions against target cells [34]. A series of experiments showing surface proximity between CD38 and CD16 suggests that signaling via CD38—despite lacking the canonical receptor structure—is enabled by functional and physical associations with another professional signaling structure, such as CD16 in NK cells [35, 36].

Monoclonal CD38 Antibodies–Applications in Organ Transplantation and Beyond

Several CD38 antibodies have been developed for the treatment of multiple myeloma, where they have been an established option with an acceptable safety profile for many years [37]. Recently, three CD38 antibodies—felzartamab, daratumumab, and isatuximab—have been tested in organ transplantation, particularly for the treatment of AMR and desensitization in broadly HLA-sensitized recipients.

Felzartamab (MOR202; IgG1λ) has shown efficacy in relapsed or refractory multiple myeloma [38], and is now being developed

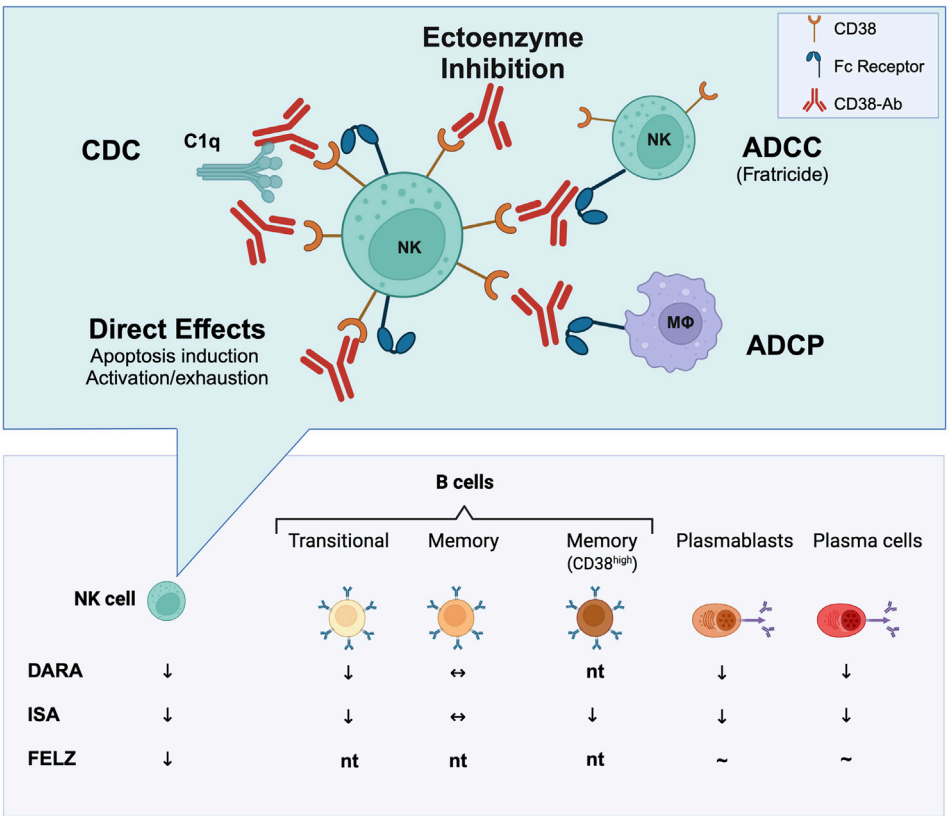

FIGURE 1 | Immune cell depletion through CD38 targeting. In transplant studies (e.g., rejection treatment or recipient desensitization), CD38 antibodies such as daratumumab (DARA), isatuximab (ISA), and felzartamab (FELZ) have been shown to deplete various immune cell (sub)types in peripheral blood, bone marrow and/or transplanted kidneys, most consistently natural killer (NK) cells. Isatuximab, in particular, has demonstrated the ability to deplete $CD38^{+}$ memory B cells, plasmablasts, and plasma cells. Conversely, felzartamab has not been convincingly shown to deplete CD138-positive cells in peripheral blood, though its effects on distinct B cell subsets and plasma cells in the bone marrow have not yet been tested (nt). CD38 antibodies may exert functional effects through multiple mechanisms. For NK cells—key effector cells involved in antibody-mediated rejection—these mechanisms potentially include inhibition of the ectoenzymatic function of CD38 or depletion of target cells through Fc-mediated processes. These processes encompass antibody-dependent cellular cytotoxicity (ADCC), leading to NK cell fratricide; antibody-dependent phagocytosis (ADCP); complement-dependent cytotoxicity (CDC) via the attachment of the key complement component C1q; as well as apoptosis or NK cell exhaustion upon activation. Created with Biorender.com.

for autoimmune diseases, including membranous nephropathy [39] and IgA nephropathy (ClinicalTrials.gov identifier, NCT05065970), as well as AMR in kidney transplants, with encouraging results in a phase II trial [20, 21].

Daratumumab (IgG1κ), the first monoclonal CD38 antibody approved for multiple myeloma treatment [40, 41], has been explored off-label in AMR, with several case reports and series published to date [42–50]. In addition, studies have been conducted in transplant recipient desensitization, and outside transplantation, such as in autoimmune diseases [51–53]. A recently proposed indication in the transplant setting may be FSGS recurrence, as suggested by recent case series in which daratumumab, was successfully used in combination with CD20 antibodies rituximab or obinutuzumab [54, 55].

Isatuximab (SAR650984; IgG1κ) was developed for multiple myeloma [56]. It has since been studied in transplant recipient desensitization [57, 58], though no data currently exist on its use in established AMR.

The CD38 antibody CID-103 was recently considered for AMR treatment but placed on clinical hold by the FDA. Mezagitamab, is being investigated for systemic lupus erythematosus [59] and IgA nephropathy (ClinicalTrials.gov identifier, NCT05174221), with a phase 3 trial for ITP underway (NCT06722235). Additionally, CM313 has demonstrated rapid platelet count increases in ITP by inhibiting ADCC on platelets while maintaining long-term efficacy via plasma cell clearance [60]. However, none of these antibodies are currently being evaluated in the organ transplant context.

Molecular and Cellular Effects of Targeting CD38

Analyses of CD38 antibody mechanisms, primarily from preclinical myeloma models suggest that target cell depletion involves Fc-dependent immune effector processes, including

complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent phagocytosis (ADCP) [27] Additional mechanisms may include interference with CD38's ectoenzymatic activity, apoptosis induction, or triggering CD38-dependent activation, potentially affecting immune cells like NK cells, where overactivation may cause exhaustion or cell death. Possible modes of action of CD38 antibodies, illustrated using NK cells as target cells, are shown in **Figure 1**. Different CD38 antibodies use distinct mechanisms. Felzartamab primarily mediates ADCC and ADCP, with minimal CDC or apoptosis [61, 62], whereas daratumumab is the most effective CDC inducer [27]. The relative contribution of direct apoptosis induction may also vary, with isatuximab showing the strongest pro-apoptotic activity [63]. Importantly, the lysis of target cell depends on the levels of CD38 expression. Beyond that, CD38 expression levels can determine the molecular mechanisms primarily underlying induction of cell death [64].

In transplant settings, daratumumab and isatuximab, as shown in **Figure 1**, have demonstrated effects on various components of the B cell-driven alloimmune response, depleting plasmablasts, plasma cells, transitional B cells, and memory B cell subsets [44, 58]. These effects likely explain the observed modest reductions in alloantibody levels, consistent with similar findings in daratumumab case studies and series [42–50]. In contrast, felzartamab's impact on B cell immunity is less defined. A phase 2 trial in late AMR suggested it may not significantly reduce HLA antibody levels [20], but detailed phenotypic and functional studies are awaited.

CD38 monoclonal antibodies rapidly reduce peripheral blood NK cell counts [65], potentially via complement-mediated lysis, NK cell activation leading to exhaustion, and/or FcγRIIIA-mediated fratricide [66]. For isatuximab, transcriptome analyses of NK cells cocultured with myeloma cells revealed deregulated expression of 70 genes linked to chemotaxis, cytolysis, and defense response, reflecting activation via Fc binding and CD38 transmembrane signaling. Labeled NK cell experiments suggested activation followed by exhaustion, rather than fratricide, partially drives NK cell depletion post-isatuximab. Additional studies suggested CD38/SLAMF7-mediated phagocytosis by M2-like macrophages [64]. Whether similar mechanisms apply to felzartamab remains unclear.

CD38 antibodies bind distinct epitopes, leading to variations in their capacity to inhibit CD38's enzymatic function [27, 31]. This is relevant, as inhibition of the CD38 ectoenzymatic domain has recently been linked to improved T cell metabolic fitness and enhanced T cell cytokine production [67]. The absence of enzymatic inhibition by felzartamab may offer a therapeutic advantage in transplantation by potentially lowering the risk of T cell-mediated rejection (TCMR), a concern linked to other CD38 antibodies like daratumumab [42, 68]. Moreover, CD38 antibodies, including daratumumab, may deplete regulatory B and/or T cells, potentially driving T cell expansion [69]. However, the immunologic consequences of these effects in transplantation remain uncertain. A reported case of early severe TCMR in a kidney transplant recipient treated with daratumumab for myeloma before transplantation underscores this concern [68]. However, in the felzartamab trial transcriptome analyses did not reveal exacerbated TCMR under treatment [20, 70]. Nonetheless, one of the 24-week follow-up biopsies revealed tubulo-interstitial infiltrates classified as Banff IA TCMR after 6 months of treatment. This finding mirrors discrepancies seen with daratumumab, where T cell infiltration occurred despite a negative molecular TCMR score on the Molecular Microscope platform [44].

It remains unclear how differences between CD38 antibodies affect their pharmacodynamic utility, efficacy, and safety in AMR, including risks of infection, malignancy, or TCMR. It is also uncertain whether these differences influence the primary mechanism of action, such as NK cell versus plasma cell depletion.

Rationale Behind Targeting CD38 in AMR?

The rationale for using CD38 antibodies in AMR lies in the strong expression of CD38 on plasma cells, key producers of alloantibodies. Depleting plasma cells with CD38 antibodies may reduce DSA levels, mitigating rejection. A nonhuman primate study by Kwun et al. [42] showed that rhesus macaques sensitized through sequential skin grafts and treated with daratumumab (combined with plerixafor/anti-CXCR4) had significantly reduced DSA levels and prolonged renal graft survival. Clinically, daratumumab reduced HLA antibodies and improved AMR outcomes in a combined heart/kidney transplant recipient and a highly sensitized heart transplant candidate, facilitating heart graft access [42].

A second rationale for targeting CD38 in AMR, supported by Doberer et al. [44] involves the effect of CD38 monoclonal antibodies on NK cells. This was further confirmed in a phase 2 trial of felzartamab for late AMR, where no meaningful reduction in DSA levels was observed [20]. The effect on NK cell counts is significant, as NK cells are involved in AMR, with studies showing their prevalence in capillaries and association with AMR-related transcripts [71, 72]. Functional polymorphisms in NK cell receptors, such as FcγRIIIA, have also been linked to microvascular inflammation (MVI) in the presence of DSA [73, 74]. NK cell abundance has been identified as a strong predictor of graft outcomes [75]. With rodent studies showing that NK cell depletion can reduce DSA-triggered graft injury [76].

First Clinical Results of CD38 Targeting in AMR

In recent years, two CD38 monoclonal antibodies, felzartamab and daratumumab, have been used off-label or, in the case of felzartamab, systematically evaluated in AMR through a clinical trial. The results obtained provide an initial look at the potential of these treatments for this complex condition. **Table 1** summarizes anecdotal cases, case series, and both completed and ongoing trials. The promising findings, including those from a recently published phase 2 trial in late-stage AMR, will be discussed below.

TABLE 1 | CD38 targeting in AMR after organ transplantation.

First author, year (ref)	Identifier[a]	CD38 antibody (schedule)	Design	Patients (organ)	AMR phenotype	Key results
Case reports and case series						
Kwun [42]	-	Daratumumab (8 weekly infusions; second 4-month course) plus eculizumab	Case report	1 (heart/kidney)	Rerfractory late AMR plus TCMR (PC-predominant infiltration) Preformed/*de novo* HLA-DSA	Reduction of AMR activity and DSA MFI Depletion of circulating PC, decrease in PC infiltrate Reversal of graft dysfunction Recurrence of rejection after a first treatment course; response to a second course
Spica [43]	-	Daratumumab (6 weekly infusions)	Case report	1 (kidney)	Refractory early AMR ABO-Ab+	Reversal of AMR and ABO Ab reduction Reversal of graft dysfunction
Doberer [44]	-	Daratumumab (IV, 9 months)	Case report	1 (kidney)	Late chronic active AMR HLA-DSA+	Reversal of (histologic/molecular) AMR & DSA reduction Depletion of circulating/intragraft NK cells and bone marrow PC Stabilization of renal function
Süsal [45]	-	Daratumumab (SC, four doses) plus immunoadsorption	Case report	1 (kidney)	Early AMR ABO-Ab+/HLA-DSA+	Reversal of AMR morphology and DSA/anti-A blood group antibody reduction Reversal of graft dysfunction
Zhu [46]	-	Daratumumab (IV; 2–3 months weekly plus PP/IVIG; followed by maintenance with daratumumab alone)	Case series	2 (kidney)	Refractory late/chronic AMR	Resolution of AMR activity (follow-up biopsy in one patient) & DSA reduction Stabilization of renal function Development of TCMR in one patient
De Nattes [48]	-	Daratumumab (IV, 1-weekly/7 weeks); following 1 week immunoadsorption	Case report	1 (kidney)	Early AMR after desensitization HLA-DSA+	Reversal of (histologic/molecular) AMR activity & DSA reduction Stabilization of renal function
Lemal [47]	-	Daratumumab (IV, single dose) Plus PP ± IVIG	Case series	3 (kidney)	Active AMR HLA-DSA+	Resolution of AMR activity and DSA reduction Reversal of graft dysfunction
Vicklicky [50]	-	Daratumumab (SC; 11 injections over 6 months)	Case report	1 (kidney)	Early AMR (subclinical) after desensitization HLA-DSA+	Reversal of (histologic/molecular) AMR & DSA reduction Decrease in dd-cfDNA
Osmanodja [49]	-	Daratumumab (IV; 6–9 months	Case series	2 (kidney)	(Refractory) chronic active AMR HLA-DSA+	Histologic resolution of AMR & DSA reduction Decrease in dd-cfDNA Depletion of circulating NK cells
Guo [77]	-	Daratumumab (IV; 6–19 months), followed by tocilizumab	Case series	7 (kidney)	Late AMR (mixed rejection: n = 5) HLA-DSA+	Stabilization of renal function; reduction in i-IFTA, partial remission of MVI in 4/6 patients at 24–48 months
Systematic trials						
Mayer [20]	NCT05021484	Felzartamab (IV; 0–20 weeks)	Phase 2 trial, randomized, placebo-controlled	22 (kidney)	Late AMR HLA-DSA+	Primary outcome (safety and tolerability): Acceptable safety profile; felzartamab: 8/11 patients with infusion-related reactions Secondary endpoints: Reduction of morphologic/molecular AMR activity (resolution of histologic AMR activity in 9/11 versus 2/11 subjects); NK cell depletion/no effect on DSA levels; dd-cfDNA reduction. (Partial) recurrence of AMR after cessation of a 6-month treatment course
Ongoing trials registered at ClinicalTrials.gov						
-	NCT05913596	Daratumumab (IV; 0–22 weeks)	Single-arm	15	Chronic active AMR HLA-DSA+	Recruiting (Primary outcome measure: percent change in DSA levels)

(Continued on following page)

TABLE 1 | (*Continued*) CD38 targeting in AMR after organ transplantation.

Case reports and case series						
First author, year (ref)	**Identifier[a]**	**CD38 antibody (schedule)**	**Design**	**Patients (organ)**	**AMR phenotype**	**Key results**
-	NCT06685757	Felzartamab (IV; up to 12 months)	Phase 3 part 1: 6 months placebo-controlled; part 2: open label)	120	Late AMR HLA-DSA+	Recruiting (Primary outcome measure: percentage of participants who achieve biopsy-proven histologic resolution)

Abbreviations: ABO-Ab, ABO, blood group antibody; AMR, antibody-mediated rejection; dd-cfDNA, donor-derived cell-free DNA; DSA, donor-specific antibody; IV, intravenous; IVIG, intravenous immunoglobulin; MFI, mean fluorescence intensity; NK, natural killer cell; PC, plasma cells; PP, plasmapheresis; SC, subcutaneous; TCMR, T cell-mediated rejection.
[a]ClinicalTrials.gov identifiers.

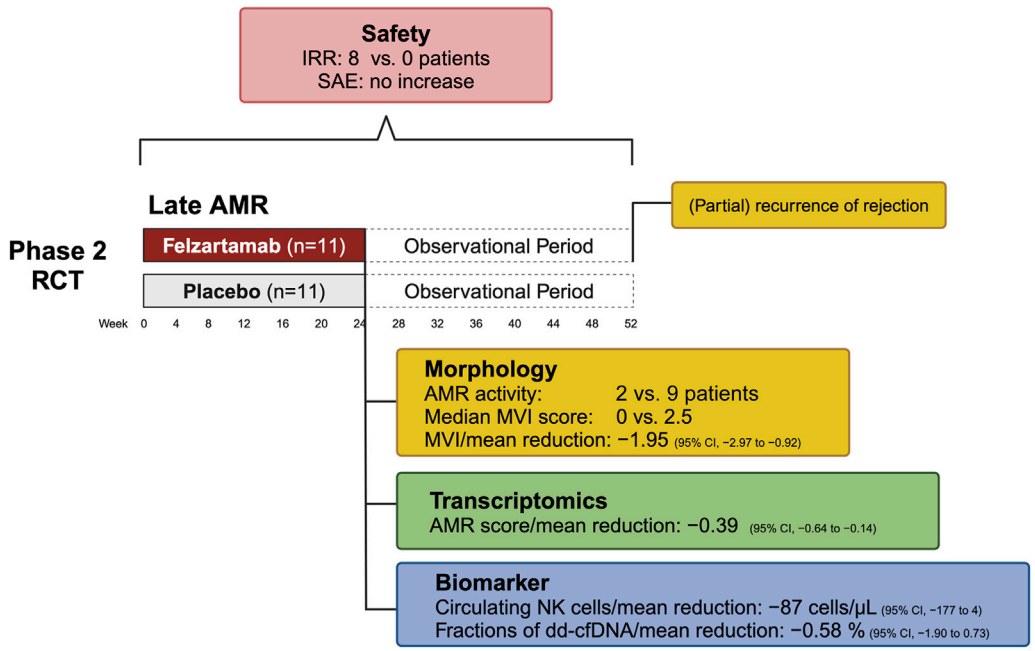

FIGURE 2 | Key results from the phase 2 trial of felzartamab in late antibody-mediated rejection. Abbreviations: AMR, antibody-mediated rejection; CI, confidence interval; dd-cfDNA, donor-derived cell-free DNA; IRR, infusion-related reactions; MVI, microvascular inflammation; NK cell, natural killer cell; RCT, randomized controlled trial.

Felzartamab in Late AMR

A recent randomized, placebo-controlled phase II pilot trial evaluated a six-month treatment course of the CD38 antibody felzartamab versus placebo in late DSA positive AMR ≥180 days after kidney transplantation, demonstrating significant reductions in AMR activity as observed in 24-week follow-up biopsies [20]. Key secondary outcomes underscored felzartamab's effectiveness, with 82% of patients achieving resolution of AMR activity by week 24, compared to 20% in the placebo group, alongside notable reductions in MVI scores [20]. Major results of the felzartamab trial are shown in **Figure 2**.

Transcriptomic studies showed that felzartamab consistently reduced molecular AMR activity scores by selectively suppressing interferon gamma-inducible and natural killer cell transcripts, with minimal effects on AMR-induced endothelial transcripts [70]. However, while therapy reduced AMR activity in all subjects who had pretreatment activity, the suppression was often incomplete in those with very high activity. While MVI recurred in only a subset of patients at week 52, molecular recurrence was nearly universal after treatment discontinuation. Interestingly, molecular analyses indicated that felzartamab provided sustained parenchymal benefits, slowing the progression of molecular injury even after the treatment period [70]. Importantly, resolution of AMR activity was linked to rapid and substantial reductions in donor-derived cell-free DNA (dd-cfDNA) —a marker of ongoing allograft injury—though levels approached baseline after treatment cessation [20].

While the results suggest that targeting CD38 may have the potential to slow the progression to kidney failure, the trial was not powered to assess the impact of felzartamab on long-term graft outcomes [78]. However, preliminary data, including an

apparent stabilization of eGFR slope, hinted at potential clinical benefit [20]. An analysis of the iBox prognostication system designed to predict death-censored allograft survival [79] revealed that the probability of graft survival decreased by 7.6% per year in the placebo arm, and increased by 6.4% in the felzartamab arm [80]. Remarkably, in an analysis evaluating the two trial periods (6 months treatment; 6 months observation) this treatment effect was observed only during the exposure period [80]. However, it should be noted that the actual value of the iBOX scoring system, or similar models, in predicting survival for such a new intervention, needs to be validated using real survival data.

The treatment with felzartamab was associated with an 80% reduction in $CD16^{bright}$ NK cells and 20%–30% decreases in immunoglobulin levels, though no significant changes were observed in immunodominant DSA levels [20]. While causality has yet to be confirmed, these findings suggest that depleting FcR-expressing NK cells may disrupt the pathogenic effects of DSA in the allograft microvasculature, potentially mitigating DSA-mediated injury. Despite the trial's limited sample size and short treatment duration, the results are promising, especially when compared to previous trials like the IMAGINE study, which was prematurely halted due to lack of clinical efficacy (ClinicalTrials.gov identifier, NCT03744910). From a commercial perspective, the CD38 antibody landscape is highly competitive, with several therapies targeting plasma cells or immunoglobulin kinetics. However, felzartamab's distinct mechanism—focusing on $CD38^+$ FcR-expressing NK cells and to a lesser extent $CD38^+$ plasma cells—could provide a unique clinical differentiation.

Felzartamab demonstrated an acceptable safety profile [20]. Adverse events were more frequent in the felzartamab group (119 events) compared to the placebo group (81 events), though serious adverse events were less common in the felzartamab group (9% vs 36%). Mild to moderate infusion-related reactions (IRR) occurred in 73% of felzartamab-treated patients, despite premedication. Infections were more common in the felzartamab group (91%) than in the placebo group (64%), with nasopharyngitis being the most frequent. However, no serious infection-related adverse events were observed. Additionally, no safety signals were seen regarding COVID-19, with only mild or moderate infections in the felzartamab arm, while the placebo arm had two COVID-19-related serious adverse events [20]. Interestingly, a previous report in membranous glomerulopathy showed robust immune responses in felzartamab-treated patients following SARS-CoV-2 vaccination [81].

Anti-drug antibodies (ADA) were not observed [20, 82]. It is worth noting that felzartamab has primarily been studied in European populations, and potential racial differences in ADA development warrant further exploration [82].

Felzartamab - Regulatory Designation and Future Prospects

Felzartamab received Orphan Drug Designation for AMR treatment from the U.S. Food and Drug Administration (FDA) in March 2024 and the European Commission in December 2024. The FDA also granted Breakthrough Therapy Designation in October 2024. In a phase 2 trial, felzartamab showed an acceptable safety profile and promising efficacy, though AMR recurrence occurred after therapy discontinuation. Ongoing studies are exploring longer or individualized treatment regimens (based on dd-cfDNA monitoring), with a phase 2 extension trial (NCT05021484) assessing repeated felzartamab courses in patients with recurrent or persisting AMR. Further confirmation of its safety and efficacy in larger patient cohorts is needed. Additionally, a phase 3 trial (TRANSCEND) in late AMR has been launched in the US and is expected to start in Europe (NCT06685757).

Daratumumab in AMR–Anecdotal Cases, Case Series, and Ongoing Trials

A series of case reports and small case series highlight the efficacy of CD38 targeting in managing AMR after kidney transplantation in diverse scenarios, including early, late, and treatment-refractory rejection (**Table 1**). Kwun et al. [42] reported a case of DSA-positive AMR following kidney and heart transplantation, showing reductions in both AMR activity and DSA-MFI. Spica et al. [43] reported successful resolution of early AMR after ABO-incompatible transplantation, with a notable decrease in anti-A blood group antibodies. In a case of chronic active AMR, Doberer et al. [44] demonstrated persistent resolution of rejection activity, accompanied by plasma cell and NK cell depletion as well as reduced DSA levels and altered DSA production by antibody secreting cells isolated from bone marrow aspirates. Süsal et al. [45] described early, DSA-positive AMR occurring 5 days post-ABO and HLA-incompatible transplantation, again with reductions in anti-A blood group titers and DSA levels. Zhu et al. [46] presented the course of daratumumab plus PP/IVIG in two patients with refractory chronic active AMR showing reduced DSA-MFI, while Lemal et al. [47] reported AMR resolution and DSA-MFI reductions in three AMR cases. In another case, de Nattes et al. [48] reported on a sensitized patient who after successful transplantation under desensitization showed AMR in a 3-month biopsy. Rejection was successfully reversed using daratumumab, as also supported by molecular analysis [48]. Viklicky et al. [50] described histologic and molecular AMR resolution with decreased DSA-MFI, and Osmanodja et al. [49] reported reductions in AMR activity, NK cell depletion, and moderate DSA-MFI reductions in two cases of refractory DSA-positive AMR. In the latter two reports, also a substantial decrease in dd-cfDNA levels was documented [49, 50]. These findings collectively underscore the potential of CD38 targeting in resolving histologic (and as shown in two cases also molecular) AMR activity and mitigating immune-mediated injury. While not proven in a rigorous study, daratumumab may affect alloantibody levels to a certain degree. Given the proposed deregulation of T cell immunity it is notable that clinically relevant TCMR was reported in one of the reported cases [46], while in another case subclinical $CD3^+$ T cell infiltrates were noted but not associated with molecular TCMR-related transcript sets [44]. A prospective

open-label, single-arm trial of daratumumab in DSA-positive chronic active AMR, with DSA MFI reduction as the primary outcome, is underway in China (ClinicalTrials.gov identifier: NCT05913596) (**Table 1**). Finally, a recent case series including seven patients with late or chronic active AMR suggests the potential of sequential therapy with CD38 mAb followed by tocilizumab to enhance DSA reduction [77].

While the aforementioned anecdotal reports and case series provide interesting initial insights, we emphasize that systematic trials will ultimately be necessary to establish the efficacy of daratumumab in AMR, whether as monotherapy or in combination with other therapies. Such studies may clarify the ability of daratumumab to decrease DSA, further assess its safety—particularly its potential role in triggering TCMR (which was not a safety issue with felzartamab in the phase 2 trial)— and determine the clinical significance of differences in the mechanisms of action among CD38 antibodies. Variations in efficacy or safety could stem from differing effects on immune cell subpopulations due to variable complement-fixing ability or ectoenzyme interference in relation to ADCC or ADCP induction.

CD38 ANTIBODIES FOR DSA- AND C4D-NEGATIVE MVI?

In the Banff 2022 scheme, a distinct MVI subcategory, that is, MVI, C4d-negative and DSA-negative, was defined [12]. Gene expression patterns associated with this phenotype were found to be similar to those observed for AMR [83]. However, in addition to potential antibody-triggered mechanisms, including non-HLA specificities, mechanisms independent of DSA—such as missing-self NK cell activation or alloantigen-dependent NK or monocyte activation—have been proposed [10]. Given the potential key role of NK cells in these cases, one might speculate that CD38-targeting therapy could be beneficial, where other treatments may be less effective. As an example, a recent cohort study suggested that in DSA-MVI, tocilizumab, in contrast to DSA + rejection, failed to modify the course of eGFR, possibly due to persistent NK cell activity [84]. Future studies may be of interest to explore whether CD38 targeting could be effective in such cases.

CD38 ANTIBODIES FOR THE PREVENTION OF AMR IN PRE-IMMUNIZED PATIENTS?

CD38 antibodies may aid in the transplantation of highly immunized patients in two ways. First, depletion of HLA antibody-producing plasma cells can be expected to gradually decrease the levels of preformed deleterious alloantibodies and increase the chance to receive a suitable organ. On the other hand, pre-emptive depletion of the effector cell population ($CD38^+$ NK cells) may also be clinically beneficial to limit ADCC and ADCP in the early posttransplant phase. The latter concept is supported by a report by Schrezenmeier et al. [85] who documented successful prevention of rejection with daratumumab (single dose shortly before transplantation, thereafter continued treatment), combined with imlifidase, intravenous immunoglobulin and rituximab-based desensitization to allow T- and B-cell cytotoxic crossmatch-positive and ABO-incompatible living donor transplantation in a 35-year-old female patient systemic lupus erythematosus (SLE) and antiphospholipid syndrome. The patient exhibited an extreme level of HLA sensitization and was running out of vessels. Two follow-up biopsies showed no features of AMR, perhaps not only the effect of transient antibody depletion, but also a result of a continuous depletion of NK effector cells [85].

Several studies have explored the use of CD38 monoclonals, particularly daratumumab and isatuximab, for recipient desensitization to reduce preformed HLA antibodies. Case reports and small case series [42, 47, 86] have supported ongoing trials addressing this issue. Two trials, one using isatuximab and the other daratumumab, have been published. In an open-label phase 1/2 study, Vincenti et al. [57] investigated the safety, pharmacokinetics, and preliminary efficacy of isatuximab in patients awaiting kidney transplantation. The study included 23 patients who received isatuximab 10 mg/kg weekly for 4 weeks then every 2 weeks for 8 weeks. Treatment was well tolerated and resulted in decreases in $CD38^+$ plasmablasts, plasma cells, and NK cells and significant reductions in HLA-specific IgG-producing memory B cells. Treatment decreased HLA antibodies to a certain extent, an effect that was maintained for 26 weeks after the last dose. Overall, calculated panel reactive antibody (cPRA) values were only minimally affected, but six patients received transplant offers, of which four were accepted. In a prospective 2-phase monocenter open-label trial (DARDAR study), Pilon et al. [87] investigated the safety and efficacy of daratumumab in kidney transplant candidates >95% cPRA. In the first (safety) phase (9 patients), they used 4-weekly escalating doses of daratumumab. Phase 2 tested desensitization with 8 weekly infusions (14 patients). Treatment-emergent adverse events were mostly infusion-related, with no serious adverse events reported. The study showed significant, though transient and incomplete reductions in cPRA levels and the number and MFI of HLA antibodies at 6 months. The modest effect on HLA antibodies was temporary, with levels returning to baseline after 12 months. The authors highlighted this as a limitation for the clinical use of daratumumab for desensitization.

Torija et al [58] further analyzed 26 highly sensitized patients from the two CD38 antibody desensitization trials [57, 87], confirming the significant depletion of plasmablasts, long-lived plasma cells, and other B cell subsets, including B cell precursors and class-switched memory B cells. They identified key phenotypes, particularly CD38-negative class-switched memory B cells, differentiating successful serologic responders from low- or non-responders [58].

Strategies to enhance HLA antibody reduction by targeting CD38, such as combining daratumumab with belatacept,

are under investigation (ClinicalTrials.gov identifiers: NCT04827979; NCT05145296).

CONCLUSION

Antibody-mediated rejection (AMR) remains a significant challenge in kidney transplantation, as current treatments show low levels of evidence and inconsistent outcomes. CD38-targeted therapies, including daratumumab and felzartamab (in a recent phase 2 trial), have shown promise in AMR management. Felzartamab demonstrated an acceptable safety profile and resolution of rejection in many recipients, though the effects were transient, with partial recurrence of rejection. Modulation of dd-cfDNA and gene signatures indicating an injury/repair response in the felzartamab trial suggest potential long-term graft benefits. However, the value of dd-cfDNA as a non-invasive monitoring tool for detecting treatment responses and AMR recurrence still needs to be proven in larger trials. The mechanisms underlying CD38-targeted therapy efficacy remain unclear, and the dual-action model targeting plasma cells and NK cells may oversimplify its therapeutic mode of action. Ongoing studies, including a phase 3 trial, are crucial to confirm the impact on AMR and assess the long-term benefits of CD38-targeting therapies, including their broader potential in kidney transplantation and recipient desensitization.

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REVIEW
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Belatacept in Kidney Transplantation: Reflecting on the Past, Shaping the Future

Johan Noble[1,2], Juliette Leon[3], Arnaud Del Bello[4], Dany Anglicheau[3], Gilles Blancho[5,6], Simon Ville[5,6], Lionel Couzi[7,8], Philippe Grimbert[9,10], Yannick Le Meur[11], Bruno Moulin[12], Nassim Kamar[4], Lionel Rostaing[1], Florence Herr[13†], Antoine Durrbach[13†] and Dominique Bertrand[14†]*

[1]Nephrology, Hemodialysis, Apheresis and Transplantation, Centre Hospitalier Universitaire (CHU) Grenoble-Alpes, La Tronche, France, [2]University Grenoble Alpes, Centre National de la Recherche Scientifique (CNRS), Institut National de la Santé et de la Recherche Médicale (INSERM), Centre Hospitalier Universitaire (CHU) Grenoble Alpes, Institute for Advanced Biosciences (IAB), Grenoble, France, [3]Service des Maladies du Rein et du Métabolisme, Transplantation et Immunologie Clinique, Hôpital Necker, Assistance Publique Hôpitaux de Paris (AP-HP), Paris, France, [4]Département de Néphrologie et Transplantation d'Organes, Centre Hospitalier Universitaire (CHU) Toulouse, Institut National de la Santé et de la Recherche Médicale (INSERM) UMR 1291, Toulouse Institute for Infectious and Inflammatory Diseases (INFINITY), Université Paul Sabatier, Toulouse, France, [5]Service de Néphrologie - Immunologie Clinique, Centre Hospitalier Universitaire (CHU) Nantes, Nantes Université, Nantes, France, [6]Institut de la Transplantation Urologie-Néphrologie (ITUN), Institut National de la Santé et de la Recherche Médicale (INSERM), Center for Research in Transplantation and Translational Immunology, UMR 1064, Nantes, France, [7]Department of Nephrology, Transplantation, Dialysis and Apheresis, Bordeaux University Hospital, Bordeaux, France, [8]Centre National de la Recherche Scientifique (CNRS)-UMR 5164 ImmunoConcEpT, Bordeaux University, Bordeaux, France, [9]Assistance Publique Hôpitaux de Paris (AP-HP), Service de Néphrologie et de Transplantation Rénale, Fédération Hospitalo-Universitaire, Innovative Therapy for Immune Disorders, Centre Hospitalier Universitaire (CHU) Henri Mondor, Créteil, France, [10]Immunorégulation et Biothérapie, University of Paris-Est-Créteil, Créteil, France, [11]Department of Nephrology, University Hospital La Cavale Blanche, Université de Bretagne Occidentale, Brest, France, [12]Service de Néphrologie-Dialyse-Transplantation, Hôpitaux Universitaires de Strasbourg, Strasbourg, France, [13]Service de Néphrologie, Transplantation, Hôpital Creteil, Assistance Publique Hôpitaux de Paris (AP-HP), France UMR1186, Institut Gustave Roussy, Université Paris-Saclay, Villejuif, France, [14]Department of Nephrology, Transplantation and Hemodialysis, Rouen University Hospital, Rouen, France

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***Correspondence**
Johan Noble,
jnoble@chu-grenoble.fr

[†]These authors have contributed equally to this work

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Calcineurin inhibitors (CNIs) are a cornerstone of post-transplant immunosuppressive regimens. However, their use is associated with adverse effects, most notably chronic nephrotoxicity, which remains a leading cause of long-term allograft dysfunction. Belatacept, a selective costimulation blocker, offers a promising alternative to CNIs by aiming to reduce nephrotoxicity while maintaining efficacy in preventing acute rejection. While its use in *de novo* transplantation has been associated with improved graft and patient survival, it has also been linked to a higher incidence of acute rejection. Early post-transplantation conversion to belatacept has demonstrated significant improvements in renal function (eGFR gains ranging from +8.8 to +38.2 mL/min/1.73 m^2 at 1 year post-conversion) but carries a higher risk of opportunistic infections. Late conversion protocols, typically initiated beyond 6 months post-transplantation, have shown sustained—although less pronounced—eGFR improvements and better long-term graft survival compared to CNI-based regimens. Additionally, belatacept appears to reduce the incidence of donor-specific antibodies. Future directions for the use of

Abbreviations: AR, Acute Rejection; BPAR, Biopsy Proven Acute Rejection; CKD, Chronic Kidney Disease; CNI, CalciNeurin Inhibitor; DSA, Donor-Specific Antibody; eGFR, estimated Glomerular Filtration Rate; HLA, Human Leukocyte Antigen; IFN-α, Interferon-α; KT, Kidney Transplantation; MMF, Mycophenolate Mofetil; mTORi, mammalian Target Of Rapamycin inhibitors.

belatacept need further exploration, including its role in rescuing poor renal function, its combination with low-dose CNIs, mTOR inhibitors, or tocilizumab, and its application in desensitization protocols. By potentially striking a balance between efficacy and safety, belatacept may redefine the future landscape of transplant immunosuppression.

Keywords: belatacept, kidney transplantation, opportunistic infections, donor-specific antibodies, eGFR

INTRODUCTION

Calcineurin inhibitors (CNIs), particularly tacrolimus, are the most commonly used immunosuppressive agents to prevent rejection following solid-organ transplantation. Tacrolimus, in combination with mycophenolate mofetil (MMF) and steroids, forms the foundation of maintenance therapy for the majority of transplant recipients. This regimen has proven to be highly effective, with biopsy-proven acute rejection (BPAR) rates of approximately 8%–12% within the first year after kidney transplantation (KT) [1, 2]. However, tacrolimus is associated with several adverse effects, including an increased risk of diabetes, hypertension, and dyslipidemia. Moreover, tacrolimus contributes to both acute and chronic nephrotoxicity. Acute nephrotoxicity, which is reversible, results from hemodynamic changes due to afferent arteriolar vasoconstriction. In contrast, chronic nephrotoxicity is irreversible and leads to progressive decline in kidney function, characterized by interstitial fibrosis, tubular atrophy, chronic glomerulopathy, and vascular thickening.

The challenge to preserving long-term function is to find an immunosuppressive regimen that is as effective as tacrolimus in BPAR prevention but is not associated with chronic nephrotoxicity. Belatacept is the most advanced therapy in this field. Belatacept is a biotherapy derived from CTLA4-Ig (2 additional point mutations) with a higher avidity for CD80/CD86. It inhibits T-cell activation by impairing the CD28 pathway, the second signal for T-cell activation. CD28 is expressed by naive T cells and is involved in T-cell activation, proliferation, and survival in the presence of the TCR/CD3 signaling. Belatacept also interacts with CD80/CD86 on B-lymphocytes, impairing the maturation of naïve B cells in a

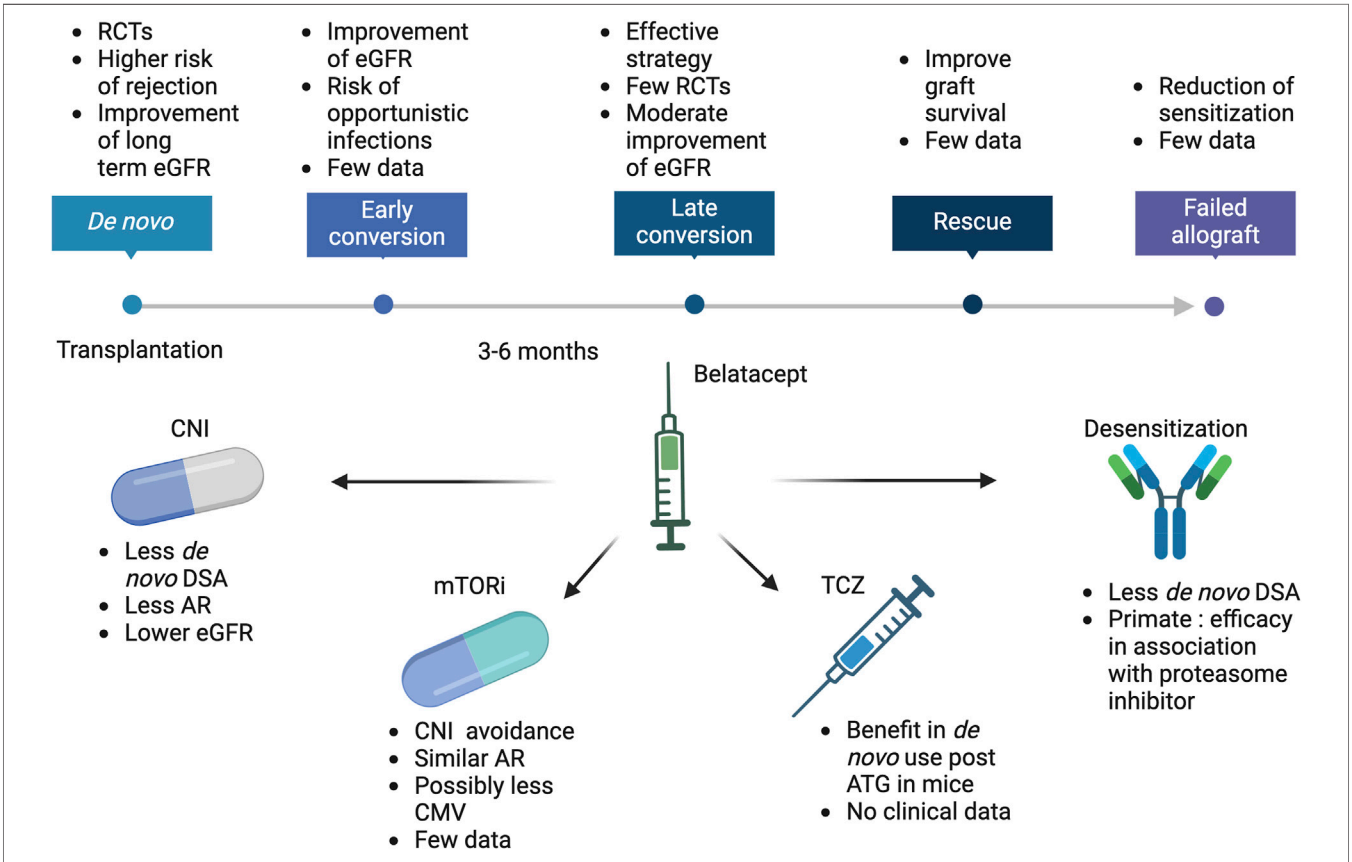

FIGURE 1 | Belatacept current and future use in kidney transplantation. ATG, Antithymoglobulin; AR, Acute rejection; CMV, Cytomegalovirus; CNI, calcineurin inhibitors; DSA, Donor specific antibodies; eGFR, estimated glomerular filtration rate; mTORi, mammalian Target of Rapamycin inhibitors; RCTs, randomized controlled trials; TCZ, Tocilizumab.

transitional phenotype. Belatacept has been developed to replace CNI in *de novo* KT and to be used in combination with MMF and steroids. Phase II and III studies have demonstrated a significant improvement in renal function compared to cyclosporine A. In standard kidneys from brain-dead donors or living donors, the gain is up to 21 mL/min/1.73m2 at 3 years and is associated with an increased graft and patient survival [3]. In extended criteria donors, the gain is +11 mL/min/1.73 m^2 at 3 years post-KT [4]. It also reduces the risk of *de novo* diabetes mellitus and improves cardiovascular risk factors [5]. The gain in renal function in *de novo* KT patients has led to exploring the use of belatacept as a replacement for CNI-treated patients to improve their renal function. The results of conversion strategies and the emerging use of belatacept are presented and discussed in this review (**Figure 1**).

BENEFIT ON RENAL FUNCTION

Early Conversion to Belatacept

The majority of studies have enrolled patients after 6 months post-transplantation. Few early conversions, i.e., before 6 months, have been reported. Initially, KT recipients were switched early to belatacept in the context of severe renal dysfunction. Two studies assessed the results in patients with very low eGFR (8 ± 12 mL/min/1.73 m^2 (n = 25 patients) and 16 ± 12 mL/min/1.73 m^2 (n = 20 patients) after a median time of 71 [15–161] and 42 [18–74] days post-KT respectively [6, 7]. The benefit in terms of eGFR at 1-year post conversion ranged between +16.6 mL/min/1.73 m [2] and +38.2 mL/min/1.73 m [2]. In the first study (Le Meur et al.), 48% of patients had a baseline eGFR <15 mL/min/1.73 m [2] and 29.1% were on dialysis. At 1 year, only 3 patients were still on dialysis. Graft and patient survival at 1 year were 83.3% and 96% respectively. In the second study (Wojciechowski et al.), 75% of patients required dialysis post-KT and before conversion. At 1 year post conversion, graft survival was 95% and no patient was still on dialysis. Patient survival was 100% at 1 year.

Some other non-randomized studies reported results on stable transplant patients in larger numbers of patients (60–453 patients) [8–12]. eGFR at the time of conversion in these patients ranged between 19.4 to 27 mL/min/1.73 m^2. The gain of eGFR at 1 year post conversion ranged from +14.4 mL/min/1.73 m^2 to +18.6 mL/min/1.73 m^2. Graft and patient survival at 1 year were 83.3% and 97.2% (Bertrand et al.) and 100% and 90.9% (Moein et al.) respectively.

In a randomized controlled study, Tawhari et al. assessed the impact of early belatacept conversion (3 months) in 27 KT recipients with stable renal function (mean eGFR at conversion was 68.5 ± 18 mL/min/1.73 m [2]) [13]. Nine patients received belatacept with MMF, 8 received belatacept with low-dose tacrolimus, and 10 had no belatacept conversion. The evolution of eGFR at 2 years was +8.8 mL/min/1.73 m [2] in the belatacept plus low-dose tacrolimus patients, −0.38 mL/min/1.73 m^2 in the tacrolimus plus MMF group and −6.60 mL/min/1.73 m^2 in the belatacept plus MMF group. The rate of graft and patient survival was not different between groups (96.3% and 92.5% respectively at 2 years).

Overall, in the early post-KT period, conversion to belatacept appears to be associated with an important improvement in renal function, with acceptable graft and patient survival. The gain in GFR appears to be even higher in patients with delayed graft function in the very early phase. Further randomized studies are required to confirm the optimal use of belatacept during this period.

Late Conversion to Belatacept

The renal function benefit of late conversion protocols has been demonstrated in several studies. In a randomized phase II trial of 173 patients comparing belatacept conversion to CNI at 19–20 months post-KT [14], the increase in eGFR at 36 months was +8.9 mL/min/1.73 m^2 in the belatacept group compared to +1.1 mL/min/1.73 m^2 in the CNI group (p = 0.01) [15, 16].

Budde et al. conducted a prospective randomized controlled study of belatacept conversion (n = 223) versus CNI maintenance (n = 223) at 6 months post-KT [17]. At 24 months, patient and graft survival (>97%)were similar in the 2 groups. At 24 months, the mean eGFR gain was +5.2 mL/min/1.73 m^2 in the belatacept group and −1.9 mL/min/1.73 m^2 in the CNI group (delta 7 mL/min/1.73 m^2).

A recent retrospective study by Divard et al. compared 243 kidney recipients with a propensity-matched cohort of patients on a CNI-based regimen [18]. The median time to conversion was 1 year in the belatacept group, and the follow-up was 7 years. Graft survival was higher (78%) at last follow-up in the belatacept group versus 63% in the CNI group. The eGFR at 7 years was higher in the belatacept group, 26 mL/min/1.73 m^2 versus 20.2 mL/min/1.73 m^2 in the CNI group. Interestingly, a retrospective study evaluated the effect of conversion to belatacept in patients with severe vascular lesions (cv ≥ 2) and poor kidney function (eGFR between 25 and 27 mL/min/1.73 m^2). The conversion to belatacept (n = 69) was found to be associated with a better graft survival at 3 years (84%) compared to patients who remained on CNI (n = 70, 65.1%) [19]. Fewer *de novo* DSA (7.4% versus 23.4%) but more opportunistic infections (OPIs) (7.6/100 person-years versus 1.0/100 person-years) were noted, while the rate of rejection and patient survival were similar.

Finally, the majority of patients switched to belatacept do not appear to have corticosteroids in their immunosuppressive treatments. A recent study compared 199 late-switched patients to belatacept without reintroduction of steroids versus 313 patients on concomitant steroids at the time of conversion [20]. The absence of steroids was not associated with an increased risk of PBAR or worse graft survival while the use of steroids was independently associated with worse patient survival.

RISK OF REJECTION

Studies have shown that belatacept-based regimens are associated with an increased incidence of BPAR. The risk of acute rejection (AR) associated with the use of belatacept in KT varies depending on whether it is used in *de novo*, in early conversion, or in late conversion. Belatacept-resistant AR in KT involves different

subsets of memory T cells, $CD4^+$ $CD28^+$ T effector-memory, $CD8^+$ CD28null, and $CD4^+$ $CD57^+$ PD1-. These cells, particularly $CD8^+$ T cells, exhibit high levels of IFN-γ production and granzyme B expression, indicating a robust cytotoxic response that is less susceptible to costimulatory blockade by belatacept but which can be regulated by mTOR inhibitors [21, 22]. Additionally, dysregulation of FOXP3+ regulatory T cells has also been implicated in belatacept-resistant AR [23].

Early conversion from CNI to belatacept also carries a risk of BPAR which varies from 5 to 22% at 2 years but was lower compared to *de novo* use [6, 7, 13]. Late conversion (after 6 months) to belatacept generally shows the lowest BPAR, varying from 4% to 8% [17, 18, 24].

Overall, the risk of AR is higher with *de novo* use and decreases with delayed conversion. The absence of antithymoglobulin use and the shorter delay between KT and belatacept conversion have been associated with an increased risk of BPAR [25]. To minimize the risk of AR during conversion, many authors have proposed an overlapping strategy with belatacept and a stepwise decrease of CNI within 1 or 2 months. The adopted scheme of CNI tapering varies, but generally involves a gradual reduction of CNI over a period of weeks to months, tailored to individual patient needs and clinical response. Nevertheless, this strategy is associated with a transient overimmunosuppression by inhibiting the first and second signals of T cell activation.

IMPACT OF BELATACEPT ON ANTI-HLA ANTIBODIES AND ANTIBODY-MEDIATED REJECTION

Despite a higher BPAR rate, the BENEFIT and BENEFIT-EXT trials showed a lower incidence of *de novo* DSA (1.4% and 3.8% in the more intensive belatacept group and 3.5% and 1.1% in the less intensive treatment group) and chronic rejection compared to a the cyclosporine groups (12.1% BENEFIT and 11.2% BENEFIT-Ext) [26]. Additionally, patients treated with belatacept had a significantly lower rate of IgM to IgG DSA conversion (22%) versus 65% in the cyclosporine group [27]. Compared to cyclosporine, the hazard ratio was 0.10, $p < 0.001$ for the more intensive belatacept group and 0.25, $p < 0.001$, for the less intensive group. These results correlate with the accumulation of transitional B cells in belatacept-treated patients suggesting an inhibition of their differentiation [28]. Samson et al. showed in a model of human germinal center formation in immunodeficient mice that belatacept inhibits the formation of these germinal centers [29]. They also showed a decrease in T follicular helper cells and B cells in the germinal centers in mice treated with belatacept, and a decrease in all types of immunoglobulin secretion. Belatacept is able to prevent the antibody response within the germinal centers [30].

Recently, 294 KT recipients on *de novo* belatacept (associated with 1 year of low-dose tacrolimus) were compared to 300 KT recipients who received long-term tacrolimus-based immunosuppression [31]. The rate of *de novo* class I and class II DSA at 1 year was not statistically different between the 2 groups (less than 4%). In subgroup analyses, based on the Eplet mismatch risk on DR/DQ, belatacept use was associated with a lower risk of immune events in intermediate-risk patients. At the last follow-up, the decrease in the DSA hazard ratio was 0.4 for the belatacept group.

For preexisting DSA in these cohorts, 100% and 94.5% of patients in the belatacept-treated groups had a decrease or stabilization of their DSA MFI compared to 71% in the cyclosporine groups [32].

Less data were available for conversion strategies. In the randomized conversion study by Budde et al., the prevalence of *de novo* DSA at 24 months was 1% in the group receiving belatacept and 7% in the CNI continuation group, whereas Kumar et al. did not find a significant decrease in DSA MFI post conversion in 19 patients switched at 44 months post KT [17].

OPPORTUNISTIC INFECTIONS AND TUMORS

The impact of belatacept on the risk of infection remains an essential area of investigation. In the BENEFIT and BENEFIT-EXT trials, infection rates, including serious infections and viral infections, did not significantly differ between groups [33, 34]. However, an increased risk of post-transplant lymphoproliferative disorder (PTLD) was observed, particularly in Epstein-Barr virus (EBV)-seronegative recipients. In randomized conversion studies, Budde et al. observed similar rates of infection between treatment groups, with one case of PTLD reported in the belatacept cohort [17]. Grinyó et al. reported no instances of PTLD in their phase 2 study [14]. These results support belatacept as a viable alternative for stable KT recipients on CNI therapy, provided careful monitoring and selection of EBV-seropositive patients.

Rescue conversion to belatacept in KT recipients is associated with a specific profile of OPIs. Several studies have documented the incidence of OPIs following belatacept conversion at a rate of 5.2–9.8 cases per 100 person-years [9, 10, 12, 35]. The most frequent OPIs were cytomegalovirus (CMV) infection and pneumocystis pneumonia, but other rare but severe infections include JC virus–induced progressive multifocal leukoencephalopathy and other viral or fungal infections. The comparative risk of OPIs is higher in belatacept-treated patients than in those maintained on CNI-based regimens, particularly for CMV reactivation and fungal infections [19]. Similarly, the incidence of pneumocystis pneumonia is higher in belatacept recipients without sufficient prophylaxis [9]. Several factors influence the risk of OPIs in patients switched to belatacept including baseline eGFR below 25 mL/min/1.73 m^2 at the time of conversion, previously treated episodes of AR, duration of pre-existing CNI therapy and the overall immunological vulnerability of these patients [9]. OPIs contribute to substantial morbidity and mortality in this population, with infection-related deaths reported in up to 26.5% of cases and graft loss in 11.8%. Hospitalizations due to infections are also markedly higher in belatacept-treated patients, particularly in those who switch early [12]. Despite these risks, the overall graft and patient survival

rates are acceptable, highlighting the need for robust infection prevention strategies.

Early conversion is associated with a substantially increased risk of CMV DNAemia and disease [12]. For instance, CMV DNAemia was reported in 31.6% of early converters compared to 11.5% of late converters [12]. In the *de novo* use of belatacept, Karadkhele et al. showed in high-risk CMV D+/R-recipients that belatacept-treated patients had a higher incidence of CMV viremia (50% of patients) compared to those treated with tacrolimus within 2 years of transplantation [36]. In the setting of rescue conversion, studies by Chavarot et al. and Bertrand et al. highlighted the heightened risk of CMV post-conversion [9, 37]. In both studies, valganciclovir was given 6 months post-transplantation to high-risk patients (D+/R-) and 3 months to intermediate risk patients (D+/R+ and D−/R+). Chavarot et al. reported that 17.9% of patients developed CMV disease after conversion, with a median onset of 9 months post-conversion [37]. The cumulative incidence of CMV disease was 6.6 per 100 person-years in belatacept-treated patients compared to 0.91 per 100 person-years in CNI-treated controls, representing a sevenfold increase. Bertrand et al. corroborated these findings by identifying CMV disease in 42.9% of OPIs in belatacept-treated patients [9]. CMV disease occurred primarily in high-risk (D+/R−) recipients, often after early conversion. Mortality associated with CMV disease was notable, accounting for 22.2% of deaths in patients with CMV disease.

Concomitant treatment could also play a role in the risk of OPIs following conversion. Chavarot et al. showed that steroids were independently associated with an increased risk of severe infections, including CMV disease [20]. The possible role of mTOR inhibitors in combination with belatacept has been highlighted as a strategy to mitigate CMV risks. In their recently published review, Zuber et al. emphasized the multifactorial nature of CMV risk, the importance of individualizing prophylaxis strategies, and the need for vigilance in high-risk patients [38].

Belatacept-treated KT recipients demonstrate a markedly reduced response to vaccination, including SARS-CoV-2 mRNA vaccines [39–41]. This reduced immunogenicity, both humoral and cellular, highlights critical challenges in protecting this vulnerable population during pandemics such as COVID-19 [42].

FUTURE POTENTIAL USE OF BELATACEPT

Belatacept in Combination With Tacrolimus

To address the increased rates of AR associated with standard belatacept regimens compared to CNI-treated patients, a combined strategy with short-term tacrolimus use in addition to belatacept has emerged in KT recipients. A cohort analysis of 50,244 patients including 417 patients receiving belatacept plus tacrolimus, 458 receiving belatacept, and 49,369 receiving tacrolimus has shown that the rate of AR was similar in tacrolimus and tacrolimus plus belatacept-based regimens and lower than in the belatacept regimen alone [43]. In contrast, eGFR and NODAT were higher and lower, respectively in the tacrolimus plus belatacept-treated patients than in the tacrolimus-treated patients. Results from a non-randomized study compared the modified belatacept-tacrolimus regimen (n = 87) with standard belatacept (n = 97) and tacrolimus treatments (n = 205) [44]. Patients also received Basiliximab induction, MMF, and corticosteroids. In the modified regimen, tacrolimus was administered for 3 months before tapering. At 3 months, the AR rates were similar for belatacept-tacrolimus (15%) and tacrolimus (17%), but nearly twice as high for belatacept (38%). However, the AR rate at 12 months for belatacept-tacrolimus (33%) was between that of tacrolimus (20.5%) and belatacept (50.5%). The rates of Banff grade IIB or III AR were 5%, 4%, and 13%, respectively. Despite higher AR rates, graft and patient survival at 3 years were similar between groups. To overcome the relapsed rate of AR, the tacrolimus exposure was extended to 9 months before being tapered within 2 months [44]. The 12-month AR rate for belatacept-extended tacrolimus was lower than in the historical tacrolimus cohort (16% vs. 20.5%), with 4% of patients experiencing Banff grade IIB or III AR. Over 3 years, the mean estimated GFR was higher for both belatacept-tacrolimus regimens than for standard tacrolimus treatment. Viremia rates for CMV and BK virus were similar between regimens suggesting that a belatacept-based regimen with transient tacrolimus use may yield AR rates comparable to those of standard CNI-based regimens without increasing infectious risks. Moreover, in a recent retrospective study analyzing the risk of de novo DSA based on the donor-recipient eplet mismatch showed that the risk was lower in the group of patients that received belatacept plus a transient exposure to tacrolimuns (n = 294) compared to the patients that received a tacrolimus-based regiment (n = 294) (hazard ratio [HR] = 0.4). The rate of antibody mediated rejection and acute rejection were also lower (HR = 0.2 and 0.45 respectively) [31].

Belatacept in Combination With mTOR Inhibitors

Mammalian target of rapamycin (mTOR) is a protein kinase that has a central role in the regulation of cell metabolism, immune function, proliferation and migration. Sirolimus and everolimus are 2 mTOR inhibitors (mTORi) approved for the prevention of organ rejection in transplant recipients. The combination of belatacept with mTORi is an interesting association, allowing to remove CNI-related nephrotoxicity and adding the potential benefits of mTORi, such as antitumor and potential anti-CMV activity [45].

A randomized controlled study conducted by Ferguson et al. compared the evolution of belatacept *de novo* associated with MMF (33 patients), with sirolimus (26 patients) and with a standard group receiving tacrolimus with MMF (30 patients) [46]. At 1 year, the rate of BPAR was 4% in the mTORi group, and the mean eGFR was 61.8 mL/min/1.73 m^2. The safety profile, along with patient and graft survival was similar between groups. The recovery, post-antithymoglobulin injection, of peripheral blood $CD4^+$, $CD8^+$, memory CD4+ and regulatory T cells was also similar between the different groups.

In 2014, Kirk et al. assessed the outcome of 20 KT recipients from non-HLA identical living donors who received alemtuzumab induction therapy followed by *de novo* belatacept and sirolimus [47]. Patients were randomized to receive or not receive unfractionated donor bone marrow. Three patients were switched to MMF because of sirolimus-related side effects. At 1 year, no clinical or histological rejection occurred and the mean eGFR was 89 ± 3.5 mL/min/1.73 m^2. Safety was also excellent with no admissions for infection or malignancy. Interestingly, 10 patients reduced their immunosuppressive therapy and seven of these experienced no rejection on belatacept monotherapy. Safety was good: five patients had spontaneously resolving EBV viremia and 1 patient had a CMV viremia that resolved after increasing the prophylaxis dose.

From a cellular point of view, memory T cells may lose the expression of CD28, and thus escape the effect of belatacept and are implicated in the high rate of rejection in *de novo* studies reported above. After induction with a depleting agent, there is a marked increase in effector memory and terminally differentiated effector memory cells CD28-$CD57^+CD8^+$ T cells. *In vitro* and *in vivo* studies have shown that mTORi are able to suppress the expansion and the differentiation of these cells and thus reduce the risk of belatacept-resistant rejection [48, 49]. These cells have been shown to be more frequent in patients with belatacept-resistant rejection with increased expression of the mTOR pathway [22]. In $CD4^+CD57^+$ T cells, the mTOR pathway was not downregulated in belatacept-resistant cells as compared to belatacept-sensitive cells [49]. Taken together, these data suggest an interesting additional effect of mTORi in targeting belatacept-resistant $CD8^+$ and $CD4^+$ T cells.

The association of belatacept and mTORi may also be considered in post-KT conversion from the belatacept-MMF regimen to the belatacept-mTORi regimen. Very recently, Del Bello et al. reported their experience in 35 patients who were switched from MMF to mTORi in combination with belatacept [50]. They showed a lower incidence of CMV DNAemia in this group (incidence of 0.035/month of exposure) as compared to a propensity-matched cohort of belatacept–MMF treated patients (incidence of 0.072/month of exposure).

Belatacept in Combination With Tocilizumab

The use of a depleting agent may be beneficial in combination with the use of belatacept *de novo* to prevent belatacept-resistant rejections, as it reduces the rate of rejection when associated with CNI [51]. However, in clinical practice, antithymoglobulin failed to prevent these rejections [52]. In a mouse model, Muckenhuber et al. showed that antithymoglobulins induce an important pro-inflammatory cytokine release, including IL-6, and that blocking IL-6 in addition to a *de novo* belatacept regimen prevents the occurrence of belatacept-resistant rejection and prolongs graft survival [53]. This combination promoted intragraft immune regulation and increased regulatory T cells within the graft.

Additionally, Herr et al. showed that the $CD4^+$CD57+PD1- memory T cell population, associated with belatacept-resistant rejection, had more IRF7 transcript (associated with Interferon-α (IFN-α) and IL-6 regulation) [54]. Inhibition of IL-6, along with type I IFN-α, reduced the proliferation of these belatacept resistant cells.

Use of Belatacept as a Desensitizing Molecule

In *de novo* studies, belatacept is associated with a lower rate of *de novo* DSA occurrence. Non-human studies have also shown the effect of belatacept in impairing the class switching of B cells. In a situation of high risk of immunization patients returning to dialysis, several teams continue immunosuppressive therapy for variable periods of time to prevent sensitization that impairs access to another transplantation, despite the associated increased risks of toxicity and infection [55]. To reduce sensitization, Badell et al. tested in a randomized study the efficacy of using belatacept in this setting in 60 patients, compared to immunosuppressive discontinuation in 7 patients. They found that belatacept reduced the incidence of *de novo* DSA and prolonged its onset, with a comparable safety profile [56].

For patients who are already sensitized, several strategies have been proposed to reduce or eliminate anti-HLA antibodies. The majority of strategies target B cells or long-lived plasma cells. Rituximab, which mainly targets B cells has failed to demonstrate significant efficacy. Proteasome inhibitors are effective in targeting antibody-producing cells but a rebound of antibodies is often seen [57]. The association of belatacept in this setting may be of interest because of its effect on germinal centers and since long-lived plasma cells re-express CD28 [58–60]. In non-human sensitized primate models, this strategy was effective in preventing DSA rebound as compared to standard immunosuppression with tacrolimus and MMF [61–64]. The "dual targeting" combination of belatacept and proteasome inhibitor on germinal centers was tested to desensitize 4 highly-sensitized heart transplant candidates and in antibody-mediated rejection post KT [65, 66]. This strategy was able to reduce anti-HLA antibodies and DSA. After discontinuation of proteasome inhibitors, belatacept was able to prevent antibody rebound in the majority of patients. Circulating cell analysis showed a reduction in naïve and memory B cells and of T follicular helper cells.

CONCLUSION

In summary, belatacept is emerging as a valuable therapeutic option in KT, demonstrating advantages such as improved renal function and a favorable long-term safety profile compared to CNI-based regimens. However, its association with an increased risk of acute rejection, particularly in *de novo* protocols or early conversion, highlights the need for individualized patient selection and close monitoring. Future studies are essential to refine the optimal use of belatacept to ensure the best balance between efficacy and safety in different transplant populations.

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SYSTEMATIC REVIEW AND META-ANALYSIS
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Ex-Vivo Perfusion of Limb Vascularized Composite Allotransplants: A Systematic Review of Published Protocols

*Tessa E. Muss[1], Eleni M. Drivas[1], Amanda H. Loftin[1,2], Yinan Guo[1], Yichuan Zhang[1], Christopher D. Lopez[1], Alisa O. Girard[1,3], Isabel V. Lake[1], Bashar Hassan[1,4], Richa Kalsi[5], Byoung Chol Oh[1] and Gerald Brandacher[1,6]**

[1]Department of Plastic and Reconstructive Surgery, Vascularized Composite Allotransplantation (VCA) Laboratory, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [3]Division of Plastic and Reconstructive Surgery, Cooper University Health Care, Camden, NJ, United States, [4]Division of Plastic Surgery, American University of Beirut, Beirut, Lebanon, [5]Department of General Surgery, University of Maryland Medical Center, Baltimore, MD, United States, [6]Department of Visceral, Transplant and Thoracic Surgery, Innsbruck Medical University, Innsbruck, Austria

Vascularized composite allotransplantation (VCA) has revolutionized restorative surgery of devastating injuries. Unfortunately, these grafts undergo significant injury during prolonged cold ischemia and subsequent reperfusion. *Ex-vivo* machine perfusion (EVMP) is a technique that has shown significant promise in solid organ transplant, but study of its utility in VCA has been limited. A systematic review was conducted to identify preclinical publications investigating perfusion in limb VCAs. Articles published through June 2023 were screened. 29 articles met inclusion criteria, comprising 370 VCA limbs from swine, rats, canines, and humans. EVMP was conducted under normothermic (n = 6), near-normothermic (n = 11), sub-normothermic (n = 3), or hypothermic (n = 13) conditions. While each study used a unique perfusate recipe, most were based on a premade medium. Many incorporated additives, including antibiotics and red blood cells. The duration varied from 3 to over 24 h. Multiple studies showed improved or equivalent biomarkers, histology, and outcomes for normothermic or near-normothermic EVMP (n = 4) and hypothermic EVMP (n = 8) compared to static cold storage, suggesting that EVMP may be a superior storage method to SCS. While there is no definitive evidence regarding the optimal temperature, perfusate composition, or perfusion time for VCAs, each perfusion factor should be chosen and adapted based on the individual goals of the study. This review offers a summary of the current literature to serve as an accessible reference for the design of future protocols in this field.

Keywords: vascularized composite allotransplantation, vascularized composite allograft, composite tissue transplantation, machine perfusion, machine preservation

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***Correspondence**
Gerald Brandacher,
brandacher@jhmi.edu

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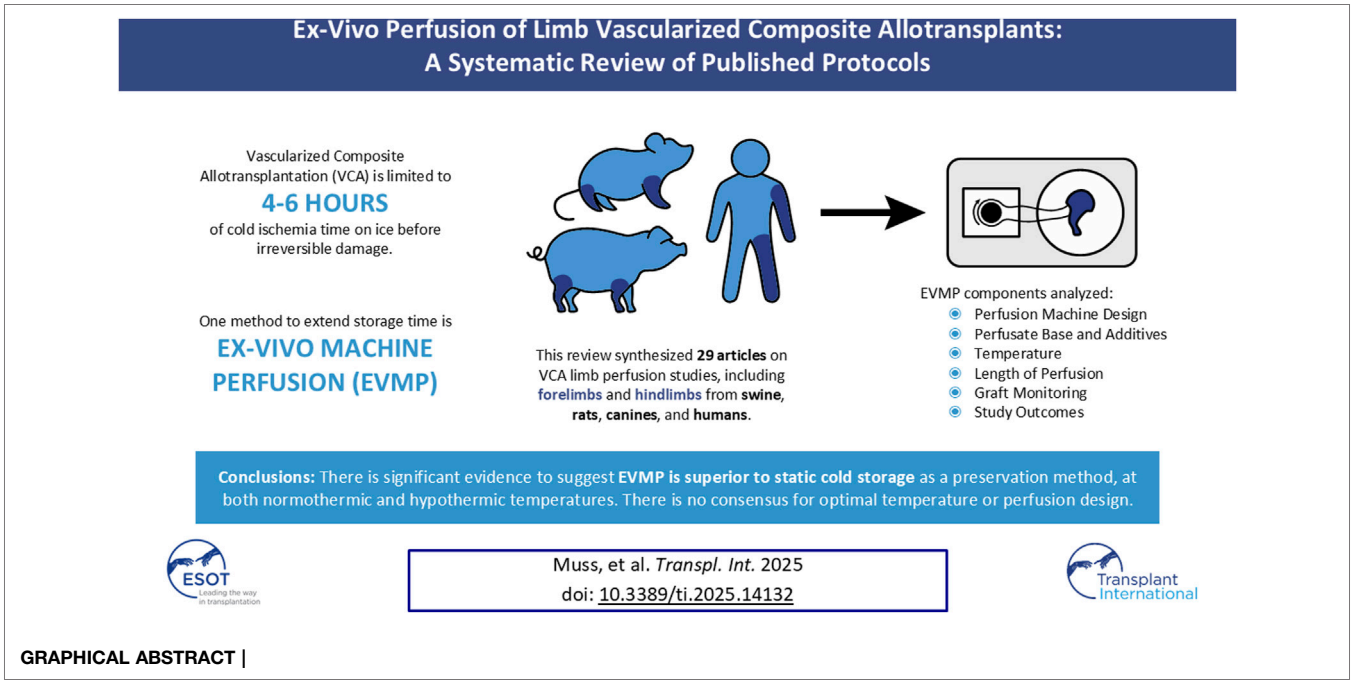

GRAPHICAL ABSTRACT |

INTRODUCTION

Vascularized composite allotransplantation (VCA) is a pioneering reconstructive approach wherein transfer of a multi-tissue allograft is used to return form and function to a site of severe tissue injury or loss [1]. In the last 25 years, more than 150 patients have undergone successful VCA, including hand, face, uterus, abdominal wall, penis, scalp, and vascularized parathyroid gland transplantation [2, 3]. Despite the life-enhancing role of VCA, these procedures carry considerable ethical and psychosocial burdens, as well as high rates of postoperative complications [4–10]. A significant challenge facing VCA is the requirement for lifelong immunosuppression and incremental allograft monitoring. While many VCAs have seen long-term success without chronic rejection, VCA procedures initially yield a disproportionate incidence of acute rejection relative to all other transplant procedures [11–16]. Graft inflammation and staged rejection are strongly influenced by allograft ischemia, temperature changes, and mechanical trauma associated with organ recovery and preservation, even under traditional static cold storage conditions [17, 18]. Interruption of allograft perfusion, and therefore cellular respiration, causes the accumulation of toxic substances and free radicals, which trigger apoptosis and tissue necrosis [19]. Sudden reperfusion increases the production of reactive oxygen species and triggers innate and adaptive immunologic responses that may impair both short- and long-term organ function [19–22]. The low ischemic tolerance of these grafts furthermore significantly limits their accessibility and utility. In response, continued advancement in VCA necessitates novel preservation strategies that decrease reperfusion injury, enhance aerobic cellular respiration, and improve outcomes.

Ex-vivo machine perfusion (EVMP) is an innovative technique designed to prolong preservation time and improve the function of solid organ transplants, and therefore has become an area of interest in VCA [23]. In solid organ transplantation, EVMP has enabled safe transportation while prolonging preservation time and expanding the donor pool [24]. Further, this highly modifiable system has enabled non-acceptable organs to be reconditioned for successful transplantation [25, 26]. A central asset of this technique is the ability to modify fluid pressure, flow rate, and temperature, enabling normothermic and near-normothermic tissue perfusion [27]. Independent from standard cold preservation, EVMP reduces the tissue damage and subsequent functional impairments associated with prolonged cold ischemia times and reperfusion injury [28–30]. Within the past decade, use of EVMP in animal models and solid organ transplantation has made promising strides toward improved post-transplant function and expansion of organ donor pools [30–33].

Given the disproportionate burden of tissue injury and rejection in VCA, application of EVMP has the capacity to revolutionize transplant protocols and outcomes in the field. Still, application of this technology in VCA is neoteric and nuanced. The complexities of perfusing a diversity of tissues, each with unique metabolic needs, warrant careful investigation of perfusate composition and preservation methodologies. Currently, only a modest cohort of studies have been published that document protocols and outcomes of this technique in experimental VCA models.

Despite a clear need for improved methods of VCA preservation, there is a paucity of literature evaluating

successful alternative transplant perfusion protocols. The purpose of this study is to conduct a systematic review of the literature on EVMP for VCA. Specific aims include identification of all current literature on EVMP in VCA, characterization of these studies in terms of perfusion protocols, perfusate composition, monitoring, and outcomes, and comparison of these protocol attributes and outcomes to assess optimal preservation of allografts. Synthesis of results will contribute to an optimized EVMP technique in VCA and guide future research in this evolving field.

METHODS

Literature Search

A comprehensive literature search of manuscripts listed in PubMed, Scopus, EMBASE, Cochrane Library, and ClinicalTrials.gov databases was conducted in June 2023 in compliance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [34]. Titles, Abstracts, Keywords, and Mesh terms (PubMed only) were searched using the following terms: ((vascularized composite allotransplantation) OR (vascularized composite allotransplant) OR (vascularized composite allograft) OR (vascularized allograft) OR (vascularized allogeneic tissue) OR (vascularized composite tissue transplantation) OR (vascularized composite tissue transplant) OR (composite tissue allotransplantation) OR (composite tissue allotransplant) OR (composite tissue allograft) OR (composite tissue allografting) OR (composite tissue transplantation) OR (composite tissue transplant) OR (reconstructive transplant)) AND ((machine perfusion) OR (machine preservation) OR (*ex vivo* perfusion) OR (extracorporeal perfusion) OR (extracorporeal circulation)). The following filters were used in each database to fit within the inclusion criteria: "Full text" in PubMed, "Article" in Scopus, and "Article" and "Article in Press" in EMBASE. The "Trials" tab was used in Cochrane Library, and no filters were applied for ClinicalTrials.gov.

Predetermined inclusion criteria for selecting studies were [1]: preclinical articles studying normothermic, near-normothermic, sub-normothermic, and hypothermic perfusion [2]; perfusion of limbs within VCA [3]; randomized control trials, prospective and retrospective case-control and cohort studies, cross-sectional cohort studies, case reports, and technique papers. Exclusion criteria were [1]: reviews without presentation of new data [2]; abstracts, conference papers, editorials, or comments [3]; articles about solid-organ perfusion [4]; articles about non-limb perfusion; and [5] articles reporting little data on perfusion technique or outcomes.

Papers meeting exclusion criteria, duplicate publications, and articles unrelated to limb perfusion were eliminated. Remaining works were sought for retrieval as full texts, and their reference lists screened for additional relevant articles meeting inclusion criteria that were missed in the electronic search. Two independent authors (TEM and AHL) conducted the search, screening, and eligibility assessment to agree upon a comprehensive list of included articles. Controversies were resolved by discussion with a third reviewer (YG and YZ).

Variables and Outcomes of Interest

The following variables were recorded for each included study: model species, tissue undergoing perfusion, perfusion device, perfusion temperature, perfusion flow type and rate, perfusion pressure, perfusion duration, perfusate composition (where this data was available), monitoring techniques, post-perfusion findings, and post-replant outcomes.

RESULTS

Study Design

Initial literature search yielded 776 unique articles, of which 29 met inclusion criteria (see **Figure 1**) [17, 35–62]. Despite the search terms specific to vascularized composite allotransplantation, the majority of these articles were focused on solid organ perfusion and were therefore excluded from the study. All included studies were randomized control trials published between 1985 and 2023 and cumulatively represent perfusion of 370 vascularized composite grafts (see **Table 1**). All grafts were limbs, of which 20 (5.4%) were human. The remainder were animal models, with the majority were harvested from swine (223, 60.3%), followed by rat (81, 21.9%) and canine (46, 12.4%). Among swine studies, 218 (97.8%) limbs were forelimbs. Eleven (36.7%) studies compared outcomes of perfused limbs against limbs placed in static cold storage. Twelve (40.0%) studies investigated outcomes after replantation (141 limbs). Most perfused grafts underwent cannulation of a single artery (335, 90.5%), although grafts perfused via two arteries were investigated by a single institution (35, 9.5%). Study comparison groups and outcomes are summarized in **Table 1**.

Perfusion Technique

Perfusion was achieved under varying temperature conditions: normothermic (NT, 38°C–39°C) in 6 studies, near-normothermic (NNT, 27°C–35°C) in 11 studies, sub-normothermic (SNT, 20°C–22°C) in 3 studies, and hypothermic (HT, 4°C–12°C) in 13 studies (see **Table 2**). Pump-controlled perfusate flow was pulsatile (7 studies), continuous (12 studies), or intermittent (cyclically paused and resumed, 1 study), although 9 studies provided insufficient detail to determine flow pattern. Seven studies discussed a technique to initiate perfusion, requiring up to 1 h to reach target pressure, flow, and temperature parameters. Perfusion was performed for 3–6 h (9 studies), 12 h (10 studies), 18 h (1 study), 24 h (5 studies), or longer (4 studies), with the longest perfusion achieved via normothermic pulsatile perfusion for 44 h [41]. While perfusate gas composition varied widely, all studies applied oxygen to the perfusion circuit.

Perfusate Composition

Among the studies, 29 unique perfusate recipes were used and four studies experimented with different perfusate recipes (see **Table 3**). Twenty studies (69.0%) used a premade medium, including STEEN (6 studies), Perfadex (3 studies), Ringer's solution (3 studies), Lactated Ringer's solution (3 studies), Custodiol HTK (2 studies), Phoxilium (1 study), Dulbecco's Modified Eagle's Medium (1 study), University of Wisconsin

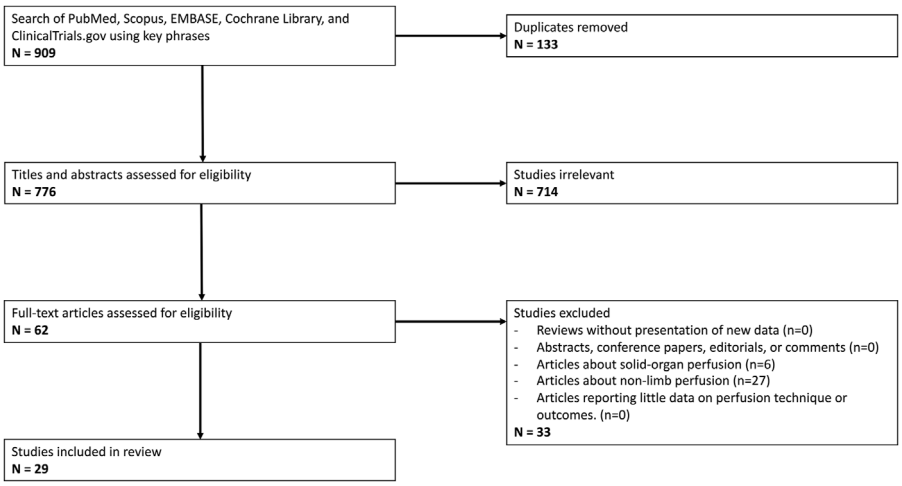

FIGURE 1 | PRISMA Flow Diagram outlining inclusion and exclusion criteria, number of abstracts screened, and full texts retrieved.

solution (1 study), Fluosol-43 (1 study), PromoCell skeletal muscle cell growth medium (1 study), and HAM's solution (1 study). (see **Table 4**). Seventeen studies (58.6%) incorporated antibiotics into the perfusate, including Cefazolin (4 studies), Vancomycin (4 studies), Meropenem (3 studies), Penicillin-streptomycin (3 studies), Piperacillin-Tazobactam (2 studies), and unnamed coverage for skin flora (1 study). One study added antifungal coverage with Amphotericin B [58], and another study wrapped the limb in an antiseptic-diluted sodium hypochlorite solution dressing for the duration of perfusion [38]. Fourteen studies (48.3%) included either red blood cells or whole blood in the perfusate, whereas the remaining 15 studies (51.7%) used acellular perfusate. Common yet inconsistently used additives were metabolic carbohydrates (e.g., glucose, dextrose, dextran; 20 studies), buffer (e.g., sodium bicarbonate, trometamol, potassium dihydrogen phosphate; 20 studies), steroids (e.g., methylprednisolone, hydrocortisone, dexamethasone; 19 studies), heparin (19 studies), insulin (17 studies), calcium (15 studies), and albumin (15 studies). Many protocols included either continuous (4, 13.8%) or periodic (12, 41.4%) partial plasma exchange, with a maximum of 13 exchanges [41].

Graft and Perfusate Monitoring

During perfusion, grafts were often monitored via capillary refill, skin or muscle temperature, skin color, neuromuscular electrical stimulation, and compartment pressure (see **Table 5**). All but three studies used sequential tissue samples for histological staining, single-muscle fiber contractility testing, TUNEL apoptosis assay, and/or quantification of various markers of ischemia-reperfusion injury and hypoxia. Change in graft weight during perfusion was noted in 20 studies. Perfusate levels of potassium, lactate, myoglobin, and creatine kinase were monitored and reported in 20, 20, 9, and 6 studies, respectively.

Perfusion Outcomes

While the designs and objectives varied between studies, multiple studies showed improved biomarkers, histology, and outcomes for EVMP limbs compared to static cold storage (SCS) at 4°C. Four studies [35, 40, 52, 59] showed equivalent or improved outcomes in NT or NNT EVMP compared to SCS, of which one involved transplantation [52]. Eight studies [44–49, 56, 57] showed equivalent or improved outcomes in HT EVMP compared to SCS, including six which involved transplantation [45, 47–49, 56, 57].

Human Limb Studies

Of note, four articles [37, 44, 59, 61] utilized human limbs for machine perfusion studies. Three studies [37, 44, 59] looked at upper limbs, all of which showed hemodynamically stable perfusions up to 24 h, with improved histology as compared to SCS in one study. The fourth human limb study [61] looked at traumatic lower extremity amputations; lower limbs were perfused for 12–15 h at SNT temperatures, with successful replantation in both cases.

DISCUSSION

EVMP is an innovative and evolving approach to solid organ preservation and reconditioning for transplantation, with great potential for clinical application to VCA. The current literature in VCA EVMP is focused mainly on upper or lower extremities, but is expanding to include a variety of perfusion protocols and subsequent structural and immunological outcomes.

Cellular Composition of Perfusate

In transplantation, perfusion media plays a crucial role in maintaining the viability and function of the graft. These media can broadly be categorized into two types: cellular and

TABLE 1 | Articles included in systematic review, n = 30.

Author (Year)	Institution	Species (details)	Limb (total #)	Cannulated arteries	Intervention (# limbs)	Comparator (# limbs)	Outcomes	Conclusion
Amin [17]	University of Manchester, UK	Swine (Landrace, 80 kg)	Fore (5)	2: brachial artery (dominant) and radial artery (collateral)	NT perfusion (5)	(0)	Cytokine concentration and leukocyte count at perfusion t = 0 and t = end (6 h)	At 6 h, there was a cumulative increase in pro-inflammatory cytokines and significant leukocyte diapedesis and depletion from the graft
Amin [35]	University of Manchester, UK	Swine (Landrace, 80 kg)	Fore (35)	2: brachial artery (dominant) and radial artery (collateral)	Experiment 1: NT at 70 mmHg (10) SNT at 70 mmHg (5) SNT at 50 mmHg (5) HT at 30 mmHg (5) Experiment 2: 2 h SCS + Optimal condition perfusion (5)	Experiment 1: Each other Experiment 2: SCS (8 h) (5)	Experiment 1: Hemodynamic and biochemical stability, to identify optimal perfusion conditions for Experiment 2 Experiment 2: Reperfusion with matched blood from unrelated donor for 4 h without immunosuppression: hemodynamic and biochemical stability	Experiment 1: NT perfusion had best outcomes and was deemed to have "optimal conditions" Experiment 2: 2 h SCS + NT perfusion was superior to 8 h SCS.
Gok [36]	UMich	Rat (275 ± 25 g)	Hind (25)	1: femoral artery or common iliac artery	NNT perfusion using: Experiment 1: Femoral artery cannulation (5) Experiment 2: Hemofilter (5) Experiment 3: 6 h NNT perfusion (5)	Experiment 1: NNT perfusion using common iliac artery cannulation (5) Experiment 2: No hemofilter (Experiment 1 limbs) Experiment 3: Contralateral limbs: No perfusion (5)	Experiment 1: Flow rate, perfusion pressure, barotrauma Experiment 2: Lactate and potassium clearance Experiment 3: Hemodynamic and biochemical stability, histology	Experiment 1: Common iliac artery cannulation offers better hemodynamics and less shear stress Experiment 2: Lactate and potassium were maintained at low levels using a hemofilter Experiment 3: Using the common iliac artery and a hemofilter, metabolic outcomes were good without barotrauma, however muscle cells were more damaged than in controls
Werner [37]	UMich	Human (3M: 2F, 37–69y, BMI 22.5–43.9 kg/m^2)	Upper (5)	1: brachial artery	NNT perfusion (5)	(0)	Hemodynamic and biochemical stability, histology, muscle contractility	Human limb allografts appeared viable after 24 h NNT perfusion
Ozer [38]	UMich	Swine	Fore (8)	1: brachial artery	NNT perfusion with autologous blood for 24 h (4)	SCS for 6 h at 4°C (4)	Hemodynamic and biochemical stability, histology; Post-perfusion transplantation to	Limb survival up to 24 h

(Continued on following page)

TABLE 1 | (*Continued*) Articles included in systematic review, n = 30.

Author (Year)	Institution	Species (details)	Limb (total #)	Cannulated arteries	Intervention (# limbs)	Comparator (# limbs)	Outcomes	Conclusion
							recipients (12 h monitoring)	
Ozer [39]	UMich	Swine (40 ± 5 kg)	Fore (7)	1: brachial artery	NNT perfusion with autologous blood for 12 h (4)	SCS for 6 h at 4°C (3)	Hemodynamic and biochemical stability, histology; Post-perfusion transplantation to recipients (7) (12 h monitoring)	Achieved transplantation of limbs after 6 h NNT perfusion with promising contractility and biochemical stability
Constantinescu [40]	Bern University Hospital, Switzerland	Swine (Large white, 37.5 ± 5.5 kg)	Fore (16)	1: axillary artery	NNT 12 h (8)	Contralateral limbs: SCS at 4°C (8)	Hemodynamic and biochemical stability, histology	Perfused limbs demonstrated superior biochemical stability and muscle contractility compared to controls
Fahradyan [41]	Cleveland Clinic	Swine (Yorkshire, 45 kg)	Fore (20)	1: subclavian artery	12h group: NT perfusion for 12 h (5) >24h group: NT perfusion until vascular resistance increased: Systolic pressure >115 mmHg, compartment fullness, weight gain, O2 decrease by 20% (5)	Contralateral limbs: SCS at 4°C (10)	Muscle contractility, compartment pressure, tissue O2 saturation, indocyanine green angiography, thermography	Outcomes of prolonged NT perfusion (>24 h) are not significantly different from 12 h NT perfusion
Duraes [42]	Cleveland Clinic	Swine (Yorkshire, 45 kg)	Fore (36)	1: subclavian artery	NT perfusion for 12 h (18), with evolving protocol of WIT, CIT, perfusate contents, and perfusate temperature	Contralateral limbs: SCS at 4°C for 12 h (18)	Muscle contractility, compartment pressure, tissue O2 saturation, indocyanine green angiography, thermography	Perfusion preserved limb physiology and function for up to 12 h. Limbs with best outcomes: Colloid + washed RBC perfusate at 39°C for 12 h
Haug [43]	BWH	Swine (Yorkshire, 40 kg)	Fore (8)	1: axillary artery	HT perfusion for 12h, using either modified STEEN (2), balanced electrolyte Phoxilium (2), or dextran-enriched Phoxilium (PHODEX) (2)	SCS at 4°C for 12 h (2)	Hemodynamic and biochemical stability, histology, HIF1a	PHODEX is an affordable substitute for STEEN, with exception to elevated creatine kinase and lactate dehydrogenase
Haug [44]	BWH	Human (2M: 1F, 24–51y, BMI 22.3–29.1 kg/m^2)	Upper (6)	1: brachial artery	HT perfusion for 24 h (3)	Contralateral limbs: SCS for 24 h (3)	Hemodynamic and biochemical stability, histology, HIF1a	HT perfusion extended preservation time to 24 h
Kueckelhaus [45]	BWH and Germany	Swine (Yorkshire, 38.4 ± 1.5 kg)	Fore (7)	1: Unspecified	HT perfusion for 12 h using portable perfusion machine and subsequent heterotopic replantation (3)	SCS at 4°C for 4 h and subsequent heterotopic replantation (4)	Hemodynamic and biochemical stability, histology, cytokine levels	Perfused limbs were superior to SCS limbs after transplantation
Kueckelhaus [46]	BWH and Germany	Swine (Female Yorkshire, 50–60 kg)	Hind (10)	1: femoral artery	HT perfusion using portable perfusion machine (5)	SCS for 12 h (5)	Hemodynamic and biochemical stability, histology	Successful perfusion via portable device, superior to SCS.

(Continued on following page)

TABLE 1 | (*Continued*) Articles included in systematic review, n = 30.

Author (Year)	Institution	Species (details)	Limb (total #)	Cannulated arteries	Intervention (# limbs)	Comparator (# limbs)	Outcomes	Conclusion
Krezdorn [47]	BWH and Germany	Swine (Female Yorkshire, 35–45 kg)	Fore (8)	1: axillary artery	HT perfusion for 24 h and subsequent replant onto same animal (4)	SCS at 4°C for 4 h and subsequent replant onto same animal (4)	Hemodynamic and biochemical stability, histology, 7-day monitoring of animals	Perfused limbs were comparable to SCS limbs and may reduce muscle damage and systemic reactions on replantation
Krezdorn [48]	BWH	Swine (Female Yorkshire, 35–45 kg)	Fore (8)	1: axillary artery	HT perfusion at 10°C for 2 h and subsequent replantation onto same animal (3) Or HT perfusion at 10°C for 12 h and subsequent replant onto same animal (3)	SCS at 4°C for 2 h and subsequent replant onto same animal (2)	Hemodynamic and biochemical stability, histology, PCR of target genes	Perfused limbs demonstrated downregulation of genes involved in glycolysis, angiogenesis, and DNA damage compared with SCS limbs
Kruit [49]	Radboud University Medical Center, Netherlands	Swine (Female Dutch Landrace, ~69 kg)	Fore (24)	1: brachial artery	HT perfusion for 18 h and subsequent replant onto the same animal (6)	SCS at 4°C–6°C for 4 h and subsequent replant onto the same animal (6) Sham surgery in contralateral limbs (12)	Hemodynamic and biochemical stability, histology, nerve stimulation, 12 h monitoring of animals	Muscle contraction comparable between perfused, SCS, and sham limbs, perfused limbs had greater edema than SCS limbs. There was no correlation between muscle function and histology
Domingo-Pech [50],	Spain	Canine (Mongrel)	Hind (21)	1: iliac artery	Perfusion for 24 h (9) Perfusion for 24 h and subsequent replantation onto same animal (6)	Limb harvest and immediate replant (6)	Hemodynamic and biochemical stability, histology, 6 h monitoring of animals	Edema was managed with peripheral vasodilators, steroids, and cool perfusate temperature
Usui [51]	Japan	Canine (Mongrel, 10–15 kg)	Hind (46)	1: femoral artery	Intermittent perfusion with fluorocarbon at room temp (9) or HT (6); Continuous perfusion with fluorocarbon at room temp (6) or HT (5); Continuous perfusion with Lactated Ringer's at HT (5) All limbs were replanted	Limb harvest and immediate replantation (15)	6 h monitoring of animals	Fibrocarbon inhibited anaerobic metabolism and creatine phosphokinase leak from the limb and was more pronounced under continuous and HT perfusion conditions
Muller [52]	Bern University Hospital, Switzerland	Swine (Large white, 39 ± 5.5 kg)	Fore (61)	1: unspecified	6 h SCS/12 h perfusion (7) 12 h SCS/5 h perfusion (6) No SCS/12 h perfusion/ replantation (11) 6 h SCS/12 h perfusion/ replantation (8)	Contralateral limbs SCS for 18 h (10) Contralateral limb biopsies at euthanasia (19)	Hemodynamic and biochemical stability, histology, inflammatory markers, 7-day monitoring of replanted animals	No significant difference in markers for ischemia/ reperfusion injury
Adil [53]	University of Toronto	Rat (Male Lewis, 300–430 g)	Hind (4)	1: femoral artery	Decellularization perfusion for 5 days (4)	(0)	Hemodynamic and biochemical stability, histology	Successful decellularization

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TABLE 1 | (*Continued*) Articles included in systematic review, n = 30.

Author (Year)	Institution	Species (details)	Limb (total #)	Cannulated arteries	Intervention (# limbs)	Comparator (# limbs)	Outcomes	Conclusion
Burlage [54]	MGH	Rat (Lewis, 250–300 g)	Hind (39)	1: femoral artery	HT perfusion with BSA for 6 h (4) HT perfusion with BSA/PEG for 6 h (4) HT perfusion with HBOC-201 for 6 h (4) HT perfusion with HBOC-201 for 6h, then transplant (13)	SCS 6h, transplant (4) SCS 24h, transplant (5) Direct transplant after harvest (5)	Hemodynamic and biochemical stability, histology	Lower edema with HBOC-201 perfusate compared to BSA and BSA/PEG, decreased energy charge ratios in SCS compared to HBOC-201
Figueroa [55]	Cleveland Clinic	Swine (Yorkshire, 45 kg)	Fore (24)	1: subclavian artery	NNT perfusion with HBOC-201 (6) NNT perfusion with RBC perfusate (6)	SCS at 4°C (12)	Hemodynamic and biochemical stability, histology	No significant differences between HBOC-201 and RBC-perfused limbs
Gok [56]	UMich	Rat (Male Lewis, 250 ± 2.5 g)	Hind (25)	1: unspecified	HT perfusion with HTK for 6h, then transplant (5)	No intervention (5) Sciatic nerve transected and directly repaired (5) Limb harvest and immediate transplant (5) HTK flush, 6h SCS, then transplant (5)	Hemodynamic and biochemical stability, histology, muscle contractility after 12 weeks	No significant differences in myocyte injury in HT perfusion group compared to controls, decreased muscle force in HT perfusion after 12 weeks compared to controls
Goutard [57]	MGH	Rat (Lewis, 250 ± 50 g)	Hind (32)	1: femoral artery	HT perfusion 3 h (4) 12h SCS, HT perfusion 3 h (4) 18h SCS, HT perfusion 3 h (4) 12h SCS, HT perfusion 3h, transplant (4)	Direct transplant (4) SCS 12–48h, transplant (16)	Hemodynamic and biochemical stability, histology, 21-day monitoring of animals	No differences in survival for 0–24 h SCS, frequent delayed graft failure for 48h SCS, increased edema in 18 h SCS perfusion compared to 12h SCS, improved clinical appearance 12 h SCS perfusion transplants compared to 12 h SCS only
Mayer [58]	Humboldt Univerty, Berlin, Germany	Swine	Fore (60)	1: unspecified	NNT perfusion (60)	(0)	Hemodynamic and biochemical stability	Viability of flaps for up to 27 h
Rezaei [59]	Cleveland Clinic	Human (Adult DBD)	Upper (20)	1: brachial artery	NT perfusion 48 h at 38°C (10)	SCS at 4°C (10)	Hemodynamic and biochemical stability, histology	Improved histology and decreased edema in perfusion compared to SCS
Stone [60]	University of Manchester, UK	Swine (Landrace, 80 kg)	Fore (10)	1: brachial artery	NT limb + kidney perfusion 5 h (5) NT limb only perfusion 5 h (5)	(0)	Hemodynamic and biochemical stability, histology, inflammatory markers, thermal imaging	Addition of a kidney rapidly stabilized lactate, bicarbonate, and pH levels, more homogenous global perfusion

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TABLE 1 | (*Continued*) Articles included in systematic review, n = 30.

Author (Year)	Institution	Species (details)	Limb (total #)	Cannulated arteries	Intervention (# limbs)	Comparator (# limbs)	Outcomes	Conclusion
								in kidney group compared to limb only
Taeger [61]	University Hospital Regensburg, Germany	Human (Adult traumatic amputations)	Lower (2)	1: femoral artery	HT perfusion followed by reattachment to patient (2)	(0)	3-month follow-up	Successful replantation in both patients
Valdivia [62]	Hannover Medical School, Germany	Rat (Lewis, 227–400 g)	Hind (30)	1: femoral artery	HT perfusion 4 h with lentiviral vectors (15) HT perfusion 4 h (15)	(0)	Hemodynamic and biochemical stability, histology, cytokine levels, bioluminescence detection, cell phenotyping	No significant tissue damage from lentiviral vector use

C, continuous flow; Fore, forelimbs; h, hours; Hind, hindlimbs; HT, hypothermic; N_2, nitrogen; NR, not reported; NT, normothermic; NNT, near-normothermic, P, pulsatile flow; q#time, to indicate frequency a medication was administered; SCS, static cold storage; SNT, sub-normothermic; Upper, upper limbs.

acellular. Despite both being designed to preserve the organ, their composition and mechanisms vary significantly.

Cellular media often incorporate contents like red blood cells (RBC) or hemoglobin-based oxygen carriers which facilitate the transport of oxygen to the tissue. The inclusion of cellular components aims to create an environment that is similar to *in vivo* conditions, which may especially benefit organs or tissues with high metabolic rates. The presence of cellular elements can also enhance oxygen transport and provide essential nutrients, thereby reducing ischemic injury. Werner and Ozer both adopt cellular media and show its efficacy in preserving the viability of human and swine limbs for up to 24 h [37, 38]. However, cellular media may pose challenges such as inflammation and increased risk of thrombosis. Amin has observed a cumulative increase in pro-inflammatory markers at 6 h in swine forelimb perfusion [17]. Additionally, cellular blood-based perfusate is limited by blood bank accessibility, blood refrigeration, and the short shelf life of blood products, limiting its utility in military and emergency settings [63, 64]. Blood-based perfusates also carry risk of infection and coagulation, as well as HLA-sensitization and transfusion-related reactions [64–66].

By contrast, acellular media lacks cellular components and therefore generally relies on the dissolving of oxygen. Several studies in porcine lung EVMP suggest that acellular perfusates are a suitable alternative to blood-based perfusate [67–69]. Therefore, acellular perfusates have gained increasing interest as a more accessible and low-maintenance approach, evidenced by nearly half of the studies in this cohort using acellular perfusate. Importantly, while simpler and easier to manage, the absence of specialized oxygen carriers like RBCs may limit the efficiency of O_2 transport. Thus, acellular media often need additional oxygenation such as adding synthetic oxygen carriers or pumping with oxygen [70].

Base Medium

The base medium (see **Table 4**) can be roughly categorized into 3 different types: 1) cell culture, 2) electrolyte balance, 3) preservation and perfusion. They share many common functions, including basic functions like maintaining osmotic balance, cellular homeostasis, and regulation of pH. Some of the media contains nutrients like amino acids, glucose, or specialized carbohydrates, which can provide cells with additional substrates for metabolism support during preservation. Certain media like HTK has tryptophan which can protect the graft against oxidative stress during ischemic conditions [71].

Supplements and Additives

There are a variety of supplements that can be added to tailor the perfusate to specific experimental conditions. Electrolytes are a common inclusion, especially sodium chloride, which is necessary to maintain the osmotic balance. Additionally, calcium and magnesium compounds serve important roles in cellular signaling and enzymatic functions. Potassium is important in maintaining a high intracellular-to-extracellular gradient via the Na + K + ATPase pump, as most total body potassium is stored within muscle.

The base media chosen also contains different additives that can help modulate the perfusate. Cell culture media like DMEM usually contain general nutritional components for cellular division. By contrast, STEEN and Perfadex include unique components like albumin and D_{40}, which is specialized for specific organs like lungs. Fluosol-43 is designed to promote tissue oxygenation [72]. University of Wisconsin solution (UW) contains potassium lactobionate and raffinose, where the former compound is critical for minimizing cellular edema and the latter one is crucial in providing carbohydrate sources for metabolism. Custodiol HTK include histidine and tryptophan, amino acids that can help in maintaining pH balance and protecting cells during ischemic or hypothermic conditions.

Perfusion Time

The duration of perfusion is a pivotal factor that may influence cellular viability, organ functionality, and the risk of ischemic injury. Even brief periods of ischemia can lead to significant tissue

TABLE 2 | Details of perfused limbs.

Author (Year)	WIT	CIT (target)	Perfusion device	Flow type	Relative perfusate temp	Actual perfusate temperature (target) (°C)	Perfusion initiation technique	Perfusate flow rate (% of *in vivo* baseline measurements)	Perfusion pressure (target) (mmHg)	Vascular resistance	Gas content	Perfusion duration (h) (target)
Amin [17]	25 ± 2.7 min	124.6 ± 6.2 min (120 min)	Centrifugal pump	NR	NT	37.1 ± 0.1 (38)	Pressure increase 5 mmHg Q5 min	119.8 ± 12.75 mL/min/Kg; 356 ± 131.5 mL/min	MAP: 69.5 ± 0.4; (70)	Decreased until t = 1 h, stable thereafter	95% O_2/5% CO_2	6
Amin [35]	NR	NR	Centrifugal pump	NR	NT NNT HT	NR (38) NR (28) NR (10)	Pressure increase 5 mmHg Q5 min	102.3 ± 34.8 mL/kg/min	MAP: 65.6 ± 6.7	NT at 70 mmHg: 0.4 ± 0.3 mmHg/min/mL, stable, uniform	95% O_2/5% CO_2	6
Gok [36]	NR	NR	Peristaltic roller pump (Masterflex L/S peristaltic pump	P	NNT	NR (30–35)	Flow at t = 0 0.1 mL, increased incrementally to 2.5 mL/min over first 20 min	Experiment 3: 0.9 ± 0.24 mL/min	Experiment 3: 33.74 ± 14.83	Gradual decrease	95–100% O_2; adjusted to maintain pO_2 225–400 mmHg/0%–5% CO_2	6
Werner [37]	76min	NR	Roller pump (Shiley Roller Pump)	P	NNT	32.0 ± 0.2 (30–33)	NR	310 ± 20 mL/min (6%–10%)	Systolic: 93 ± 2	0.4 ± 0.3 mmHg/min/L	40–60% O_2/5–10% CO_2/Remaining% N_2	24
Ozer [38]	NR	NR	Perfusion pump (Waters Medical Systems, Minneapolis, MN)	P	NNT	NR (27–32)	NR	80 mL/h	MAP: 60–80	Increased until t = 1 h, decreased after t = 2 h	95% O_2/5% CO_2	24
Ozer [39]	NR	NR	RM3 pulsatile perfusion pump (Waters Medical Systems, Minneapolis, MN)	P	NNT	NR (27–32)	NR	80–120 mL/h	MAP: 60–80	High at t0 = 3 h, later normalized	95% O_2/5% CO_2	12
Constantinescu [40]	1 h	NR	Turbine pump (MEDOS Deltastream Blood Pump, Model DP2; Medos Medizintechnik AG, Stolberg, Germany)	C	NNT	NR (32)	NR	100–150 mL/min (50%)	MAP: 33.73 ± 2.06	NR	21% O_2; arterial pO2 128.81 ± 8.82 mmHg	12
Fahradyan [41]	NR	NR	Roller pump (Terumo Sarns 8000) fitted with a pulse module (Terumo Sarns)	P	NT	NR (38)	Flow and temp were gradually increased during first hour	12 h group: 0.77 ± 0.1 L/min >24 h group: 0.43 ± 0.03 L/min	12 h group: Systolic: 107.25 ± 31.02 Diastolic: 44.69 ± 21.10 >24 h group: Systolic: 111.14 ± 12.48 Diastolic: 64.25 ± 14.15	12 h group: +6.4% ± 18.4% >24 h group: +33.3% ± 23.6%	12 h group: 100% O_2/7% CO_2/93% N_2 >24 h group: 100% O_2 1 L/min	12 h group: 12 >24 h group: 24–44
Duraes [42]	12h 39°C colloid/wRBC: 112 ± 68 min	12h 39°C colloid/wRBC: None	Roller pump (Terumo Sarns 8000) fitted with a pulse module (Terumo Sarns)	P	NNT NT	n = 1 (N/A) n = 7 (32) n = 8 (39)	NR	NR	NR	NR	100% O_2 + 7% CO_2/93% N_2	6–12 (12)
Haug [43]	77.5 ± 5.24 min	NR	Peristaltic machine pump (Master Flex Pump L/S, Cole-Parmer, Illinois, USA)	C	HT	(10)	NR	20 mL/min	24.48 ± 10.72	NR	377.22 ± 89.58 mmHg	12

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TABLE 2 | (*Continued*) Details of perfused limbs.

Author (Year)	WIT	CIT (target)	Perfusion device	Flow type	Relative perfusate temp	Actual perfusate temperature (target) (°C)	Perfusion initiation technique	Perfusate flow rate (% of *in vivo* baseline measurements)	Perfusion pressure (target) (mmHg)	Vascular resistance	Gas content	Perfusion duration (h) (target)
Haug [44]	Median: 90min (65–155 min)	Median: 67 min (37–148 min)	Peristaltic machine pump (Master Flex Pump L/S, Cole-Parmer, IL)	C	HT	Median: 9.43 (Range 4.8–14.3) (10)	NR	Median: 30.4 mL/min	30	NR	385.4–609.7 mmHg, median 555.8 mmHg	24
Kueckelhaus [45]	NR	NR	NR	C	HT	10 ± 1.9 (10)	NR	NR	30	NR	Oxygenator used	12
Kueckelhaus [46]	NR	NR	Peristaltic pump	C	HT	(10–12)	NR	NR	30	NR	Oxygenator used	12
Krezdorn [47]	26.2 ± 14.4 min	NR	Pump	C	HT	(8)	NR	Fluctuating	29.4 ± 0.6		8.2 ± 0.7mL/100 mL	24
Krezdorn [48]	NR	NR	NR	NR	HT	(10)	NR	NR	NR	NR	Oxygenated	12
Kruit [49]	NR	NR	Centrofugal pump (BP-50 Bio-Pump Centrifugal Blood Pump, Medtronic)	NR	HT	(8–10)	NR	16 ± 1.7 mL/min	<30	NR	95% O_2/5%CO_2	18
Domingo-Pech [50]	NR	NR	Sarns low velocity pump	NR	HT	"Cold"	NR	NR	>100	NR	Oxygenated	24
Usui [51]	NR	NR	NR	C or I	SNT HT	(~20) (4)	NR	NR	50	NR	Oxygenated, >400 mmHg in Fluorocarbon group	C: 6 h I: 20min perfusion for 3 or 5 cycles
Muller [52]	NR	Group 1: 6.2 ± 0.03 h (6) Group 2: 12.9 ± 1.5 h (12) Group 4: 6.2 ± 0.2 h (6)	MEDOS DataStream blood pump, model DP2 (Medos Medizintechnik AG, Germany)	NR	NNT	(32)	NR	100–150 mL/min	NR	NR	Oxygenated	Group 1: 12.1 ± 0.2 (12) Group 2: 4.9 ± 1.9 Group 3: 12.0 ± 0.3 (12) Group 4: 12.0 ± 0.1 (12)
Adil [53]	NR	NR	Peristaltic pump	C	NT	NR	NR	1 mL/min	NR	NR	NR	120
Burlage [54]	10–15 min	NR	Rotating pump (Drive Mflex L/S, Cole-Parmer, IL)	C	HT	NR	NR	HBOC-201: median 0.4 mL/min	30–40	Decreased within 1st hour, stable afterwards	Oxygenated	6
Figueroa [55]	HBOC-201: 35.50 ± 8.62 min RBC: 30.17 ± 8.03 min	NR	Roller pump (Terumo Sarns 8000)	C	NNT	HBOC-201: 33.23 ± 1.11 RBC: 33.12 ± 1.69 (38)	Temperature raised from 27°C to 38°C over 1 h	HBOC-201: 325 ± 25.00 mL/min RBC: 444.73 ± 50.60 mL/min	HBOC-201: 78.50 ± 10.75 RBC: 85.70 ± 19.90 (MAP 90)	HBOC-201: 214.80 ± 69.80 mmHg/min RBC: 190.90 ± 58.33 mmHg/min	Oxygenated	HBOC-201: 22.50 ± 1.71 RBC: 28.17 ± 7.34
Gok [56]	30 min avg	NR	Peristaltic roller pump (Masterflex L/S peristalitic pump, Cole-Palmer, IL)	C	HT	(10–15)	NR	NR	20–40	NR	Oxygenated	6
Goutard [57]	NR	NR	Roller pump (Drive Mflex L/S, Cole-Palmer, IL)	C	SNT	(21)	NR	0.8 mL/min	30–50	Decreased over 3 h	Oxygenated	3
Mayer [58]	NR	NR	NR	NR	NNT	NR	NR	NR	NR	NR	Oxygenated	NR

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TABLE 2 | (*Continued*) Details of perfused limbs.

Author (Year)	WIT	CIT (target)	Perfusion device	Flow type	Relative perfusate temp	Actual perfusate temperature (target) (°C)	Perfusion initiation technique	Perfusate flow rate (% of *in vivo* baseline measurements)	Perfusion pressure (target) (mmHg)	Vascular resistance	Gas content	Perfusion duration (h) (target)
Rezaei [59]	59.6 ± 20.9 min	NR	Roller pump	C	NNT	35.1 ± 1.7 (38)	Flow gradually increased over 1 h	0.41 ± 0.06 L/min	MAP 90	187.3 ± 26.7 mmHg x min/L	Humidified 100% O_2	41.6 ± 9.4 h (48)
Stone [60]	20.6 ± 3.0 min	195.4 ± 13.7 min (180 min)	NR	NR	NT	(38)	Pressure 55 mmHg, increased by 5 every 5min to reach target, limbs added after 1 h renal perfusion	Limb/Kidney: 496 ± 78.29 mL/min Limb Only: 232 ± 106.6 mL/min	75	NR	95% O_2, 5% CO_2	5
Taeger [61]	NR	NR	ECMO Pediatric Set (Quadrox, Maquet, Germany)	P	SNT	(20)	NR	NR	NR	NR	100% O_2	Patient 1: 15 h 49 min Patient 2: 12 h 27 min
Valdivia [62]	NR	NR	NR	NR	HT	NR	NR	NR	NR	NR	Oxygenated	4

CIT, Cold ischemia time, time in cold storage. I, intermittent; WIT, Warm ischemia time, time from limb procurement until cold flush. Numbers formatted as mean ± standard deviation (SD).

damage. Shorter perfusion times, generally around 6 h, are beneficial for minimizing logistical challenges and reducing the risk of complications. However, perfusion times ranging between 6 and 24 h can allow for better equilibration with the perfusion solution and potentially offer a broader window for assessing organ viability prior to transplant or replant. Extended perfusion durations that exceed 24 h are usually employed for experimental settings. While they allow for in-depth monitoring and potentially improved transplantation outcomes, these extended durations are logistically complex and pose an elevated risk of complications like delayed graft function. The decision regarding duration of perfusion requires thorough consideration of the aforementioned factors and should be tailored to the type of organ, logistical challenges, and overall objective of the perfusion.

Limitations and Suggestions for Future Research

This systematic review presents with several limitations. Literature search was conducted with the assumption that all relevant studies would be discoverable via six large databases and a predetermined set of search terms. Additionally, non-English studies, abstracts, posters, conference presentations, and unpublished data were excluded from this study. In consideration of the small cohort of included studies, it is possible that we excluded other research that would offer valuable insight into the development of research in VCA EVMP. Specifically, the exclusion of non-English papers may have unintentionally limited this review, and further insights might be gleaned from supplementary examination of non-English VCA EVMP articles. Additionally, this review excludes articles published after June 2023. As VCA research is rapidly evolving, multiple studies may have been published on this topic in the intervening time.

The conclusions drawn from this review are limited by the quality and design of published research in VCA EVMP. As the swine forelimb represents the dominant model in this review, outcomes of these studies may not be generalizable to humans or other models with more complex forearm and hand anatomy. Future investigations in EVMP of monkey or ape limbs and subsequent functional testing may help to bridge this gap in knowledge. Additionally, the included studies are not representative of the breadth of VCA (e.g., face, calvarium, abdominal wall, and genital transplantation). As such, these studies may not be applicable to preservation of these structures.

While this paper details the technical aspects and limitations of VCA EVMP, these are not the only barriers to clinical translation. VCA is performed by a limited number of institutions, and on a significantly smaller scale than solid organ transplants. The low numbers of yearly VCAs are cost-prohibitive for a standardized perfusion machine, and severely limit the sample size for any potential clinical trials. VCAs also carry unique ethical considerations, including vulnerability of recipients, as well as racial and socioeconomic disparities [73]. These logistical and ethical barriers further hinder the successful clinical translation of EVMP in VCA.

TABLE 3 | Perfusate content.

Author (date)	TCV	Base Medium	pRBCs	Albumin	Heparin	Antibiotics	Glucose	Buffer	Methyl-prednisolone	Insulin	Calcium	Other Additives or Notes	Perfusate exchange (volume and freq)
Amin [17]	1.1 L	500 mL Ringer's	pRBCs: 500 mL Hct: 20%	BSA	5000 iU	500 mg meropenem	15% 10 mL/h	8.4% $NaHCO_3$ 10 mL/h	500 mg	Actrapid, 10 mL/h, C	Calcium in Nutriflex, $CaCl_2$ in Ringer's	Nutriflex 10 mL/h, continuous infusion	X
Amin [35]	1.1 L	500 mL Ringer's	pRBCs: 500 mL Hct: 20%	BSA	5000 iU	500 mg meropenem	15% 10 mL/h	8.4% $NaHCO_3$ 10 mL/h	500 mg	Actrapid, 10 mL/h, C	Calcium in Nutriflex, $CaCl_2$ in Ringer's	Nutriflex 10 mL/h, continuous infusion	X
Gok [36]	>25 mL	STEEN (25 mL)	Swine RBCs to Hb 6–9 g/dL	X	2000 U	5 mg cefazolin	Glucose in STEEN	$NaHCO_3$ given in 0.5-1 m Eq increments to maintain >5.0 mmol/L	10 mg	X	15 mg calcium gluconate	X	Continuous plasma filtration at 6 mL/h, replaced with equal volume Plasma-Lyte A, with 30 m Eq/L $NaHCO_3$ and 1000 U/L heparin
Werner [37]	250–300 mL	X	Hb 4–6 g/dL	Albumin	Sodium heparin	Yes; skin flora coverage	Dextrose given to maintain >100 mg/dL	$NaHCO_3$	200 mg at t = 0 and with each PPE	Regular insulin given if glucose >300 mg/dL	$CaCl_2$	Tromethamine	PPE q3-5 h
Ozer [38]	NR	X	1:2 pRBCs: plasma, 20–30 mmHg colloid pressure	X	10,000 U	Limb in antiseptic-diluted sodium hypochlorite solution dressing	Dextran; 1 mL D_{50} given if < 4.5 mmol/L, Q2h	X	X	2U given if glucose >14 mmol/L, Q2h	X	Leukocyte and platelet fractions removed	Continuous PPE at 80 mL/h
Ozer [39]	300 mL	Dulbecco's Modified Eagle's Medium (200 mL)	Hct 10%	X	10,000 U	X	Dextrose in DMEM, 1 mL D_{50} given if < 4.5 mmol/L, Q2h	NaHCO3 in DMEM	X	2U given if glucose >14 mmol/L, Q2h	$CaCl_2$ in DMEM	Leukocyte and platelet fractions removed	PPE 160 mL q2h (fresh contained 10% hemoglobin with similar parameters of plasma oncotic pressure)
Constantinescu [40]	NR	X	Hb 5 mg/mL	X	10,000 U	X	20 mL 10% glucose given if potassium >5.5 mmol/L	X	40 mg	15IU Actrapid given if potassium >5.5 mmol/L	X	Circuit primed with 250 mL colloid solution	X
Fahradyan [41]	2.5 L	X	Washed RBCs, Hct 10%–15%	Albumin	10,000 U	500 mg vancomycin	Maintenance D_{50}	$NaHCO_3$ (12 h group) or THAM (>24 h group) given if pH < 7.1	500 mg	Regular insulin 1U/h	X	X	400 mL PPE at t = 6 h and q3h thereafter
Duraes [42]	>0.5 L	X	Whole blood, Washed RBCs (Hct 10%–15%) or none	Albumin	10,000 U	500 mg vancomycin	Glucose	THAM to correct base deficit	500 mg	Regular insulin 1U/h	X	X	500 mL PPE q3h
Haug [43]	NR	(1) Modified STEEN, (2) balanced electrolyte Phoxilium, or (3) Phoxilium enriched with dextran (PHODEX)	Acellular	HSA in STEEN	2,500 U/L	X	Glucose in STEEN, 0.1% D_{50}	$NaHCO_3$ in STEEN and Phoxilium	125 mg	0.0075% Insulin R	$CaCl_2$ in STEEN and Phoxilium	X	PPE at t = 1 h and t = 6 h
Haug [44]	NR	STEEN (XVIVO Perfusion AB, Göteborg, Sweden)	Acellular	HSA in STEEN	2,500 U/L	X	Glucose in STEEN, 0.1% D_{50}	$NaHCO_3$ in STEEN	125 mg	0.0075% Insulin R	$CaCl_2$ in STEEN	X	PPE at 1, 6, 12, and 18 h
Kueckelhaus [45]	5.6 L	5.6 L Perfadex (XVIVO Perfusion AB, Göteborg, Sweden)	Acellular	X	X	X	D_{40} in Perfadex	4 mL THAM at t = 0	500 mg at t = 0	30U at t = 0	X	X	4 mLD_{50}, 30 U insulin, and 500 mg methylprednisolone were replenished at 7 h
Kueckelhaus [46]	5.6 L	5.6 L Perfadex (Vitrolife, Göteborg, Sweden)	Acellular	X	X	X	D_{40} in Perfadex	4 mL THAM at t = 0	500 mg at t = 0	30U at t = 0	X	X	4 mLD_{50}, 30 U insulin, and 500 mg methylprednisolone were replenished at 7 h
Krezdorn [47]	4 L	4 L STEEN (XVIVO Perfusion AB, Göteborg, Sweden)	Acellular	HSA in STEEN	X	X	Glucose in STEEN, 4 mL D_{50}	$NaHCO_3$ in STEEN	500 mg	0.3 mL	$CaCl_2$ in STEEN	X	PPE at 1, 6, 12, and 18 h

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TABLE 3 | (*Continued*) Perfusate content.

Author (date)	TCV	Base Medium	pRBCs	Albumin	Heparin	Antibiotics	Glucose	Buffer	Methyl-prednisolone	Insulin	Calcium	Other Additives or Notes	Perfusate exchange (volume and freq)
Krezdorn [48]	NR	5.6 L "Modified" Perfadex (XVIVO Perfusion AB, Göteborg, Sweden)	Acellular	X	X	X	D_{40} in Perfadex	KH_2PO_4 in Perfadex	X	X	X	X	X
Kruit [49]	1 L	1 L University of Wisconsin solution	Acellular	X	X	X	X	KH_2PO_4 in UW	40 mg	X	X	X	X
Domingo-Pech [50]	727 mL	27.5% Lactated Ringer's solution	27.5% preserved whole blood	X	3 mg/kg initial dose	Piperacillin	20.6% Rheomacrodex (LMW dextran)	10.3% $NaHCO_3$	Prednisolone	X	$CaCl_2$ in LR	13.7% Mannitol	PPE q4h with addition of sodium heparin 5%, 3 mg/kg, Prednisolone 20mg, Piperacillin Na 1g, Nitroglycerin 5 mg
Usui [51]	NR	(1) Fluorocarbon (Fluosol-43) diluted in Lactated Ringer's or (2) Lactated Ringer's alone	Acellular	X	X	X	Glucose in Fluosol-43	$NaHCO_3$ in Fluosol-43	X	X	$CaCl_2$ in Fluosol-43 and LR	X	X
Muller [52]	NR	Heparinized autologous blood	NR	X	Heparinized	X	X	X	X	X	X	Initially flushed with synthetic colloid hydroxyethyl starch solution	X
Adil [53]	5 L	Sodium dodecyl sulfate	Acellular	X	X	X	X	X	X	X	X	X	X
Burlage [54]	500 mL	PromoCell skeletal muscle cell growth medium or HBOC-201	Acellular	10 g BSA	1 mL heparin	2 mL penicillin-streptomycin	X	X	100 µL hydrocortisone, 8 µg dexamethasone	100 µL	X	5 mL L-glutamine	X
Figueroa [55]	2500 mL	HBOC-201 or washed RBC	Hct 10%–15%	800 mL	5000U/L	500 mg vancomycin	X	X	500 mg	1U/L	2300 mg calcium gluconate	X	400 mL exchange every 3 h starting at 6 h
Gok [56]	NR	Custodiol HTK	Acellular	2.5 g	1000U	5 mg Cefazolin	X	$NaHCO_3$ 1mEq	X	X	X	X	Hemofiltration 0.1–0.3 mL/min
Goutard [57]	200 mL	Modified STEEN solution	Acellular	BSA	2000U/L	4 mL/L Penicillin-streptomycin	Glucose in STEEN	$NaHCO_3$ in STEEN	16 mg/L dexamethasone, 200 mg/L hydrocortisone	20U/L	$CaCl_2$ in STEEN	X	X
Mayer [58]	NR	HAM's solution	Acellular	BSA	X	Penicillin-streptomycin, amphotericin B	Glucose in HAM's solution	X	X	X	$CaCl_2$ in HAM's solution	L-glutamine	Hemofiltration
Rezaei [59]	2500 mL	pRBC, FFP	1200mL pRBC, 900 mL FFP	350 mL 25% albumin	5000U	250 mg vancomycin, 250 mg cefazolin	X	X	500 mg	As needed	X	X	500 mL every 3 h starting at 6 h
Stone [60]	NR	Ringer's solution	Hct 25%–30%	BSA	4000U	500 mg meropenem	30 mL 15% glucose	50 mL $NaHCO_3$	13.2 mg dexamethasone	X	$CaCl_2$ in LR	40 mL 20% mannitol GTN infusion 10 mL/hr Nutriflex infusion 10 mL/hr	X
Taeger [61]	42–50 L	Heparinized Custodiol HTK or lactated Ringer's	Erythrocyte concentrates	X	Heparinized	4g –0.5 g piperacillin-tazobactam every 3 h	X	X	X	X	$CaCl_2$ in LR	X	X
Valdivia [62]	NR	STEEN solution and Sterofundin ISO	Acellular	HSA in STEEN	X	500 µL/mL cefazolin	Glucose in STEEN	$NaHCO_3$ in STEEN	X	X	$CaCl_2$ in STEEN	0.06% sodium hydrogen carbonate	X

BSA, bovine serum albumin; D_{50}, Dextrose 50% in normal saline; HSA, human serum albumin; MW, molecular weight; $NaHCO_3$, sodium bicarbonate; NR, not reported; pRBCs, packed red blood cells; PPE, partial perfusate exchange; q#time, to indicate frequency a medication was administered; TCV, total circulating volume; THAM, trometamol or tris-hydroxymethyl aminomethane; X, not used or tested.

TABLE 4 | Contents of base media used in perfusate preparation.

Base Medium	Contents
STEEN	Albumin, D_{40}, glucose, KCl, NaCl, $CaCl_2$, $MgCl_2$, NaH_2PO_4, $NaHCO_3$, NaOH
Perfadex	D_{40}, NaCl, KCl, MgS, Na_2HPO_4, KH_2PO_4, glucose monohydrate
Phoxilium	$CaCl_2$, $MgCl_2$, NaCl, $NaHCO_3$, KCl, Na_2HPO_4
Dulbecco's Modified Eagle's Medium	Amino acids, vitamins, $CaCl_2$, $Fe(NO_3)_3$, $MgSO_4$, KCl, $NaHCO_3$, NaCl, NaH_2PO_4, dextrose
University of Wisconsin (UW) solution	Potassium lactobionate, KH_2PO_4, $MgSO_4$, raffinose, adenosine, glutathione, allopurinol, hydroxyethyl starch
Fluosol-43	FC-43, Pluronic F-68, NaCl, KCl, $CaCl_2$, $MgCl_2$, $NaHCO_3$, glucose, hydroxyethyl starch
Lactated Ringer's solution	NaCl, KCl, $CaCl_2$, sodium lactate
Ringer's solution	NaCl, KCl, $CaCl_2$, $NaHCO_3$, +/- other minerals
Custodiol HTK	NaCl, KCl, $MgCl_2$, $CaCl_2$, histidine, tryptophan, mannitol, potassium hydrogen 2-ketoglutarate
PromoCell skeletal muscle cell growth medium	Amino acids, vitamins, fetal calf serum, fetuin, EGF, bFGF, insulin, dexamethasone
HAM's solution	Amino acids, vitamins, glucose, NaCl, KCl, $CaCl_2$, $MgCl_2$, $CuSO_4$, $FeSO_4$, Na_3PO_4, $ZnSO_4$

TABLE 5 | Limb monitoring and common outcome measurements.

Author (date)	Limb monitoring	Graft weight	Potassium (mmol/L)	Lactate (mmol/L)	CK (U/L)	Mb (ng/mL)
Amin [17]	- Capillary refill, skin temp, and color Q15-60 min - Samples: Skin, muscle, vessel, at t < 0, t = end	X	6.3 ± 0.5	X	X	X
Amin [35]	- Capillary refill, skin temp, and color Q15-60min - Samples: Skin, muscle, vessel, at t < 0, t = end	NT at 70 mmHg: –0.3% ± 1.7%	NT at 70 mmHg: 7.0 ± 1.7	NT at 70 mmHg: 15.1 ± 4.8	X	X
Gok [36]	- Samples: 100 mg gastrocnemius sample, flash frozen, and stored at –80°C - Metabolomics profiling	+3.1% ± 0.4%	Increased; 6.3 ± 1.2	Increased; 4.3 ± 1.3	X	X
Werner [37]	- Palm skin temp Qh - Median and ulnar nerve electrical stimulation Q2h - Samples: Flexor carpi radialis samples at 0, 12, and 24 h of perfusion	–0.4% (–7%-+7%)	Varied, 3.0–5.5	Steadily increased from 5 to 15	X	43 K at t = 0; 92 K at t = 24 h
Ozer [38]	- Capillary refill - Skin temp - Functional electostimulation Qh; Single-fiber contractility testing - Samples: Muscle biopsies, 10 mm x 5 mm	+20% after perfusion; decreased to +15% after transplantation	Stable, no change after transplantation	Increased steadily during perfusion, no change after transplantation	X	X
Ozer [39]	- Functional electostimulation Qh; Single-fiber contractility testing - Samples: Muscle biopsies, 10 mm x 5 mm	Significant gain after perfusion; No significant gain after transplantation	Stable	Gradual increase during perfusion, normalized after transplantation	X	X
Constantinescu [40]	- Capillary refill Qh - Electrical stimulation of 3 proximal nerve bundles - Skin and muscle color Qh; Compartment pressure - Samples: Muscle, nerve, vessel biopsies at t = end; Immunofluorescence staining	Maximum of +1.32%	4.27 ± 1.38	16.83 ± 2.46	X	X
Fahradyan [41]	- Peripheral perfusion via ICG angiography, t = end - Muscle surface temp Qh - Muscle and motor nerve electrical stimulation and contractility	12 h group: –1.28% ± 8.59% >24 h group: +7.28% ± 15.05%	12 h group: 5.7 ± 1.7 >24 h group: 6.5 ± 1.8	12 h group: 9.2 ± 4.4 >24 h group: 9.6 ± 4.7	12h group: 53 K ± 15 K >24 h group: 64 K ± 32 K	12 h group: 875 ± 294 >24 h group: 1134 ± 538

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TABLE 5 | (*Continued*) Limb monitoring and common outcome measurements.

Author (date)	Limb monitoring	Graft weight	Potassium (mmol/L)	Lactate (mmol/L)	CK (U/L)	Mb (ng/mL)
	- Flexor and extensor compartment pressure - Samples: Muscle biopsies					
Duraes [42]	- Peripheral perfusion, ICG angiography, t = end - Muscle temp - Muscle and motor nerve electrical stimulation and contractility - Flexor and extensor compartment pressure, Tissue O_2 sat - Samples: Muscle, skin, and nerve biopsies collected at 0 and 12 h	12 h 39°C colloid/ wRBC: +0.54% ± 7.35%	12 h 39°C colloid/ wRBC: 5.4 ± 1.1	12 h 39°C colloid/ wRBC: 9.4 ± 2.4	12h 39°C colloid/ wRBC: 53 K ± 15 K	12 h 39°C colloid/ wRBC: 875 ± 291.4
Haug [43]	- Samples: Muscle biopsies, hematoxylin/eosin stain, HIF-1α Western blot	SCS: +3% STEEN: +25% Phoxilium: +58% PHODEX: +36%	Decreased during first 1–2 h, increased to 6 h, stable to 12 h	Decreased during first 1–2h, increased to 6h, stable to 12 h	STEEN: +1.2 K Phoxilium: +1.5 K PHODEX: +5.5 K	STEEN: +1 Phoxilium: +121 PHODEX: +140
Haug [44]	- Samples: Muscle biopsies, HIF-1α Western blot - Cytokine analysis with ELISA	SCS: +1.4% Perfusion: +4.3%	9.6 (0 h) 5.77 (24 h)	6.9 (0 h) 2.8 (24 h)	1.4 K (0 h) 4 K (24 h)	4.4 K (0 h) 9 K (24 h)
Kueckelhaus [45]	- Samples: Muscle biopsy, histology, TEM - PCR quantification of hypoxia/ischemia markers, cytokine assay	SCS: None Perfusion: +10% ± 2%	Peaked at 3 h perfusion; SCS>perfusion after transplant	Perfusion: Increased steadily to 2.43 mM	X	Peaked at 3 h perfusion; SCS>perfusion after transplant
Kueckelhaus [46]	- Samples: Muscle biopsy, histology	SCS: None Perfusion: +44.06%	5.73 (0 h) 9.35 (12 h)	X	X	X
Krezdorn [47]	- ATP and glycogen assay - 3-Tesla MRI of muscle changes - Samples: Muscle biopsy histology	+40%	Increased during perfusion; decreased after replantation	Increased during perfusion; increased in 3 h after replantation	X	Increased during perfusion; decreased after replantation
Krezdorn [48]	- Samples: Muscle biopsies after replant, histology - PCR of genes involved in glycolysis, angiogenesis, and DNA damage	X	X	X	X	X
Kruit [49]	- Muscle core temp - Nerve stimulation, muscle contractility - Samples: Flexor and extensor muscle histology	SCS: +1.6% Perfusion: –2.7%	SCS and perfused limb potassium increased after replantation (P = 0.4), remained wnl	0.7 (18 h), remained low throughout perfusion, similar to SCS (P = 0.4)	15.6 K (18 h), higher in perfused group than SCS after replantation (P < 0.01)	X
Domingo-Pech [50],	- Samples: Muscle biopsy, H&E stain	+20–50%	X	X	X	X
Usui [51]	- *In vivo* basic metabolic panel, enzymes	Continuous perfusion with fluorocarbon: –3.4% ± 1.2% after perfusion; +26.8 ± 2.7 after replant	After replant: Immediate marked increase, stable after 30 min	After replant: Immediate marked increase, normal at 6 h only in continuous perfusion with fluorocarbon group	X	X
Muller [52]	- Samples: muscle biopsy, peripheral nerve biopsy, blood vessel biopsy, histology - Inflammatory markers, serum complement activity	X	X	X	X	X
Adil [53]	- Samples: muscle, nerve, bone, skin, vessels	X	X	X	X	X
Burlage [54]	- Samples: muscle biopsy	HBOC-201: +4.9 g BSA: +48.8 g BSA/PEG: +27.3 g	HBOC-201: 5.8 after 1 h BSA: 1.8 after 1 h BSA/PEG: 4.4 after 1 h	X	X	X

(Continued on following page)

TABLE 5 | (*Continued*) Limb monitoring and common outcome measurements.

Author (date)	Limb monitoring	Graft weight	Potassium (mmol/L)	Lactate (mmol/L)	CK (U/L)	Mb (ng/mL)
			Initially increased, stabilized after 3 h			
Figueroa [55]	- Samples: muscle biopsy - ICG angiography - Compartment pressures	HBOC-201: +23.10% ± 3.00% RBC: +13.18% ± 22.70%	HBOC-201: 6.45 ± 1.69 RBC: 6.78 ± 1.94	HBOC-201: 14.66 ± 4.26 RBC: 13.11 ± 6.68	X	X
Gok [56]	- Samples: muscle biopsy - Muscle contractility	+3.5%	X	<2	X	X
Goutard [57]	- Samples: skin, muscle	X	Decreased over 3 h	Decreased over 3 h	X	X
Mayer [58]	X	X	X	X	X	X
Rezaei [59]	- Samples: muscle biopsy Muscle and nerve functionality - Compartment pressures	+0.4% ± 12.2%	7.6 ± 0.9	20 at median time point 15 h	956 within 1 h, 49020 at endpoint	5370 initially, 34730 at endpoint
Stone [60]	- Samples: muscle, skin, vessel - Thermal imaging	X	X	Limb/Kidney: 10.9 ± 3.5 after 1h, 7.5 ± 1.7 at endpoint Limb Only: 14.6 ± 2.2 after 1 h, 13.8 ± 3.7 at endpoint	X	X
Taeger [61]	X	X	X	X	X	X
Valdivia [62]	- Samples: skin, muscle, vessels - Bioluminescence detection - Cell phenotyping	X	X	Vector: 562.3 ± 38.9 μM Non-Vector: 577 ± 26.8 μM	X	Vector: 224.9 ± 10.3 ng/mL Non-Vector: 222.9 ± 44.8 ng/mL

C, continuous monitoring; CK, creatine kinase; Hb, hemoglobin; ICG, indocyanine green; Mb, myoglobin; PPE, partial perfusate exchange; TEM, transmission electron microscopy; wnl, within normal limits.

CONCLUSION

VCA EVMP is a versatile platform through which grafts may be preserved and optimized prior to replantation or replantation. There is significant evidence to suggest that EVMP may be superior to SCS as a preservation method. While methods greatly varied throughout the literature reviewed, the major factors of each perfusion protocol remained the same: temperature, perfusate composition, and perfusion time. As in solid organ transplant perfusion, there is currently no consensus on the optimal temperature for VCA perfusion. Studies reviewed in this paper showed promising results for both HMP and NMP/NNT, and no recent evidence has definitively suggested the benefit of one temperature over the other. Rather than attempting to condense VCA EVMP down to a singular optimal perfusion protocol, perfusion factors should be chosen and adapted based on the individual needs and goals of each future study. For instance, the choice of a blood-based perfusate might be more suitable for NMP given the higher metabolic rate, or for a shorter perfusion duration given the limitations of obtaining and storing blood. An acellular perfusate might be more suitable for HMP given the lower metabolic rate, or for a longer perfusion duration to facilitate perfusate exchange. Overall, preclinical studies offer promising results regarding the feasibility of VCA preservation via machine perfusion, but additional experimental studies are needed to overcome technical barriers to clinical translation.

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

TM manuscript writing, literature review, data analysis. ED manuscript writing, literature review, data analysis. AL manuscript writing, literature review, data analysis. YG literature review, manuscript review. YZ literature review, manuscript review. CL literature review, manuscript writing, manuscript review. AG literature review, manuscript writing, manuscript review. IL literature review, manuscript review. BH literature review, manuscript writing, manuscript review. RK literature review, manuscript writing, manuscript review. BO study conceptualization, literature review, manuscript review. GB study conceptualization, literature review, manuscript review. 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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Tocilizumab-Based Treatment of Microvascular Inflammation in Kidney Transplant Recipients: A Retrospective Study

Johan Noble[1,2,3†], Giorgia Comai[4,5†], Valeria Corredetti[4†], Reda Laamech[1†], Celine Dard[6], Thomas Jouve[1,2], Diane Giovannini[7], Audrey Le Gouellec[8], Shivani Wadnerkar[3], Paolo Cravedi[3], Della Apuzzo[4,5], Daniele Vetrano[4,5], Marco Busutti[4], Chiara Abenavoli[4,5], Paolo Malvezzi[1], Lionel PE Rostaing[1,2†] and Gaetano Lamanna[4,5†]*

[1]Nephrology, Hemodialysis, Apheresis and Kidney Transplantation Department, University hospital Grenoble, Grenoble, France, [2]Univ. Grenoble Alpes, CNRS, Inserm, U 1209 CNRS UMR 5309, Team Epigenetis Immunity, Metabolism, Cell Signaling and Cancer, Institute for advanced Biosciences, Grenoble, France, [3]Precision Immunology Institute, Translational Transplant Research Center (TTRC), Icahn School of Medicine at Mount Sinai, New York, NY, United States, [4]Nephrology, Dialysis and Kidney Transplant Unit, IRCCS-Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, [5]Department of Medical and Surgical Sciences (DIMEC), Alma Mater Studiorum-University of Bologna, Bologna, Italy, [6]Établissement Français du Sang Auvergne-Rhône-Alpes, HLA and immunogenetics Laboratory, Grenoble, France, [7]Anatomopathology Department, University hospital Grenoble, Grenoble, France, [8]Université Grenoble Alpes, CNRS, Grenoble INP, CHU Grenoble Alpes, TIMC-IMAG, Grenoble, France

Chronic-active antibody mediated rejection (caAMR) is the leading causes of long-term kidney graft failure. Tocilizumab (TCZ), an anti-IL-6 receptor antibody, has been suggested as a treatment, but data are conflicting. We retrospectively studied consecutive adult kidney transplant recipients with caAMR or microvascular inflammation (MVI) without Donor-Specific Antibodies (DSA) and without C4d deposition (MVI + DSA-C4d-), who received TCZ as first-line therapy in two European centers. Estimated glomerular filtration rate (eGFR) and DSA were assessed one-year before and after TCZ initiation. The study included 64 patients who received TCZ between July 2018 and September 2023. The eGFR trajectory significantly decreased after TCZ treatment (–1.2 ± 0.2 vs. 0.03 ± 0.2 mL/min/1.73 m^2/month pre- vs. post-TCZ, respectively; p = 0.001). The percentage of patients with DSA decreased from 63.9% to 38.9% (p < 0.001), and the average MFI decreased from 9,537 to 7,250 (p = 0.001). In multivariate analysis, younger age (OR = 0.95, p = 0.02), MVI + DSA-C4d- phenotype (OR = 5.2, p = 0.01), and lower chronic glomerulopathy score (OR = 4.5, p = 0.02) were associated with TCZ response (trajectory ≥0 after TCZ). Patient survival was 98.4%, and graft survival was 93.7% at

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***Correspondence**
Johan Noble,
jnoble@chu-grenoble.fr

†These authors have contributed equally to this work

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Abbreviations: AMR, Antibody-Mediated Rejection; caAMR, chronic active AMR; cg, Chronic Glomerulopathy; CKD-Epi, Chronic Kidney Disease-Epidemiology collaboration equation; CLZ, Clazakizumab; CMV, Cytomegalovirus; DSA, Donor-Specific Antibody; eGFR, estimated Glomerular Filtration Rate; g, glomerulitis; HLA, Human Leukocyte Antigen; iDSA, immunodominant Donor-Specific Antibody; IL-6, Interleukin-6; IL-6R, Interleukin-6 Receptor; KT, Kidney Transplantation; MFI, Mean fluorescence Intensity; Ptc, Peritubular capillaritis; RCT, Randomize Clinical Trial; TCZ, Tocilizumab; UACR, Urine Albumin-To-Creatinine Ratio.

one-year. First-line TCZ therapy for caAMR or MVI + DSA-C4d- is associated with an improvement of eGFR trajectories, reduced DSA numbers and MFI and histological inflammation in glomeruli. These data suggest a potential benefit of TCZ in these settings.

Keywords: kidney transplantation, tocilizumab, microvascular inflammation, donor-specific antibody, chronic-active antibody-mediated rejection

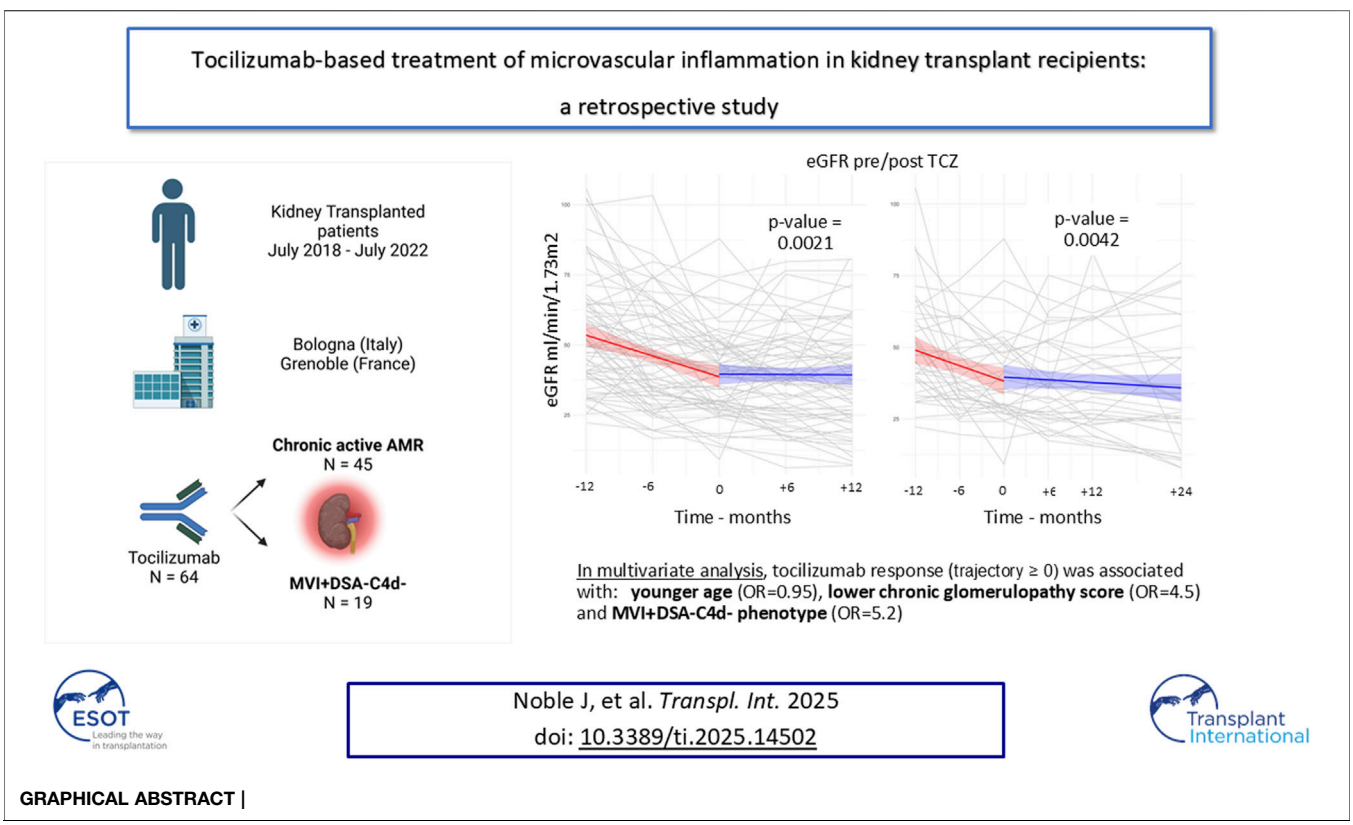
INTRODUCTION

Chronic active Antibody-mediated rejection (caAMR) is a leading cause of immune-mediated allograft failure after kidney transplantation (KT) [1–3]. The key histological features of caAMR diagnosis demonstrate signs of a recent interaction between alloantibodies and vascular endothelium, i.e., microvascular inflammation (MVI) [glomerulitis(g) and peritubular capillaritis (ptc)], with chronic features such as transplant glomerulopathy (cg > 0) and/or severe peritubular capillary basement membrane multilayering [4, 5]. caAMR is primarily driven by the development of donor-specific antibodies (DSA), which can trigger complement activation and subsequent allograft injury due to direct endothelial cell damage and the recruitment of innate immune cells such as neutrophils, macrophages, and natural killer (NK) cells, which further exacerbate inflammation and tissue damage [6–8].

CaAMR appears to be a more complex and polymorphic entity, as some patients present with microvascular inflammation without DSA or C4d staining [9]. In 2022, the 16th Banff meeting for allograft pathology defined new phenotypes in patients presenting MVI: MVI-positive, DSA-negative and C4d-negative (MVI + DSA-C4d-) and probable AMR in patients DSA + but MVI below the threshold (i.e., g + ptc <2) [10]. In 2024, Sablik et al. showed that after reclassifications of patients according to the new Banff classification, those two newly defined phenotypes exhibit a worse graft survival at 5 years of the biopsy compared to the patients classified without AMR [9]. The physiopathology of MVI + DSA-C4d- lesions may be driven by NK cells and T-cells rather than antibodies [11–13].

Treatment of these conditions remains a major unmet need [14]. Despite recent evidence from a pilot safety phase II randomized clinical study showing efficacy of anti-CD38 depleting antibody on histological remission and renal function in 9 of 11 active and caAMR patients (defined according to 2019 Banff classification), there is no consensus on the optimal treatment [15].

Interleukin-6 (IL-6) is a pleiotropic cytokine implicated in promoting the activation and expansion of B and T cells,



GRAPHICAL ABSTRACT |

especially T-follicular helper cells, and in the initiation of the acute phase inflammatory response [16]. IL-6 also inhibits the induction of regulatory T cells and promotes their conversion into Th17 cells [16]. Blockade of the IL-6/IL-6 receptor axis, a well-established concept for the treatment of autoimmune diseases, is an attractive option to treat MVI progression through its effect on antibody secretion, T-cell regulatory/effector balance, and endothelial activation by DSA [17, 18].

Experimental data from a mouse model of skin transplantation have shown that IL-6 blockade decreased antibody rebound after a second skin graft, reduced pro-inflammatory cytokine, and increased regulatory T-cells [19].

Tocilizumab (TCZ, Actemra/RoActemra, Roche/Genentech, San Francisco, CA), an anti-IL-6 receptor humanized monoclonal antibody, has been proven to decrease immunodominant DSA (iDSA), C4d, microvascular inflammation and to stabilize transplant glomerulopathy in retrospective studies [20–22]. Those study included small retrospective, heterogeneous and monocentric cohorts. To date, there are no randomized studies assessing its efficacy in caAMR or MVI + DSA-C4d- [22–25]. The INTERCEPT study is a controlled open-label multi-center randomized clinical trial (RCT) in KT recipients to compare the efficacy of TCZ in caAMR. This study is still under recruitment [26].

A phase 2 randomized trial to assess the safety and efficacy of Clazakizumab (CLZ), an anti-IL-6 antibody in late antibody-mediated rejection showed a significant decrease of DSA MFI but failed to find a significant benefit in molecular and histologic scores of rejection [27]. Besides, CLZ was able to improve the estimated glomerular filtration rate (eGFR) trajectory, but it was associated with a high risk of gastrointestinal perforation. Therefore, the phase 3 IMAGINE trial tested a lower CLZ dose to reduce toxicity in accordance with the phase 2 trial gastrointestinal complications. However, it was discontinued early due to futility [28].

This is a retrospective multicentric study in two independent European transplant centers using TCZ as first-line therapy of KT patients with MVI including caAMR and MVI + DSA-C4d-.

MATERIALS AND METHODS

Population Study

In this multicenter, retrospective study, we assessed all consecutive adult KT recipients between July 2018 and July 2022 from nephrology and KT departments of Grenoble (France) and Bologna (Italy). The study included all patients with histological signs of caAMR or MVI + DSA-C4d- according to the 2022 Banff classification and that received intravenous TCZ at a dose of 8 mg/kg monthly and/or subcutaneous at the dose of 162 mg/15 days. Patients presenting *de novo* or recurrence of glomerulopathy, acute tubular necrosis were excluded. In Grenoble, surveillance kidney biopsies were performed 1-year after starting TCZ.

Patients were monitored for renal function (eGFR calculated using the CKD-EPI formula), urine albumin-to-creatinine ratio (UACR) (g/g), adverse and severe adverse events from treatment, graft- and patient-survival rates, and DSA levels every 3 months. Patient were all followed at least 1 year following MVI treatment initiation and data were collected at last available follow-up.

TCZ was given off-label in the absence of validated therapeutic alternative. Regarding retrospective data assessment, all patients signed an informed consent form. The study was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments. All medical data were collected from Grenoble database [CNIL (French National committee for data protection) approval number 1987785v0] and from Bologna KT Center informatics repository for clinical data (protocol number 244/2023/Sper/AOUBo).

Immunosuppression Regimen

In both centers, all patients received induction with antithymocyte globulins or Basiliximab, methylprednisone (500 mg), and started peri-operatively with 1 g of mycophenolate mofetil or 720 mg mycophenolic acid.

Maintenance immunosuppression after KT consisted of tacrolimus or cyclosporine associated with mycophenolate mofetil/mycophenolic acid or everolimus. In Grenoble, steroids were rapidly tapered until withdrawal at month-3 except for highly sensitized patients (PRA more than 75%), patients who had experienced a previous acute rejection, patients with IgA nephropathy, or patients with circulating DSAs. In Bologna, steroids were rapidly tapered and maintained at prednisone 5 mg per day.

Pathological Assessment of Graft Biopsy

MVI-positive biopsies, including caAMR and MVI + DSA-C4d- were diagnosed on biopsies performed for clinical indications, i.e., rising serum creatinine and/or *de novo* proteinuria and/or *de novo* DSA detection. C4d staining was performed for all biopsies. The kidney-graft sample was processed for light microscopy by fixing in alcohol-formol-acetic acid (AFA, fluid and embedded in paraffin). Specimens were stained with trichrome blue, hematoxylin eosin and safran, periodic acid-schiff reagent, C4d staining, and immunofluorescence studies, including direct immunofluorescence for immunoglobulins, immunoglobulin light chains, C3 and C1q fractions of the complement. The results of biopsies were all re-assessed according to the 2022 Banff classification of rejection [10].

Anti-HLA Antibody Measurement

DSA were monitored at the time of each kidney biopsy, i.e., at MVI diagnosis and at 1-year post treatment. Screening of HLA Class I and II DSA in recipients was performed on sera using a Luminex platform with two different single antigen bead assay kits in the HLA department of Grenoble and of Bologna (Luminex Single Antigen assay, Immucor, Norcross, GA, United States and LABScreen, One Lambda Inc, Canoga Park, CA, United States) Screening and single-antigen assessment were performed every 6 months post-transplant and systematically at the time of a kidney biopsy. The retained mean fluorescence intensity (MFI) values corresponded to the manufacturers background corrected MFI value. Positivity for the Luminex analysis was defined as an MFI > 500.

Interleukin-6 Measurement

IL-6 was assayed on frozen sera at the time of MVI diagnosis (before any treatments) and at 1-year post treatment initiation. In Grenoble, IL-6 quantitative dosage in sera was performed using a Lumipulse G600II system (Fujirebio, Tokyo, Japan).

Statistical Analysis

The primary endpoint was the eGFR trajectory before and after TCZ initiation. To calculate and compare the trajectories of eGFR before and after TCZ, a mixed linear regression model with random intercepts was employed. The model included an interaction term between the time variable and the period variable, which allowed for the evaluation of differences in the trajectories between the "Before" and "After" periods. Five patients were diagnosed MVI-positive within the first-year post-transplantation. For the calculation of GFR trajectory, while most of studies remove patients from with end-stage renal disease from the eGFR trajectories, we assigned an eGFR value of 10 mL/min/1.73 $m^2$for patients who experienced graft loss during follow-up. This value was chosen to reflect a realistic approximation of kidney function at the time of progression to end-stage kidney disease while maintaining the continuous nature of the variable. This approach avoids the introduction of extreme imputation values (e.g., 0 mL/min/1.73 m^2 of eGFR), which could disproportionately skew the trajectory analysis, particularly for patients already in advanced stages of chronic kidney disease at baseline.

Secondary endpoints were the one-year death-censored graft survival rate, proteinuria (g/g of creatininuria) and DSA. Six patients were not classified because of a follow-up shorter than 1-year and were excluded of the analysis. Thirty-three patients did not reach 2-year of follow-up and were excluded for the 2-year eGFR trajectories analysis. Evolution of histological biopsies was analyzed only in the Grenoble-Alpes hospital where patients had follow-up biopsies at 1-year.

Continuous variables are presented as means ± standard deviations (SD) or as medians with quartiles [Q1–Q3] in cases of high dispersion. Qualitative data are reported as the numbers of patients/events and percentages. The Wilcoxon or the Kruskal--Wallis tests were used for continuous variables, the chi-squared test was used for categorical data. For paired categorical data we used the Stuart-Maxwell test. For graft survival analyses, a multivariate generalized logistic-regression model was run. A two-sided *p*-value of <0.05 was considered statistically significant. Statistical analyses were conducted using R statistical software [29].

RESULTS

Baseline Characteristics

Between July 2018 and July 2024, 64 patients presented histological lesions of MVI on a for cause kidney biopsy performed after a median period of 58 [16–130] months post-transplantation. Patients' age was 48.6 ± 14 years; female/male ratio was 0.4. Most of KT were deceased donor transplants (83.3%). The baseline characteristics of the cohort in both categories of MVI (i.e., caAMR and MVI + DSA-C4d-) are reported in **Table 1**. Chronic glomerulopathy lesions (cg score of ≥1) were present in 53.1% of patients, glomerulitis lesions (g score of ≥1) in 81.2%, and peritubular capilaritis (ptc score of ≥1) in 82.3% of patients. C4d staining was positive in 26.5% of patients. DSA were positive in 40 of patients (62.5%). eGFR at the time of biopsy was similar in caAMR and MVI + DSA-C4d- groups (41.1 ± 15 versus 37.9 ± 17, p = 0.378 respectively). We also compared the patients in both centers (**Supplementary Table S1**). Patients from Grenoble versus Bologna were significantly younger (44.8 versus 53.3 years, p = 0.02), received more often antithymoglobulin (100% versus 44.4%, p < 0.001), had less DSA at the time of biopsy (48.7% versus 84%, p = 0.01) and had earlier MVI diagnosis (median 35 months post-KT versus 154 months, p < 0.001).

Kidney Function

eGFR at the time of biopsy (before TCZ treatment) was 39.4 ± 16 mL/min/1.73 m^2. The eGFR 1-year before TCZ treatment was 54.0 ± 19 mL/min/1.73 m^2 and at 1 year after TCZ treatment was 40.8 ± 18 mL/min/1.73 m^2. Among thirty-one patients (43%) with a follow-up of 2 years post-treatment, last eGFR was 43.6 ± 18 mL/min/1.73 m^2.

The linear mixed-effect model of the eGFR trajectory showed a significant difference between the pre- TCZ and post- TCZ period: -1.2 ± 0.2 vs. +0.03 ± 0.2 mL/min/1.73 m^2/month, respectively; p = 0.001 (**Figure 1A**). When considering only the patients with 2 years of follow-up, eGFR trajectory decreased from −1.2 ± 0.2 mL/min/1.73 m^2/month before TCZ versus −0.15 ± 0.1 mL/min/1.73 m^2/months after 2 years TCZ, p < 0.001 (**Figure 1B**).

eGFR trajectory was then evaluated according to the Banff 2022 classification. In caAMR phenotype patients, the eGFR trajectory significantly decreased from −0.9 ± 0.3 mL/min/1.73 m^2/month before TCZ to −0.2 ± 0.3 mL/min/1.73 m^2/month after TCZ, p = 0.038), as shown in **Supplementary Figure S1A**. In MVI + DSA-C4d- patients, there was also a significant improvement of eGFR trajectory after TCZ introduction (−1.9 ± 0.5 mL/min/1.73 m^2/month before TCZ versus +0.5 ± 0.5 mL/min/1.73 m^2/month after TCZ, p = 0.007) (**Supplementary Figure S1B**).

In both centers, the improvement of eGFR trajectory post TCZ was significant: -1.4 ± 0.5 mL/min/1.73 m^2/month before TCZ versus +0.1 ± 0.2 mL/min/1.73 m^2/month after TCZ, p = 0.004 in Grenoble and 0.6 ± 0.3 mL/min/1.73 m^2/month before TCZ versus −0.5 ± 0.2 mL/min/1.73 m^2/month after TCZ, p = 0.03 in Bologna (**Supplementary Figure S2**).

In the whole cohort, albuminuria/creatininuria ratio declined from 1.0 ± 1.3 g/g to 0.6 ± 0.7 at 1-year after TCZ (p = 0.033; **Figure 2A**).

Prediction of Response to Treatment

To evaluate the predictors of TCZ response, patients whose eGFR did not decrease (trajectory ≥0) between baseline and 1-year post treatment were defined as "responders" to TCZ, whereas patients whose eGFR decreased after 1-year were defined as "non-responders." According to this definition, twenty-five (37.8%)

TABLE 1 | Baseline characteristics.

	Patient with TCZ as a first line therapy for MVI N = 64		
Variables	**caAMR N = 45**	**MVI + DSA-C4d-N = 19**	**p-value**
Age – years	48.8 ± 14	46.4 ± 14.7	0.562
Female Gender - N (%)	17 (37.8%)	9 (47.4%)	0.475
Pre-emptive transplantation – N (%)	2 (4.4%)	4 (21.1%)	0.037
Nephropathy– N (%)			0.778
PKD	10 (22.2%)	5 (26.3%)	
Diabetes	4 (8.9%)	2 (10.6%)	
Vascular disease	3 (6.6%)	1 (5.3%)	
Autoimmune	5 (11.1%)	2 (10.6%)	
Unknown	15 (33.3%)	7 (36.9%)	
Other	6 (13.3%)	4 (21%)	
Induction therapy			0.325
Antithymoglobulin	35 (77.7%)	17 (89.4%)	
Basiliximab	10 (22.2%)	2 (10.5%)	
Living donor – N (%)	8 (17.8%)	3 (15.8%)	0.847
Serum creatinine at the time of biopsy - µmol/L	177 ± 54.8	191 ± 130	0.234
eGFR at the time of biopsy – mL/min/1.73m^2	41.1 ± 15	37.9 ± 17	0.378
Albuminuria at the time of biopsy – g/g of creatininuria	0.9 ± 1.1	1.4 ± 1.7	0.61
Time after transplant - months	34.8 [15–89]	28.2 [5–57]	0.217
Immunosuppression at the time of biopsy			0.067
- Tacrolimus	32 (72.7%)	18 (94.7%)	
- Cyclosporine	11 (25.0%)	1 (5.3%)	
- MMF	38 (88.4%)	14 (73.7%)	
- Everolimus	2 (4.7%)	5 (26.3%)	

AMR, anitbody mediated rejection; DSA, Donor-specific antibody; cg, chronic glomerulopathy; eGFR, estimated glomerular filtration rate; MVI, microvascular inflammation.

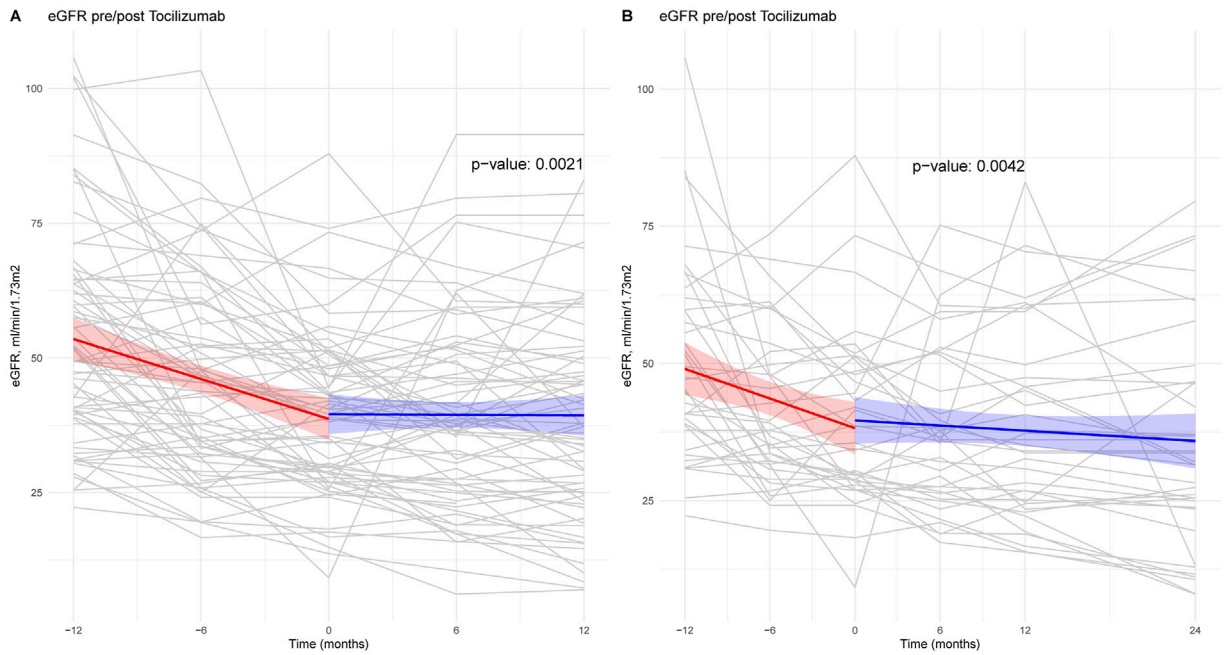


FIGURE 1 | Estimated glomerular filtration rate trajectories before versus after Tocilizumab treatment. Panel **(A)** shows the mixed linear regression between – 12 months and +12 months post-tocilizumab. Panel **(B)** shows the mixed linear regression between – 12 months and +24 months post-tocilizumab. Grey curves represent patient's eGFR evolution during each period of follow-up. Time "0" corresponds to the introduction of Tocilizumab to treat antibody-mediated rejection. The p-value for the comparison of the two models, indicating the statistical significance of the difference between the two periods: pre and post tocilizumab.

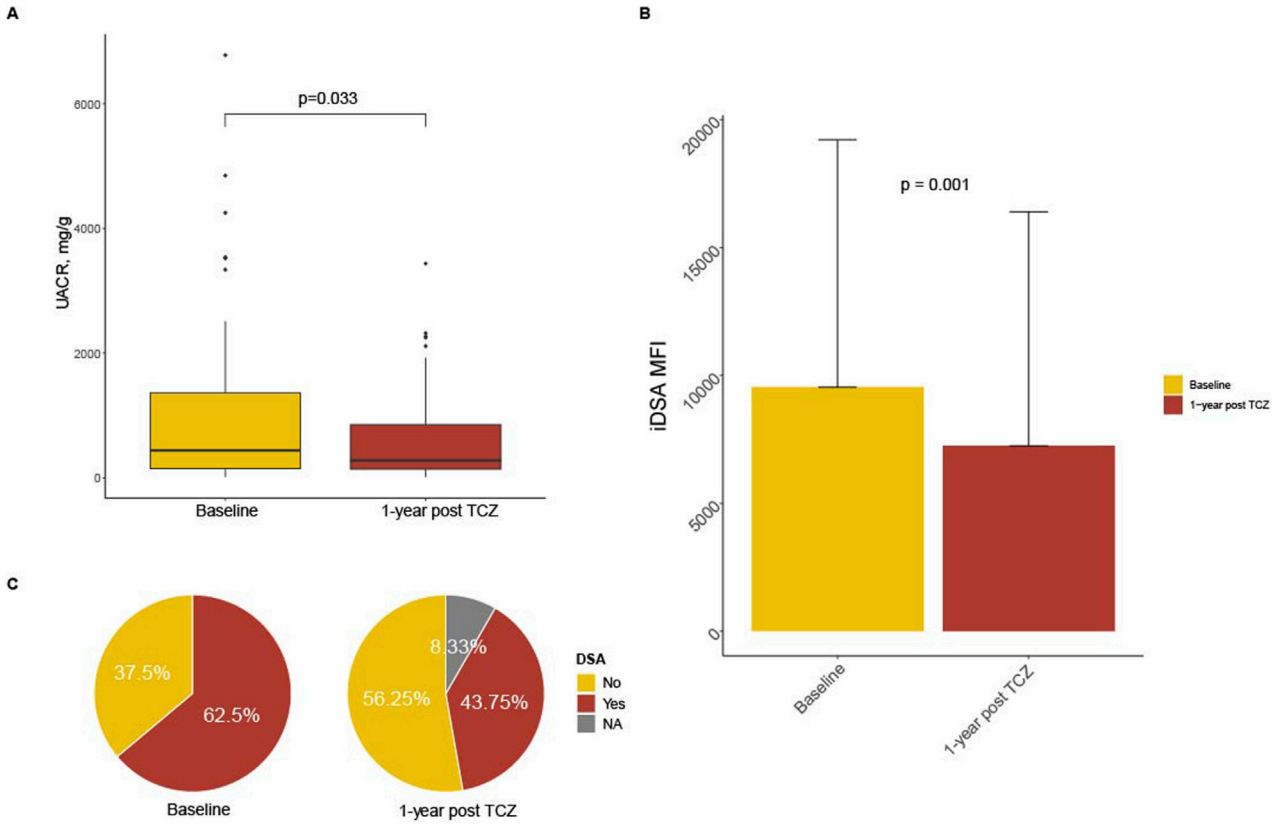


FIGURE 2 | Evolution of Donor-specific antibodies (DSA) and albuminuria post tocilizumab (TCZ). Panel **(A)** shows boxplots of urinary albuminuria over creatininuria (mg/g) at baseline and at 1-year post TCZ. Panel **(B)** shows the MFI of the immunodominant DSA (iDSA) at the time of diagnosis (baseline) and after 1-year post TCZ (median and SD). Panel **(C)** chows Pie chart of the presence of at least one DSA at the time of diagnosis (baseline) and after 1-year post TCZ treatment.

TABLE 2 | Univariate logistic regression and multivariate Cox regression analysis of factors associated with TCZ response.

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age	0.97 [0.96–0.98]	<0.035	0.95 [0.91–0.99]	0.022
Gender (reference: female)	1.13 [0.78–1.64]	0.507		
Pre-emptive transplant	0.27 [0.03–1.49]	0.148		
Donor status	2.27 [0.61–23.10]	0.160		
Induction therapy (reference: thymoglobulin)	3.26 [0.72–8.84]	0.219		
Transplant glomerulopathy score	3.45 [1.14–11.9]	0.035	4.49 [1.29–18.96]	0.025
C4D deposition (yes/no)	0.60 [0.17–1.90]	0.406		
MVI + DSA-C4d- phenotype (reference: AMR phenotype)	3.58 [1.24–10.84]	0.019	5.25 [1.55–20.71]	0.010
iDSA MFI	1.0 [0.99–1.00]	0.906		
IL6 dosage	0.97 [0.84–1.09]	0.706		
eGFR	0.98 [0.95–1.01]	0.371		
Albuminuria	1.00 [0.99–1.00]	0.353		

*Responder to TCZ are patients whose eGFR did not decrease (trajectory ≥0) between baseline and 1-year post treatment. TCZ: tocilizumab; *p < 0.05. Mean ± SD.*

patients were classified as "responders" and 41 (62%) patients were "non-responders". In univariate analysis the factors that resulted to be significantly associated with TCZ response were younger age (OR = 0.97 [0.96–0.98], p = 0.035), lower chronic glomerulopathy lesions in the initial biopsy (OR = 3.45 [1.14–11.9], p = 0.035), and the MVI + DSA-C4d- phenotype (OR = 3.58 [1.24–10.8], p = 0.019). In the multivariate analyses, younger age (OR = 0.9 [0.91–0.99], p = 0.022), lower score of chronic glomerulopathy (OR = 4.49 [1.29–18.9], p = 0.025) and MVI + DSA-C4d- phenotype (OR = 5.25 [1.55–20.71], p = 0.01) remained significantly associated with the 1-year response to TCZ (**Table 2**).

Graft and Patient Survival

At 1-year post-TCZ, patient survival was 98.4% and graft survival was 93.75%. During the follow up, one patient died (due to gastric cancer 48 months after TCZ start and one patient discounted TCZ, documented by a 4-fold increase over the baseline of AST and ALT blood levels. At last-follow-up visit post-TCZ, patient survival was 98.4% and graft survival was 85.9%.

The patients who lost their graft during the first year were significantly older compared to the rest of the cohort (61.4 ± 15 vs. 47.9 ± 14 years-old, p = 0.016), were diagnosed for MVI after a longer period post-transplantation (16.4 years [12–20] versus 4.8 [1–10.3] years, p = 0.003) and had a higher UACR at the time of biopsy (1.5 ± 0.9 vs. 0.9 ± 1.3 g/g, p = 0.024). There was no difference in the initial histological severity between patients who developed graft loss and the others. All patients who experienced transplant failure had received their kidney grafts from deceased donors, while 9.4% of grafts of the rest of the cohort were from living donors, a difference that did not reach statistical significance (p = 0.180). At last follow-up visit of 19.7 [13–32] months, 15 patients (20.8%) had lost their graft after a mean time of 13.4 ± 10 months.

DSA Changes After Starting Tocilizumab Therapy

At the beginning of TCZ treatment, the median MFI of iDSA was 9,537 [1,426–15,075] and, at 1-year post treatment, decreased to 7,250 [3,100–14,975] (p = 0.001) (**Figure 2B**). Forty (62.5%) patients had at least a positive circulating DSA before TCZ treatment and, at 1-year post treatment, 28 (38.9%) of patients had a positive DSA (p < 0.001) (**Figure 2C**). Most of those DSA were class II (84.3%). Patients referred as "responders" were more often DSA negative (72.5% versus 45.8%, p = 0.03), but the average MFI of the iDSA was not statistically different between "responders" and "non-responders": 3,400 [1,265–19,175] versus 10,000 [2,912–13,900] respectively, p = 0.648. There was no statistical difference for DSA presence or iDSA MFI between patients who lost their graft versus those with a functioning graft at 2-year post- TCZ.

Interleukin-6

Serum specimens were available for 25 patients before and at 1-year post-treatment. In these patients, IL-6 levels significantly increased at 1 year after treatment initiation (**Supplementary Figure S3**). Baseline IL-6 levels were tendentially higher (although not significant) the "non-responders" than in the "responders" (7.1 ± 10 versus 5.9 ± 3.1 pg/mL, p = 0.118). Lastly, there was no association between serum IL-6 levels at baseline and graft loss (not shown).

Histological Evolution

All patients of Grenoble had follow-up kidney biopsies. **Figure 3** shows the evolution of g scores, ptc scores, g + ptc scores, c4d staining and chronic glomerulopathy lesions (cg) between the first biopsy with microvascular inflammation (MVI) diagnosis and 1-year post TCZ treatment. After 1-year in the TCZ group, a significant decrease was observed in the g score (p = 0.014) but not in the ptc, g + ptc, c4d and cg scores. Within the g-score, 29.3% had a score of 3 before TCZ vs. 22% at 1-year post-TCZ, p = 0.032.

Tocilizumab Route of Administration

Within the cohort, 17 patients (26.6%) received TCZ subcutaneously during the first year of treatment. The evolution of eGFR post TCZ was followed in patients converted to sub-cutaneous TCZ within the first year and those who remained on intravenous infusion. In patients converted to sub-cutaneous injections, eGFR trajectory during the first year was – 0.21 ± 0.46 mL/min/1.73 m^2/month, not statistically different from patients that received only intravenous TCZ: +0.15 ± 0.29 mL/min/1.73 m^2/month, p = 0.521 (**Supplementary Figure S4**). Sub-cutaneous administration was not associated with a reduced response to TCZ (20.8% in the group of responder vs. 30% in the non-responder patients, p = 0.421).

Tocilizumab Safety

Thirteen patients (20.3%) discontinued TCZ either because of histological or clinical stabilization (n = 7, 10.9%) or because of side effects (n = 6, 9.3%) at the end of follow-up (2 discontinued during the first year). The side effects that resulted in TCZ discontinuation were infections in five patients (peritonitis, cryptococcosis disease, CMV disease, tuberculosis and *campylobacter* infection), hepatotoxicity (1) within the first year and a DRESS (Drug reaction with eosinophilia and systemic symptoms syndrome) within the second year post-TCZ.

DISCUSSION

Few therapeutic options have proven their efficacy in caAMR and MVI + DSA-C4d- patients [30–32], that still remains among the main causes of long term graft loss. Others have found an increase of IL-6 mRNA transcripts in kidney allografts with rejection but not in healthy allografts [19]. Additionally, the addition of an IL6 blocker in association with co-stimulation blocker improved graft survival and decreased the rate of rejection in a cardiac transplantation mouse model [33]. Therefore, there is an emerging interest on targeting the IL-6/IL-6R pathway, which plays several roles in allograft inflammation [17].

TCZ, an IL-6 receptor blocker, has proven efficacy in the chronic/maintenance treatment of autoimmune diseases, like rheumatoid arthritis [34, 35]. Choi *et al.* [20] were the first to study the use of TCZ as a rescue therapy for caAMR in 36 KT recipients resistant to the standard of care. Graft- and patient-survival rates were excellent, respectively 80% and 91% at 6 years after AMR diagnosis. They also reported a significant decrease in DSA, C4d deposition and microvascular infiltration after TCZ treatment whereas the cg score and renal function remained stable. The first randomized controlled study with a direct IL-6 inhibitor (Clazakizumab) versus placebo in caAMR was published by Doberer *et al* [27]. Even though Clazakizumab arm showed no improvement of molecular and histological features of AMR over placebo, Clazakizumab patients had a significant decrease of DSA MFIs, MVI score and a slower decline of eGFR trajectories. This is consistent with our

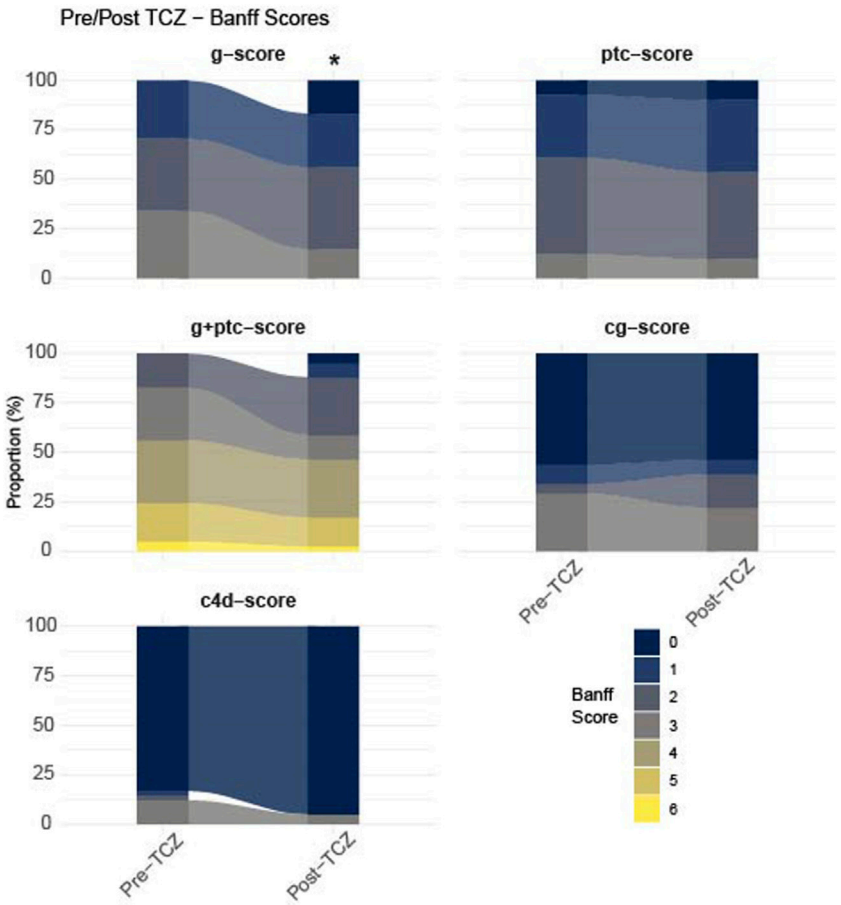


FIGURE 3 | Plot of percentage of Banff Scores in biopsies before Tocilizumab (pre-TCZ) and 1-year after TCZ treatment (post-TCZ). Banff scores are g: glomerulitis, Ptc: Peritubular capilaritis, g + ptc: addition of g and ptc scores, c4d: complement deposition, cg: chronic glomerulopathy. *p-value = 0.014.

findings showing a significant decrease of eGFR loss after TCZ initiation.

When analyzing the factors associated with TCZ response, we showed that the response to TCZ was higher in those with less cg lesions, suggesting that TCZ may be more effective if initiated early in the setting of caAMR and MVI.

Our data highlighted a more marked effect when TCZ is used in the presence of fewer chronic lesions, in agreement with a previous randomized study on 30 patients with subclinical inflammation by Chandran *et al.* [36] who described histological improvement and increased regulatory T cells post-TCZ. Taken together, these data suggest that TCZ may be considered in the early stages of graft inflammation.

Our study revealed a global stabilization of eGFR already 1 year after TCZ initiation, which was statistically confirmed at 2 years post TCZ initiation. Moreover, the positive effect of TCZ was also corroborated by proteinuria reduction and decrease of g-score at 1 year.

The two cohorts presented some differences at baseline. In the Bologna's cohort recipients were older, with longer transplant vintage at TCZ start worse chronic lesions, and the more frequent presence of DSA at the time of MVI diagnosis. Despite these differences, TCZ demonstrated the same efficacy in the two populations.

The presence of DSAs is strongly associated with kidney graft failure [37]. Here, we show a significant decrease of DSA number and of iDSA MFI at 1-year post treatment, consistently with the literature evidence of the role of IL-6 in promoting the production of HLA-DSA [38]. It can be postulated that treatment with TCZ, by lowering the titer of these antibodies, may improve graft survival. Our study is in line with other cohorts, where an overall decrease of iDSA MFI was noticed [39].

Elevated circulating IL-6 is observed in patients with inflammatory diseases [35, 40, 41] and the IL-6 levels correlate with disease activity. A pharmacological study [34] suggested that, under TCZ, serum IL6 trough level rises in rheumatic arthritis patients (1.55-fold) and in Castleman disease (23-fold). Likewise, at 1-year post treatment, we notice a 10-fold increase, which is strong evidence in favor of efficient IL-6 blockage by TCZ in KT recipients. It has been shown that the increase of IL-6 following TCZ is not attributed to increase secretion but to the inhibition of IL-6 receptor-mediated clearance by TCZ [34]. After blockade of the receptor by TCZ, the serum IL-6 level after TCZ may reflect the level of endogenous

production and therefore the baseline level of inflammation. In our TCZ cohort, a higher level of serum IL-6 was not associated with clinical outcomes.

Our study is in line with other studies concerning TCZ in caAMR patients with an overall good tolerance, as only 2 patient discontinued the TCZ treatment within the first year and 4 patients at the end of follow-up because of possible related side effects [42, 43].

It is known that rejection is primarily caused by non-adherence to therapy; therefore, a treatment that adds to the already high number of pills taken and requires in-hospital administration could increase non-compliance. Although the trend in patients that received sub-cutaneous TCZ was a lower eGFR at 1-year post treatment compared to intravenous patients, we did not find a significant difference in the eGFR trajectory. This aspect could be relevant in improving compliance and it could benefit patients with poor venous access. Long follow-up data are needed to confirm the equally effectiveness between sub-cutaneous and intravenous administration, as it was shown in rheumatoid arthritis patients [44].

Our study has some limitations to be acknowledged, including its retrospective nature. Moreover, patient's criteria of inclusion (i.e., histological diagnosis of MVI) may be heterogeneous as it includes caAMR and MVI + DSA-C4d- phenotypes associated with different renal prognosis. However, our inclusion criteria based on MVI, allowed to show that the response to TCZ was significantly associated in multivariate analysis with MVI + DSA-C4d- phenotype. These results highlight the possible efficacy of anti-IL6 therapies beyond humoral-mediated injuries but may be rejection mediated by immune cells such as NK cells or T-cells [11, 13]. TCZ have been shown to reduce NK cells and promote regulatory T [45], and to enhance NK cells cytotoxicity and cytokine production [46]. Moreover, IL-6 is involved in T cells mediated endothelial injury [47]. We may hypothesis that, by blocking IL-6, TCZ reduce NK mediated inflammation and endothelial injury in the graft, promote graft tolerance and reduce endothelium activation. The inflammation in non-humoral rejection may be driven by IL-6, which TCZ directly targets. Another limitation of this study is the absence of a matched control cohort, which restricts the strength of causal interpretations. Although we explored retrospective matching strategies using key clinical variables such as baseline eGFR, rejection severity, DSA status, and age, the small sample size and heterogeneity of our cohort—including both caAMR and MVI cases with or without DSA—made this approach unfeasible without introducing significant bias. Future prospective studies with matched control groups will be essential to confirm and extend these findings.

In a nutshell, this study suggested that first-line therapy with TCZ for patients with MVI histological feature is associated with an improvement of eGFR trajectories, and reduced DSA number and MFI. Response to TCZ was higher in younger patient, within the MVI + DSA-C4d- group and when associated with less chronic glomerulopathy lesions, suggesting that TCZ may be more effective as started early during MVI evolution, and may maintain longer the benefits for renal function. Initial IL-6 levels do not seem associated with clinical outcomes. Despite the failure of the IMAGINE trial using Clazakizumab, large randomized controlled trials on IL-6 receptor blockade such as the INTERCEPT study are needed to fully assess the efficacy of this strategy.

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 French data: CNIL (French National committee for data protection) approval number 1987785v0 and Bologna data: protocol number 244/2023/Sper/AOUBo. The studies were conducted in accordance with the local legislation and institutional requirements. This is a retrospective study. All patients signed a consent of data collection and analysis at the time of transplantation. And the studies were approved by local ethic committees.

AUTHOR CONTRIBUTIONS

Conceptualization: JN, PC, VC, RL, GC, GL, and LR; Data curation: JN; Formal analysis: JN; Methodology: TJ and PC; Visualization: JN; Writing – original draft: JN, Histology analysis: DG; HLA analysis: CD; IL-6 dosage and interpretation: AL; Writing – review and editing: JN, GC, VC, RL, CD, TJ, DG, AL, SW, PC, DA, DV, MB, CA, PM, LR, and GL. 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.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.14502/full#supplementary-material

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ORIGINAL RESEARCH
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Clinical and Economic Burden Associated With Anti-Cytomegalovirus (CMV) Prophylaxis Therapies in Adult Kidney Transplant Recipients (LECOCYT): An Observational Study

Nassim Kamar[1], Hannah Kaminski[2], Christophe Masset[3], Claire Castagné[4], Guilhem Tournaire[4], Xavier Bourge[4], Lionel Bensimon[4], Moustafa Naja[5], Stéphanie Degroote[5], Isabelle Durand-Zaleski[6] and Christophe Legendre[7] on behalf of the LECOCYT Study Group*

[1]Department of Nephrology and Organ Transplantation, Toulouse Rangueil University Hospital, Institut national de la santé et de la recherche médicale - Unité Mixte de Recherche 1291 (INSERM UMR 1291), Toulouse Institute for Infectious and Inflammatory Diseases (Infinity), University Paul Sabatier, Toulouse, France, [2]Department of Nephrology-Transplantation-Dialysis-Apheresis, University Hospital of Bordeaux, Bordeaux, France, [3]Department of Nephrology, Institut de Transplantation Urologie-Nephrologie (ITUN), Nantes University Hospital, Nantes, France, [4]MSD France, Puteaux, France, [5]ClinSearch, Malakoff, France, [6]Unité de Recherche Clinique en Économie de la Santé d'Ile de France (URC-Eco), Assistance Publique-Hôpitaux de Paris (AP-HP), Université Paris Est Créteil, Créteil, France, [7]Nephrology and Renal Transplantation Department, Necker Hospital, Paris, France

The incidence of leukopenia and neutropenia associated with cytomegalovirus (CMV) prophylaxis in kidney transplant (KT) recipients is not well established. LECOCYT, a prospective observational multicenter study, aimed to investigate the clinical and economic burdens of CMV prophylaxis during the first 6 months post-transplantation. Grade 3 or 4 leukopenia or neutropenia was assessed in CMV-seropositive donors/CMV-seronegative recipients (D+/R-) who received current anti-CMV prophylaxis, and in CMV-seronegative donors/CMV-seronegative recipients (D-/R-) who did not. The economic burden in D+/R- was also evaluated. The adjusted odds ratio for grade 3 or 4 leukopenia or neutropenia was 5.16 [95% confidence interval: 1.97–13.53] for D+/R- group. The median costs, excluding the KT procedure, for D+/R- subgroup patients who experienced at least one episode of severe leukopenia or neutropenia were approximately €4,500 (Q1 = €561; Q3 = €10,000). D+/R- patients with no episode incurred significantly lower costs, with a median of nearly €2,100 (Q1 = €182; Q3 = €6,500) (p = 0.02). D+/R- patients with severe

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***Correspondence**
Nassim Kamar,
christophe.legendre@aphp.fr

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Abbreviations: ANC, Absolute Neutrophil Count; ATIH, French Agency for Information on Hospital Care – Agence technique de l'information sur l'hospitalisation; CI, Confidence Interval; CMV, Cytomegalovirus; CTCAE, Common Terminology Criteria for Adverse Events; D-/R-, CMV-seronegative donors/CMV-seronegative recipients; D+/R-, CMV-seropositive donors/CMV-seronegative recipients; eCRF, Electronic Case Report Form; G-CSF, Granulocyte-Colony Stimulating Factor; GHM, Homogeneous group of patient's classification – Groupe Homogène de Malades; HCRU, Healthcare Resource Utilization; KT, Kidney Transplant; MPA, Mycophenolic Acid; mTOR, Mechanistic Target of Rapamycin; OR, Odds Ratio; PMSI, French Information Systems Medicalization Program – Programme de Médicalisation des Systèmes d'Information; PROs, Patient-Reported Outcomes; Q1/Q3, Quartiles 1 and 3; QoL, Quality of Life; RCT, Randomized Clinical Trial; RTQ, Renal Transplant Quality of Life – ReTransQoL; SAS, Statistical Analysis System software; SD, Standard Deviation; SF-36, Short Form 36; VAS, Visual Analog Scale; WBC, White Blood Cell.

leukopenia or neutropenia had a higher rate of outpatient consultations than those without episode (73.9% vs. 57.6%, p = 0.002), and a higher average number of consultations per patient (5.5 ± 4.1 vs. 4.5 ± 3.3, p = 0.042) than D+/R- patients without. Anti-CMV prophylaxis in D+/R- transplant recipients was significantly associated with a higher rate of severe leukopenia or neutropenia compared to no prophylaxis in D-/R- recipients.

Keywords: cytomegalovirus prophylaxis, economics, ganciclovir, valganciclovir, kidney transplant

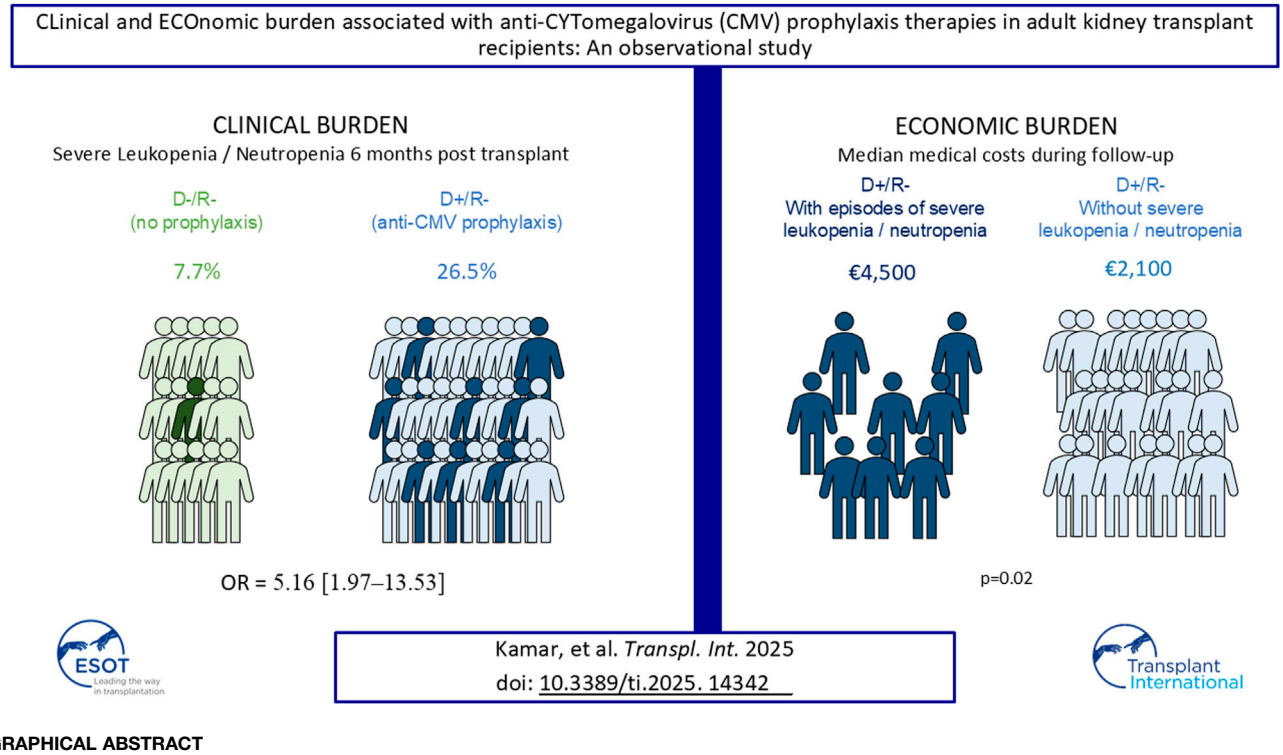
INTRODUCTION

The risk of cytomegalovirus (CMV) infection in kidney-transplant (KT) patients is driven by the serostatus of the donor and recipient. The highest risk is for seronegative recipients (R-) receiving organs from seropositive donors (D+), followed by seropositive recipients (R+). The lowest risk is for seronegative donor-recipient pairs (D-/R-) [1].

To prevent CMV infection, two main strategies are employed: prophylactic and preemptive therapies. Prophylactic therapy involves administering antivirals shortly after transplantation, typically for three to 6 months, and is recommended for high-risk (D+/R-) and intermediate-risk (R+) patients [2]. Preemptive therapy requires regular monitoring of CMV viral load in the blood and starting antiviral treatment when a specific threshold is reached, ideally before symptoms appear [2]. The choice between prophylactic and preemptive strategies can vary widely among countries due to differences in healthcare policies and guidelines, availability and cost of antiviral drugs and CMV monitoring tests [3, 4]. A 2022 survey from the European Society for Organ Transplantation revealed that 95% of participating centers give an anti-CMV prophylaxis for D+/R- patients [5]. 90% of respondents used Valganciclovir for prophylaxis [5].

Leukopenia and neutropenia are the most clinically relevant hematological toxicities among the anticipated adverse drug reactions of antiviral agents used in anti-CMV prophylaxis therapies [6]. Recent studies have shown inconsistent reporting of hematological adverse events in trials comparing prophylactic and preemptive therapies, with discrepancies ranging from no reported differences to a significant disparity of up to 30% versus 3% [7–13]. A 2023 randomized clinical trial (RCT) comparing letermovir and valganciclovir for prophylaxis in high-risk KT recipients found a higher rate of severe leukopenia or neutropenia in the valganciclovir group (64.0% versus 26.0%) [14]. In case of valganciclovir, dose reduction carries a risk of developing drug-resistant CMV strains [15].

To address the gap in the literature regarding the burdens of CMV prophylaxis in KT recipients, LECOCYT, an observational multicenter French study, aimed to primarily investigate the differences in leukopenia and neutropenia grade 3 or 4,



GRAPHICAL ABSTRACT

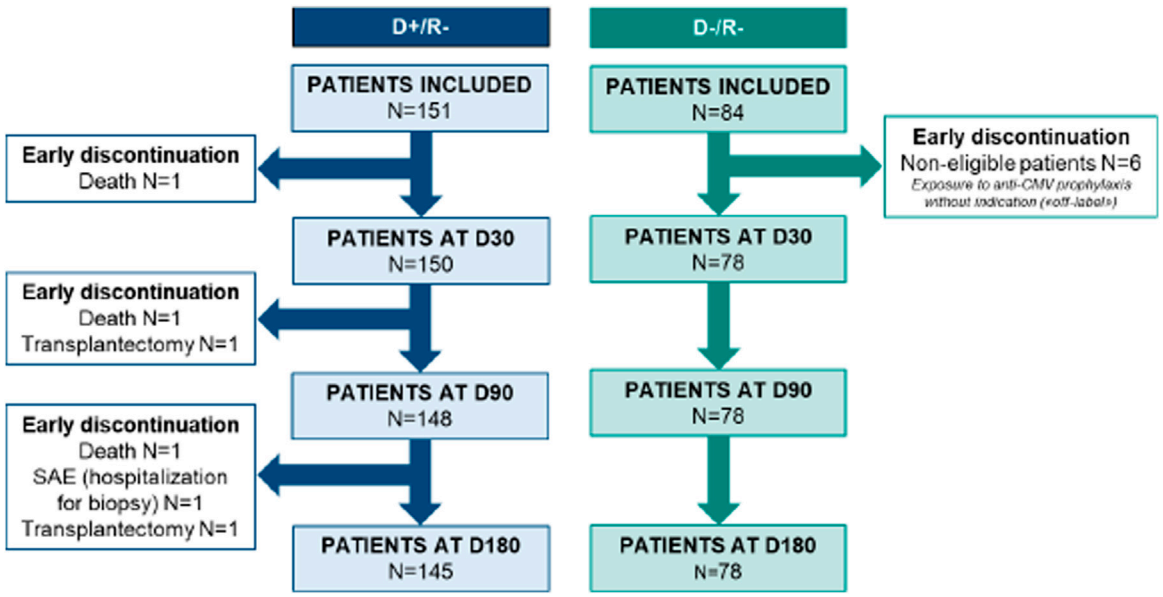


FIGURE 1 | Flow chart of inclusion and follow-up of the study.

between KT recipients who received anti-CMV prophylaxis (D+/R-) compared to untreated patients (D-/R-) over a 6-month period post-transplant, and to evaluate the clinical and economic burdens (associated with hematological toxicities).

MATERIALS AND METHODS

Study Design and Population

The LECOCYT study was a multicenter, prospective, longitudinal, observational cohort study designed to examine the characteristics and outcomes of two distinct groups. Approval was obtained from the local ethics committee with the registration number 2021-A01250-41. The first group (D+/R-), consisted of high-risk CMV transplant recipients who received antiviral prophylaxis. The second group (D-/R-) did not receive prophylaxis due to their lower risk of developing CMV-related complications, thus avoiding hematological toxicities related to prophylaxis. This second group served as a comparator to describe the clinical burden associated with antiviral prophylaxis in the first group. This study design isolated the specific outcomes associated with prophylaxis in high-risk transplant recipients (D+/R-) from the transplant procedure itself.

To assess the economic burden, comparative analyses were performed within the D+/R- group stratified into subgroups based on the presence or absence of neutropenia and leukopenia grade 3 or 4.

Twenty-two French kidney-transplant centers participated in the study; the list of centers is available in the online data **Supplementary Material S1**. We included patients 18 years or older at the time of KT, transplanted within 10 days prior to the inclusion visit from a seropositive or seronegative donor, and CMV seronegative at the time of the KT. Non-opposition to patient-level data collection was obtained. The study was registered with number ID RCB 2021-A01250-41 and conducted in accordance with the ethical principles of the Declaration of Helsinki.

The primary objective was to compare the difference of severe leukopenia/neutropenia episodes between patients who received the prophylaxis for CMV (D+/R-) and untreated patients (D-/R-) within the first 6 months post-transplantation. Secondary objectives included the clinical and economic (variable costs) burdens associated with these toxicities. Healthcare resources utilization and medical costs were measured in D+/R- patients and included the medical time required for the management of patients, the duration of hospital stay, the number of subsequent hospitalization and the use of outpatient consultations. Exploratory objectives aimed to describe the use of anti-CMV prophylaxis, clinical outcomes, comedication, and quality of life (QoL) among these patients.

Data Collection

The health and economic burden, in the D+/R- group was analyzed based on the presence or absence of neutropenia and leukopenia. An electronic Case Report Form (eCRF) was used for data collection.

Regarding hospital costs, the PMSI (*Programme de Médicalisation des Systèmes d'Informations*) is a French hospital information system designed to provide a standardized, medicalized measure of healthcare activity. For inpatient stays, this measurement relies on a coding system for procedures and diagnoses, the GHM (*Groupe Homogène de Malades*). GHM is derived from the PMSI, categorizes hospital stays into groups that are homogeneous in terms of medical characteristics and resource utilization.

TABLE 1 | Main features of the 229 study patients, including 151 CMV-seropositive donors (D+/R-) and 78 CMV-seronegative donors (D-/R-).

Variable	Patients D+/R- (n = 151)	Patients D-/R- (n = 78)	Total (N = 229)
Male, n (%)	110 (72.8)	52 (66.7)	162 (70.7)
Recipient age (years), mean (sd)	58.7 (14.9)	54.1 (13.8)	57.2 (14.6)
Body mass index, kg/m^2, mean (sd), n = 225	26.3 (4.6)	25.2 (4.7)	26.0 (4.7)
Cardiovascular disease, n (%)	29 (19.2)	16 (20.5)	45 (19.7)
Chronic obstructive pulmonary disease, n (%)	9 (6.0)	2 (2.6)	11 (4.8)
Peptic ulcer disease, n (%)	6 (4.0)	2 (2.6)	8 (3.5)
Liver disease, n (%)	2 (1.3)	1 (1.3)	3 (1.3)
Diabetes mellitus, n (%)	24 (15.9)	15 (19.2)	39 (17.0)
Solid tumor, n (%)	14 (9.3)	8 (10.3)	22 (9.6)
Dialysis history, n (%)	126 (83.4)	67 (85.9)	193 (84.3)
Duration of dialysis (months), mean (sd), n = 193	35.1 (28.5)	31.8 (22.6)	34.0 (26.6)
Pre-emptive kidney transplant, n (%)	25 (16.6)	11 (14.1)	36 (15.7)
Rank of kidney transplant			
Transplant rank equal to 1, n (%)	134 (88.7)	65 (83.3)	199 (86.9)
Transplant rank equal to 2, n (%)	15 (9.9)	11 (14.1)	26 (11.4)
Transplant rank greater than 2, n (%)	2 (1.3)	2 (2.6)	4 (1.7)
Anti-CMV prophylaxis treatment			
Valganciclovir, n (%)	138 (91.4)	-	138 (60.3)
Ganciclovir, n (%)	11 (7.3)	-	11 (4.8)
Valaciclovir, n (%)	2 (1.3)	-	0 (0.0)
Time from KT to prophylaxis initiation (days), mean (sd), n = 150	3.6 (2.7)	-	3.6 (2.7)
Time from CMV viremia analysis to transplant procedure (days), mean (sd), n = 89	2.2 (8.6)	0.9 (1.8)	1.7 (6.8)
Treatment with	92 (60.9)	48 (61.5)	140 (61.1)
Sulfamethoxazole/			
Trimethoprim, n (%)			
Immunosuppressive drugs at baseline			
Polyclonal antibodies (n, %)	41 (27.2)	21 (26.9)	62 (27.1)
Rituximab (n, %)	1 (0.7)	0 (0.0)	1 (0.4)
Basiliximab (n, %)	81 (53.6)	39 (50.0)	120 (52.4)
Belatacept (n, %)	0 (0.0)	0 (0.0)	0 (0.0)
Azathioprine (n, %)	1 (0.7)	0 (0.0)	1 (0.4)
Mycophenolic acid (n, %)	133 (88.1)	71 (91.0)	204 (89.1)
Cyclosporine (n, %)	9 (6.0)	4 (5.1)	13 (5.7)
Tacrolimus (n, %)	133 (88.1)	69 (88.5)	202 (88.2)
Everolimus (n, %)	7 (4.6)	1 (1.3)	8 (3.5)
Corticosteroids (n, %)	135 (89.4)	71 (91.0)	206 (90.0)
Eculizumab (n, %)	0 (0.0)	1 (100.0)	1 (100.0)

Abbreviations: sd, standard deviation; CMV, cytomegalovirus; KT, kidney transplantation; D+/R-, CMV-seropositive donors/CMV-seronegative recipients; D-/R-, CMV-seronegative donors/CMV, seronegative recipient; cardiovascular disease includes myocardium infarction, congestive heart failure, peripheral vascular disease and cerebrovascular accident or transient ischemic attack.

TABLE 2 | Incidence of grade 3 or 4 leukopenia and neutropenia in patients during a 6-month follow-up period.

Variable	Patients D+/R– (n = 151)	Patients[a] D–/R– (n = 78)	Unadjusted Odds Ratio (OR) [95% Confidence Interval (CI)]	Adjusted Odds Ratio (OR)[b] [95% Confidence Interval (CI)]
Leukopenia or neutropenia Grade 3 or 4 (n, %)	40 (26.5)	6 (7.7)	4.32 [1.74–10.72]	5.16 [1.97–13.53]

[a]*D-/R- is considered as the reference group for the odds ratio (logistic regression).*

[b]*Covariates: age, sex, mycophenolic acid, corticosteroids, absence of certain co-medications (mTOR, inhibitors and sulfamethoxazole/trimethoprim), proton pump inhibitors, and investigator sites.*

This classification is based on both administrative data (e.g., sex, age, length of stay) and medical data (e.g., diagnoses, procedures performed, comorbidities). Hospital procedure costs, stratified by GHM, were obtained from the French ATIH (*Agence Technique de l'Information sur l'Hospitalisation*) website. Inpatient costs were determined by linking the GHM code recorded in the electronic Case Report Form (eCRF) to the corresponding cost from the ATIH database. The weighted average procedure cost was calculated based on the length of hospital stay documented in the eCRF. Notably, hospital costs for subsequent KT-related procedures are fully covered (100%) by the French health insurance system.

TABLE 3 | Description of grade 3 or 4 leukopenia or neutropenia in patients during a 6-month follow-up period.

Variable	Patients D+/R- (n = 151)	Patients D-/R- (n = 78)	Total (N = 229)
Leukopenia or neutropenia Grade 3 or 4 (n, %)	40 (26.5)	6 (7.7)	46 (20.1)
Number of leukopenia or neutropenia episodes of grade 3 or 4, n	71	8	79
Duration of leukopenia or neutropenia episodes of grade 3 or 4 (days), mean (sd)	15.5 (15.6)	21.6 (17.0)	16.2 (15.8)
Time of diagnosis since KT procedure (months), mean (sd)	3.0 (1.4)	3.2 (1.3)	3.0 (1.4)

Abbreviations: sd, standard deviation; KT, kidney transplantation; D+/R-, seropositive donors/seronegative recipients for cytomegalovirus; D-/R-, seronegative donors/seronegative recipients for cytomegalovirus.

The following information was collected: demographic data; medical and surgical history; medication review; clinical results; laboratory analyses; healthcare resource utilization (HCRU); costs incurred, estimated from the perspective of the French health insurance. The KT procedure was excluded from the cost analysis as it is a fixed cost common to all patients.

Patient-Reported Outcomes (PROs) instruments for QoL assessment were administered in paper format, using the Renal Transplant Quality of Life (ReTransQoL) and the Short Form 36 (SF-36) questionnaires. The SF-36 includes eight scores derived from section questions, each normalized to a 0–100 scale, where higher scores mean better QoL. The RTQ, tailored for KT patients, also scores overall QoL, with higher scores representing better quality of life. QoL scores were obtained at the time of inclusion in the study and during subsequent follow-ups at D30, D90, and D180.

Exposure to anti-CMV prophylaxis therapy was defined as the initiation of antiviral treatment (valganciclovir, ganciclovir, valaciclovir or acyclovir) within the first 10 days post-KT in patients who had no detectable CMV viremia at the time of the transplant and without evidence of active infection, indicating that these medications were prescribed solely for the purpose of preventing a potential CMV infection rather than treating an existing one. Follow-up visits for KT recipients were scheduled at approximately 30 days (D30), 90 days (D90), and 180 days (D180) post-transplant, following the standard of care in France. The occurrence of severe leukopenia or neutropenia was classified according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Grades of "White Blood Cell (WBC) count decreased" (i.e., leukopenia) are defined as follows:

- Grade 3: total WBC between 2,000 and 1,000/mm^3 (or 2.0–1.0 × 10^9/L)
- Grade 4: total WBC <1,000/mm^3 (or <1.0 × 10^9/L)

Grades of "neutrophil count decreased" (i.e., neutropenia) are defined as follows:

- Grade 3: Absolute Neutrophil Count (ANC) between 1,000 and 500/mm^3 (or 1.0–0.5 × 10^9/L)
- Grade 4: ANC <500/mm^3 (or <0.5 × 10^9/L)

Graft rejection was assessed using the Banff diagnostic classification [16], and death-censored graft loss was defined as the complete loss of kidney function post-transplant, which required chronic dialysis or retransplantation.

The medical time required for patient management estimated by the physician according to usual clinical practice, as reported on a visual analog scale (VAS) from 0 to 10, was collected at each follow-up visits, with a lower score indicating less time needed for medical management. The Visual Analog Scale (VAS) is a straightforward and validated tool commonly used to assess characteristics or attitudes that are thought to exist on a continuous scale but are difficult to measure directly. Thus, it can be used to gauge a physician's perception of the time spent managing a patient in a real-world setting.

Statistical Analysis

Categorical variables were presented as frequencies and proportions, and continuous data were presented by mean, standard deviation (SD), median, minimum, maximum, and quartiles (Q1 and Q3).

The primary objective of this study was analyzed using a multivariate logistic regression model to compare the incidence of hematological toxicities and potential associated factors in the D-/R- group versus the D+/R- group by calculating OR with 95% CI. Univariate logistic regression was employed to assess the potential impact of each covariate with a p-value of ≤0.2 for inclusion in the multivariable regression. The final multivariable regression model included age, sex, mycophenolic acid, corticosteroids, absence of certain co-medications (mTOR inhibitors and sulfamethoxazole/trimethoprim), proton pump inhibitors, and investigator sites. For numerical data related to secondary or exploratory objectives, t-test or Mann-Whitney U test was used, depending on distribution and sample size. A linear mixed model was used for repeated measurements. The type 1 error rate for establishing significance was set at 0.05. To avoid unnecessary multiple comparisons, no statistical tests were applied to descriptive outcomes such as clinical profile of patients (**Table 5**). In order to mitigate the risk of false positive a hierarchical approach of outcomes was followed for the analysis. All statistical analyses were performed in SAS (version 9.4) software.

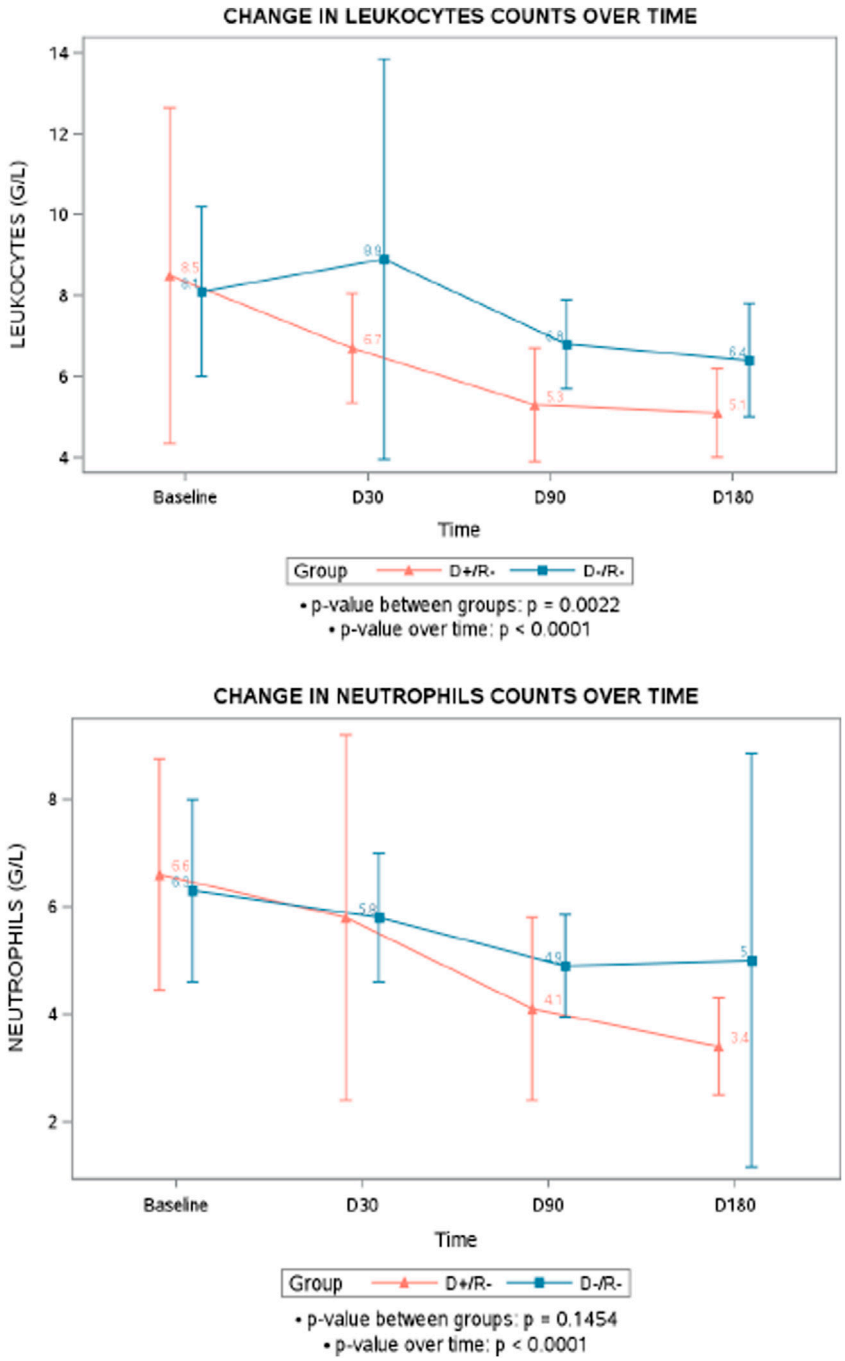


FIGURE 2 | Mean change in leukocytes and neutrophils counts over time. D+/R-, seropositive donors/ seronegative recipients for cytomegalovirus; D-/R-, seronegative donors/ seronegative recipients for cytomegalovirus.

RESULTS

Patients

Between 3 September 2021, and 9 September 2022, a total of 235 patients were enrolled with enrollment ranging from 2 to 31 patients per center. Six patients were excluded from the D-/R- group due to their exposure to off-label anti-CMV prophylaxis. Finally, 229 patients were included in the analyzable population, with 151 from the D+/R- group and 78 from the D-/R- group. There were six early terminations in the D+/R- group before the end of the study: three patients died (one in each of the periods D0–D30, D30–D90, and D90–D180, respectively), two underwent transplantectomy with no renal graft function (one in each of the periods D30–D90 and D90–D180, respectively), and

TABLE 4 | Description of utilization pattern of anti–CMV medication regimen within the 6–months post–KT.

Variable	Patients D+/R- (n = 151 patients)
Patients with anti-CMV prophylaxis treatment at baseline	151 (100.0)
Valganciclovir (%)	138 (91.4)
Ganciclovir (%)	11 (7.3)
Valaciclovir (%)	2 (1.3)
Dosage of valganciclovir at baseline (mg/day)	
Number of patients, n	138[a]
<225, n (%)	29 (21.0)
225, n (%)	29 (21.0)
450, n (%)	73 (52.9)
750, n (%)	1 (0.7)
900, n (%)	24 (17.4)
Dosage of ganciclovir at baseline (mg/day)	
Number of patients, n	11[a]
<100, n (%)	5 (45.5)
100–200, n (%)	9 (81.8)
>200, n (%)	5 (45.5)
Valganciclovir administered during the study	149
Duration of continuous treatment administration (days), mean (sd), n = 113 patients	160.8 (44.0)
Reasons for permanent end of treatment before the 6-month follow-up (n = 50 patients)	
Planned end of treatment, n (%)	34 (68.0)
Permanent interruptions due to hematological toxicities (leukopenia or neutropenia, any grade), n (%)	12 (24.0)
Permanent interruptions due to resistance to treatment, n (%)	3 (6)
Permanent interruptions due to cholestasis, n (%)	1 (2.0)
Total number of days of treatment exposure with temporary interruptions[b], mean (sd), n = 36 patients	110.8 (47.5)
Duration of treatment temporary interruptions (number of days of non-exposure), mean (sd), n = 42 interruptions	20.3 (26.5)
Temporary interruptions due to hematological toxicities, n (%)	12 (33.3)
Ganciclovir administered during the study	15
Duration of continuous treatment administration (days), mean (sd), n = 3 patients	8.0 (4.6)
Permanent interruptions due to resistance to treatment, n (%)	1 (33.3)
Total number of days of treatment exposure with temporary interruptions[b], mean (sd), n = 12 patients	12.8 (9.9)
Duration of temporary treatment interruptions (number of days of non-exposure), mean (sd), n = 14 interruptions	93.5 (130.8)

[a]*Several patients received different doses of treatment.*
[b]*Number of days of exposure excluding the time of interruption.*
Abbreviations: sd, standard deviation; CMV, cytomegalovirus; KT, kidney transplantation; D+/R-, CMV-seropositive donors/CMV-seronegative recipients; D-/R-, CMV-seronegative donors/CMV-seronegative recipients.

one experienced a serious adverse event, which was hospitalization for a biopsy (between D90 and D180) (**Figure 1**). 162/229 (70.9%) of the patients were male. The mean age of the patients was 57.2 ± 14.6 years. In the D+/R- group all patients received prophylaxis in the first 10 days post-KT, among them 91.4% (n = 138/151) received valganciclovir, 7.2% (n = 11/151) received ganciclovir and then switched to the oral form, and 2 patients (1.3%) were treated with valaciclovir (**Table 1**).

Leukopenia and Neutropenia Episodes

In the D+/R– group, 26.5% of patients (n = 40/151) experienced at least one episode of leukopenia or neutropenia grade 3 or 4, compared to 7.7% of patients (n = 6/78) in the D-/R- group over a 6-month follow-up period. The odds ratio (OR) calculated using univariate logistic regression was 4.32 [95% confidence interval: 1.74–10.72], after adjustment with the multivariate model, OR was 5.16 [95% CI: 1.97–13.53] indicating that patients in the D+/R- group who received anti-CMV prophylaxis were over five times more likely to experience at least one episode of severe leukopenia/neutropenia compared to those in the D-/R- group (**Table 2**).

A total of 79 episodes of leukopenia or neutropenia grade 3 or 4 were recorded in 46 patients, 71 episodes in the D+/R- group (n = 40 patients) and 8 episodes in the D-/R- group (n = 6 patients). The average duration of these episodes was 16.2 ± 15.8 days with their onset typically occurring around 3 months post-transplant, at a mean of 3.0 ± 1.4 months (**Table 3**).

In the D-/R- group, 1 episode of grade 4 leukopenia and 3 episodes (for 3 patients) of grade 3 leukopenia were declared. Regarding neutropenia in the same group, 3 episodes (for 3 patients) of grade 4 and 1 episode of grade 3 was declared.

In the D+/R-, 7 episodes (for 7 patients) of grade 4 leukopenia and 29 episodes of grade 3 leukopenia (24 patients had one episode, and 1 patient had 5 episodes) were declared. Regarding neutropenia in the same group, 13 episodes (11 patients had one episode and 1 patient had 2 episodes) of grade 4 and 22 episodes (for 22 patients) of grade 3 were declared.

Besides the occurrence of neutropenia or leukopenia, a general trend of decreasing counts of leukocyte and neutrophil after transplantation was observed over time ($p < 0.0001$), with the D+/R- group showing a more pronounced decline in leukocytes compared to the D-/R- group ($p = 0.0022$). However, while the neutrophil counts significantly decreased over time in the D+/R- ($p < 0.0001$), the difference between D+/R- and D-/R- was not statistically significant ($p = 0.1454$) (**Figure 2**).

The use of at least one dose of G-CSF occurred in 16 patients (10.6%) in D+/R- group versus 4 patients (5.1%) in the D-/R- group.

In the Lecocyt study, 16 patients in the D+/R- group experienced a CMV disease episode during the 6-month follow-up period, with a mean time from KT procedure to diagnosis of 107.8 days (SD: 50.4 days) and a median of 122.5 days. In the D-/R- group, only one event was reported, with diagnosis occurring 109 days after the procedure. Regarding grade 3 or 4 neutropenia/leukopenia episodes, the mean time from the procedure to diagnosis was 91.9 days (SD: 42.1) with a median of 92 days for the D+/R- group and a mean time of 95.9 days (SD: 40.7) with a median of 109 days for the D-/R- group. In general, especially in the D+/R-, the episodes of neutropenia/leukopenia occurred before the episode of CMV disease.

TABLE 5 | Clinical outcomes and infectious complications in KT Recipients within the 6–months post–KT.

Variable	Patients D+/R– (n = 151 patients)	Patients D–/R– (n = 78 patients)	Total (N = 229 patients)
Patients with at least one episode of infection requiring hospitalization[a], n (%)	30 (19.9)	9 (11.5)	39 (17.0)
Episodes of infection requiring hospitalization per patient, mean (sd), n = 50 episodes, n = 39	1.3 (0.6)	1.1 (0.3)	1.3 (0.6)
Patients with one episode of zona, n (%)	1 (0.7)	1 (1.3)	2 (0.9)
Patients with graft rejection, n (%)	10 (6.6)	3 (3.8)	13 (5.7)
Death-censored graft loss, n (%)	7 (4.6)	2 (2.6)	9 (3.9)
Patient with an episode of CMV disease during the 6-month period, n	16	1	17
Time between KT procedure and diagnostic of the episode, mean (sd), n = 17	107.8 (50.4)	109.0 (.)	107.8 (48.8)
Patients requiring hospitalization due to an episode of CMV Infection, n (%)	8 (50.0)	0 (0.0)	8 (47.1)
Death, n (%)	3 (2.0)	0 (0.0)	3 (1.3)
Causes of death			
Sudden and unexpected death, n (%)	2 (66.7)	0 (0.0)	2 (66.7)
Subdural hematoma due to a fall, n (%)	1 (33.3)	0 (0.0)	1 (33.3)

[a]*Infections other than CMV (e.g., viral, bacterial, parasitic, etc.) requiring hospitalization.*

Abbreviations: sd, standard deviation; CMV, cytomegalovirus; KT, kidney transplantation; D+/R-, CMV-seropositive donors/CMV-seronegative recipients; D-/R-, CMV-seronegative donors/CMV-seronegative recipients.

TABLE 6 | Analysis of QoL over time between D+/R- and D-/R- groups using SF-36 and RTQ questionnaires.

Variable	p-value of significance between the two groups[a] D + R- (n = 151) and D-R- (n = 78)	p-value of significance over time[a] Inclusion, D30, D90 and D180
SF-36		
Physical functioning	0.7307	<0.0001
Role limitations due to physical health	0.1808	<0.0001
Role limitations due to emotional problems	0.4930	0.0080
Energy/fatigue	0.4025	0.0024
Emotional wellbeing	0.2708	0.0085
Social functioning	0.0282	0.0032
Pain	0.5841	0.0334
General health	0.6258	0.0991
RTQ		
RTQ total score	0.0048	0.0123
Physical Health	0.0411	<0.0001
Social Functioning	0.1846	0.7980
Medical care and satisfaction	0.0364	0.0746
Treatment	0.0219	0.9777
Fear and loosing graft	0.1153	0.0013

[a]*Mixed model for repeated measures.*

Abbreviations: D+/R-, seropositive donors/seronegative recipients for cytomegalovirus; D-/R-, seronegative donors/seronegative recipients for cytomegalovirus; D30, day 30; D90, day 90; D180, day 180; SF-36, Short Form 36; RTQ, renal transplant quality of life.

Utilization Pattern of Current Anti–CMV Medication

Our study found that all D+/R- patients received prophylactic treatment within the first 10 days post-transplant. In this group, 138/151 patients (91.4%) initiated prophylaxis with valganciclovir, indicating its widespread adoption in clinical practice (**Table 4**). Ganciclovir was the initial prophylactic treatment administered to 11/151 patients (7.3%), all of whom subsequently switched to valganciclovir. The specific dosages of valganciclovir and ganciclovir are detailed in **Table 4**. The remaining 2/151 patients (1.3%) received only valaciclovir as their initial prophylactic treatment throughout the study (**Table 4**).

During the 6-month follow-up period, 113 out of 149 D+/R- patients (75.8%) who were administered valganciclovir, received continuous treatment without any interruption recorded, with a mean duration of exposure of 160.8 ± 44.0 days. Out of these 113 patients, 63 (55.8%) continued valganciclovir until the end of the study. Permanent treatment interruptions were recorded for 50/113 patients (44.2%) before the end of the 6-month follow-up period, with the primary reasons being the planned end of treatment (n = 34, 68.0%) and hematological toxicities (n = 12, 24.0%). Temporary valganciclovir treatment interruptions were observed in 36/149 patients (24.2%), including 12/36 patients (33.3%) for hematological toxicity (**Table 4**).

TABLE 7 | Comparative analysis of post-transplant healthcare costs and utilization between D+/R- patients with and without neutropenia/leukopenia episodes.

Variable	Patients D+/R- with at least one episode of Neutropenia /leukopenia[a] (n = 40)	Patients D+/R- with no episode of Neutropenia /leukopenia[a] (n = 111)	Total (N = 151)	pb
Total post-transplant costs (EUR) (index procedure not included)				0.025
Number of patients	39	91	130	
Mean (SD)	7,593 (9,424)	4,456 (6,038)	5,397 (7,327)	
Median	4,515	2073	2,595	
Min – Max	99–35,720	17–25,183	17–35,720	
Inpatients costs (follow-up procedures) (EUR)				0.090
Number of patients	27	56	83	
Mean (SD)	10,395 (9,943)	6,890 (6,450)	8,030 (7,873)	
Median	6,961	4,488	5,399	
Min – Max	470–35,720	470–25,133	470–35,720	
Outpatient consultation costs (EUR)				0.091
Number of patients	35	80	115	
Mean (SD)	228 (163)	173 (141)	190 (149)	
Median	198	165	165	
Min – Max	17–611	17–594	17–611	
Length of stay in the service of admission of index procedure (in days)				0.683
Number of patients	40	110	150	
Mean (SD)	12.7 (5.5)	12.9 (6.3)	12.8 (6.1)	
Median	11.0	12.0	11.0	
Min – Max	5–28	4–54	4–54	
Number of subsequent hospitalizations				0.050
Number of patients	24	52	76	
Mean (SD)	3.5 (3.1)	2.3 (2.0)	2.6 (2.4)	
Median	2.5	2.0	2.0	
Min – Max	1–14	1–13	1–14	
Duration of subsequent hospitalizations (days)				0.871
Number of hospitalizations (missing data)	83 (0)	114 (4)	197 (4)	
Mean (SD)	6.7 (5.3)	8.0 (9.0)	7.4 (7.7)	
Median	6.0	5.0	5.0	
Min – Max	1–32	1–47	1–47	
Number of outpatient consultation(s) per patients until D180				0.042
Mean (SD)	5.5 (4.1)	4.5 (3.3)	4.8 (3.6)	
Median	5.0	4.0	4.0	
Min – Max	1–25	1–20	1–25	

[a]*With at least one/no episode of neutropenia/leukopenia grade 3 or 4 within the first 6 months post-KT.*

[b]*Wilcoxon–Mann–Whitney test.*

Abbreviations: sd, standard deviation; D+/R-, seropositive donors/seronegative recipients for cytomegalovirus; D-/R-, seronegative donors/seronegative recipients for cytomegalovirus; D180, day 180.

During the 6-month follow-up period, 15 out of 149 D+/R- patients (10.1%) were treated with ganciclovir. Temporary treatment interruptions were reported in 12 patients (**Table 4**).

Clinical Follow-Up

Among the total of 229 patients, 39 (17%) experienced at least one episode of infection requiring hospitalization during the 6-month follow-up period: 30 (19.9%) in the D+/R- group and 9 (11.5%) in the D-/R- group. Graft rejection was experienced by 10 patients (6.6%) in the D+/R- group and 3 (3.8%) in the D-/R- group. Of these, graft loss occurred in 7 patients (4.6%) of the D+/R- group and 2 (2.6%) of the D-/R- group, respectively (**Table 5**).

Out of 229 patients, 17 (7.4%) experienced an episode of symptomatic CMV disease (which was detected at the discretion of the physician), with 16 (94.1%) of these patients being in the D+/R- group and only one patient being in the D-/R- group. The average time between KT and the diagnosis of the episode of CMV disease was 107.8 ± 48.8 days. Hospitalization due to CMV infection was required in 8 (47.1%) patients, all of whom were in the D+/R- group. In total, three deaths occurred during the study period, all within the D+/R- group. None of the deaths were related to CMV-disease or CMV-prophylaxis.

Quality of Life

The SF-36 results showed an overall significant improvement in QoL over 6 months post-transplant, with no significant differences between D+/R- and D-/R- groups, except in social functioning ($p = 0.0282$), where the D-/R- group had a better outcome. Additionally, the RTQ total score demonstrated both an overall significant improvement in QoL over the same period

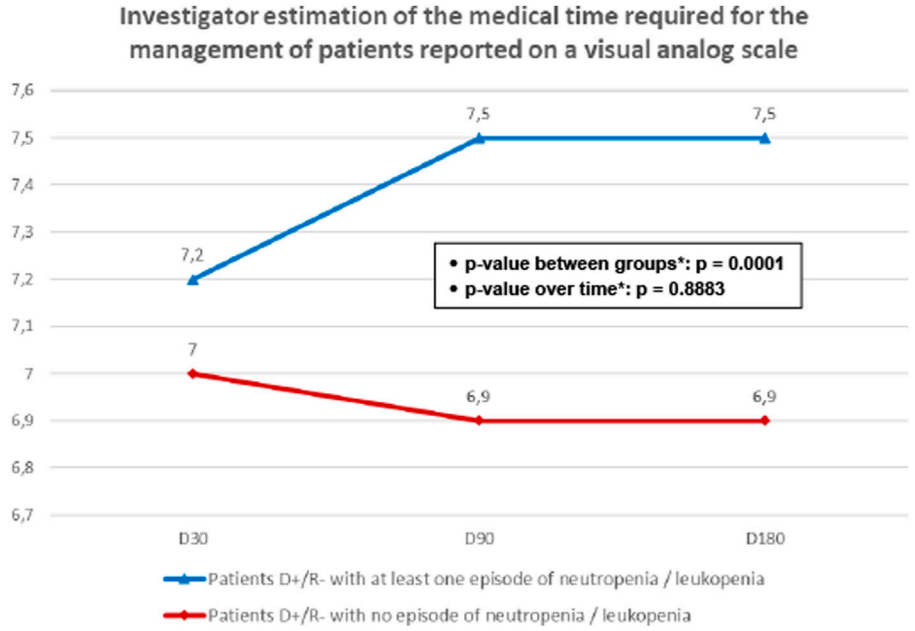


FIGURE 3 | Investigator estimation of the medical time required for the management of patients reported on a visual analog scale. D+/R-, seropositive donors/ seronegative recipients for cytomegalovirus; D-/R-, seronegative donors/ seronegative recipients for cytomegalovirus. *Mixed model for repeated measures.

and also a significant difference between the two groups (p = 0.0048), with the D-/R- group reporting a higher mean score of 75.2 (10.7) compared to 71.3 (10.6) for D+/R- group at 6 months, reflecting a better QoL (**Table 6**). Details on QoL scores are available in the online data **Supplementary Material S2**.

Healthcare and Economic Burden in D+/R- Patients

The subgroup of patients D+/R- who experienced at least one episode of neutropenia/leukopenia post-transplant (n = 40) incurred significantly higher medical costs for their medical follow-ups, with a median of approximately €4,500 (Q1 = €561; Q3 = €10,000), compared to the sub-group of those without any neutropenia/leukopenia (n = 111), which had a median cost of nearly €2,100 (Q1 = €182; Q3 = €6,500) (p = 0.02) (**Table 7**). Additionally, the subgroup of D+/R- patients with neutropenia/leukopenia had a higher number of subsequent hospitalizations following the transplant procedure (mean of 3.5 ± 3.1) compared to the subgroup of those without neutropenia/leukopenia (mean of 2.3 ± 2.0; p = 0.050); however, the duration of hospital stays did not differ significantly between the two subgroups. Furthermore, the subgroup of D+/R- patients with at least one episode of neutropenia/leukopenia had a higher mean number of outpatient consultations per patient post-transplant until D180 (5.5 ± 4.1 consultations) compared to the subgroup of D+/R- patients without episodes of neutropenia/leukopenia (4.5 ± 3.3 consultations; p = 0.042) (**TABLE 7**).

During the follow up period, patients with neutropenia/leukopenia required more medical time, as indicated by higher average scores on the VAS scale of 7.5 ± 1.1 at D90 and 7.5 ± 1.0 at D180, compared to scores of 6.9 ± 0.9 and 6.9 ± 1.1 at D90 and D180, respectively, for the subgroup without neutropenia/leukopenia. This difference was statistically significant (p = 0.0001), as shown in **Figure 3**.

DISCUSSION

In the LECOCYT study, the high-risk D+/R- group received anti-CMV prophylaxis, which was associated with a statistically significant increase in the incidence of severe leukopenia or neutropenia (grade 3 or 4) in the first 6 months following KT: 26.6% in the D+/R- group who received anti-CMV prophylaxis versus 7.7% in the untreated D-/R- group.

The adjusted OR for confounding factors was 5.16 [95% CI: 1.97–13.53], indicating that patients in the D+/R- group were more than five times as likely to develop severe leukopenia or neutropenia compared to the D-/R- group. This is likely related to the use of valganciclovir, which was the main prophylaxis treatment used by 98.7% of patients. The association between valganciclovir and higher rates of leukopenia or neutropenia is supported by a recent RCT and in a retrospective study that showed a higher incidence of leukopenia or neutropenia with valganciclovir compared to letermovir, a newer anti-CMV treatment, in adult D+/R- recipients over a 52-week period [14, 16]. Valganciclovir-based prophylaxis remains a widely used strategy for preventing CMV infection, but it has

limitations beyond its hematologic toxicities such as the development of resistance or the need for close monitoring of kidney function for dose adjustments [14].

An alternative to prophylaxis is the preemptive therapy strategy, which requires a weekly monitoring of CMV DNAemia and the initiation of treatment when the latter is detected. This strategy may be responsible of less leucopenia. However, the risk of anti-CMV resistance is higher is D+/R- kidney-transplant patients receiving preemptive therapy [17]. Furthermore, in an international survey, it has been shown that most transplant physicians prefer prophylaxis to preemptive therapy in this setting [5].

Our study captured a difference in the time required for the management of patients of D+/R- with severe leukopenia or neutropenia despite a low number of outpatient consultations reported per patient post-transplant, as leukopenia/renal function is often managed remotely in current practice in France. The additional time required for the management of D+/R- with severe leukopenia or neutropenia may result from the necessity for dose adjustments based on renal function. D+/R- patients with severe neutropenia or leukopenia, scored higher on the VAS for medical time and required more rehospitalizations and outpatient consultations ($p = 0.042$) with higher total healthcare expenditures observed for these patients ($p = 0.025$), despite the variability and sample size limitations that warrant cautious interpretation of cost differentials. Increased healthcare costs post-transplant, associated with episodes of neutropenia or leukopenia have been reported [18, 19]. Our study is the first to assess the direct costs associated with severe leukopenia or neutropenia in D+/R- adults in France.

The study faced limitations in data collection, as actual costs were unavailable. Consequently, researchers used estimated costs based on standardized reimbursement package for hospital admissions, outpatient consultations and work absenteeism. Another limitation of our study is the exclusion of R+ patients, who typically receive prophylaxis for 3 months. Additionally, data on the dosages of mycophenolic acid (MPA) administered to patients was not available. MPA is an immunosuppressive agent commonly used in organ transplant recipients to prevent graft rejection. Accurate dosing information is crucial, as it can affect both the risk of infection and the incidence of hematological adverse events, including leukopenia and neutropenia. This study was funded by MSD France whom did not play any role in data collection and did not interfere in the results interpretation. The study was supervised by an internationally recognized expert committee with a strong track record in this field, ensuring rigorous oversight throughout the process. The study was carried out in strict adherence to all applicable clinical research standards and regulations, with full efforts to maintain the integrity of the research. Despite these constraints, the research offers important insights and is notable for being the only prospective, real-world study in France that explores the clinical and economic burden associated with hematological toxicities related to anti-CMV prophylaxis in D+/R- patients.

In conclusion, the LECOCYT study found that KT recipients receiving current anti-CMV prophylaxis (D+/R-) have a higher risk of severe hematologic toxicities compared to unexposed patients (D-/R-). D+/R patients with leukopenia or neutropenia grade 3 or 4 required more medical management time and incurred in higher costs than those without episodes.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of ethics restrictions. Requests to access the datasets should be directed to christophe.legendre@aphp.fr.

ETHICS STATEMENT

The studies involving humans were approved by COMITE DE PROTECTION DES PERSONNES ILE DE FRANCE IV Saint Louis. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Participated in research design: NK, CL, ID-Z, SD, and LB; Participated in the coordination or conduct of the study: NK, CL, HK, CM, ID-Z, CC, GT, XB, and LB; Participated in the data collection: NK, CL, HK, CM, and the LECOCYT Study Group; Participated in the analysis and interpretation of the data: NK, CL, HK, CM, ID-Z, CC, GT, XB, LB, SD, and MN; Participated in the writing and revising of the manuscript: NK, CL, HK, CM, ID-Z, CC, GT, XB, LB, SD, and MN. All authors contributed to the article and approved the submitted version.

GROUP MEMBERS OF ON BEHALF OF THE LECOCYT STUDY GROUP

Gabriel CHOUKROUN, CHU Amiens; Fatouma TOURE-DIABIRA, CHU Limoges; Laeticia ALBANO, Hôpital Pasteur 2; Mathilde LEMOINE, CHU Rouen; Alexandre HERTIG, Hôpital Foch; Thibault RENOUF, CHU Rennes; Antoine DURRBACH, Hôpital Henri-Mondor; Lionel ROSTAING, CHU Grenoble Alpes – Hôpital Nord Michallon; Claire TINEL, CHU Bocage, Antoine THIERRY, CHU Poitiers; Cyril GARROUSTE, CHRU Gabriel Montpied; Martin PLANCHAIS, CHU Angers; Peggy PERRIN, Nouvel Hôpital Civil Strasbourg, Magali GIRAL, CHU Nantes; Pierre MERVILLE, CHU Bordeaux; Yannick LE MEUR, CHRU Brest; Tristan LEGRIS, Hôpitaux Universitaires de Marseille Conception; Pierre GALICHON, La Pitié Salpêtrière; Betoul

SCHVARTZ, CHU Reims; Philippe GATAULT, CHRU Tours; Christophe MARIAT, CHU Saint-Etienne – Hôpital Nord.

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CONFLICT OF INTEREST

Authors CC, GT, XB, and LB are employed by MSD France. LB holds stock in MSD. HK has been a speaker for Biotest, and a consultant for MSD, GSK, and Takeda. ID-Z reports having received consulting fees from MSD. NK has received speakers' fees and participated to advisory boards for Alexion, Astellas, AstraZeneca, Biotest, BMS, CSL Behring, Chiesi, ExeViR, Gilead, Grifols, Hansa, MSD, Glasgow Smith Kline, Pierre Fabre, Medison, Neovii, Novartis Pharma, Roche, Sanofi, Sandoz, Synklino, Takeda. Authors MN and SD were employed by ClinSearch.

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

GENERATIVE AI STATEMENT

The authors 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.2025.14342/full#supplementary-material

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12-Month Outcomes of a Prospective Randomized Trial Investigating Effects of IVIG on Top of rATG Versus rATG Alone in Pre-Sensitized Kidney Transplant Recipients: The INHIBIT Study

Ondrej Viklicky[1,2], Ivan Zahradka[1], Jan Mares[3], Janka Slatinska[1], Alena Parikova[1], Vojtech Petr[1], Matej Roder[4], Katerina Jaklova[4], Klara Osickova[1], Libor Janousek[5] and Petra Hruba[2]*

[1]Department of Nephrology, Institute for Clinical and Experimental Medicine, Prague, Czechia, [2]Transplantation Laboratory, Institute for Clinical and Experimental Medicine, Prague, Czechia, [3]Department of Data Science, Institute for Clinical and Experimental Medicine, Prague, Czechia, [4]Department of Immunogenetics, Institute for Clinical and Experimental Medicine, Prague, Czechia, [5]Department of Transplantation Surgery, Institute for Clinical and Experimental Medicine, Prague, Czechia

Intravenous immunoglobulins (IVIG) are commonly used in peri-transplant desensitization, but evidence supporting their efficacy is limited. We conducted a prospective, randomized single-center, open-label, Phase IIIb non-inferiority clinical pilot trial to compare the efficacy of IVIG (administered at a dose of 3 × 0.5 g/kg) versus no IVIG, in conjunction with rabbit anti-thymocyte globulin (5–7 mg/kg) induction, in pre-sensitized patients with donor-specific antibodies who had negative pre-transplantation Flow- and CDC-crossmatches, between July 2020 and November 2022. The primary endpoint was the rate of efficacy failure, defined as biopsy-proven rejection within 12-month post-transplant. Secondary endpoints included the incidence of rejection at protocol biopsies, evaluated by histology and biopsy-based transcripts diagnostics. Of the screened patients, 53 (72.6%) were excluded due to crossmatch positivity. Ten patients were randomized to the IVIG+, and 7 to the IVIG-arm. The trial was prematurely terminated due to futility at interim analysis. In the IVIG-arm, 3 patients (43%) experienced the primary endpoint compared to none in the IVIG+ arm ($p = 0.026$). MMDx identified one molecular ABMR in the IVIG+ and 2 in the IVIG-arm in 12-month protocol biopsies. There was one graft loss in the IVIG-arm. The results of this pilot study, although not definitive, do not support the use of IVIG-sparing regimens in HLA-incompatible kidney transplantation (NCT04302805).

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***Correspondence**
Ondrej Viklicky,
ondrej.viklicky@ikem.cz

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Abbreviations: ABMR, antibody-mediated rejection; ACR, albumin/creatinine ratio; BKV, BK polyomavirus; CDC, complement-dependent cytotoxic crossmatch; CMV, CI cytomegalovirus confidence interval; cPRA, calculated panel-reactive antibodies; DGF, delayed graft function; DSA, donor specific antibodies; EBV, Eppstein-Barr virus; eGFR, estimated glomerular filtration rate; FCXM, flow-cytometry crossmatch; HLA, human leukocyte antigen; IQR, interquartile ranges; IVIG, Intravenous immunoglobulins; KTR, kidney transplant recipient; MFI, mean fluorescence intensity; MMDX, Molecular Microscope Diagnostic System; POD, post-operative day; PRA, panel-reactive antibodies; PTDM, post-transplantation diabetes mellitus; rATG, rabbit anti-thymocyte globulin; SAB, single antigen bead; SSP, sequence specific primer; TCMR, T-cell mediated rejection.

This study is registered on ClinicalTrials.gov under the identifier NCT04302805.

Keywords: IVIG, desensitization, induction, HLA-incompatible transplantation, kidney transplantation

INTRODUCTION

Kidney transplantation across the HLA barrier is associated with an increased risk of antibody-mediated rejection (ABMR) and inferior transplantation outcomes [1, 2]. Consequently, the presence of donor specific anti-HLA antibodies (DSA) prior to transplantation is often met with reluctance to accept transplant offers. However, a too cautious approach is impractical for broadly sensitized patients, who thus often wait for many years and are at risk of never being transplanted. To address this, some centres offer HLA-incompatible (HLAi) transplantations to highly sensitized patients, after carefully weighing the associated risks and benefits [3, 4].

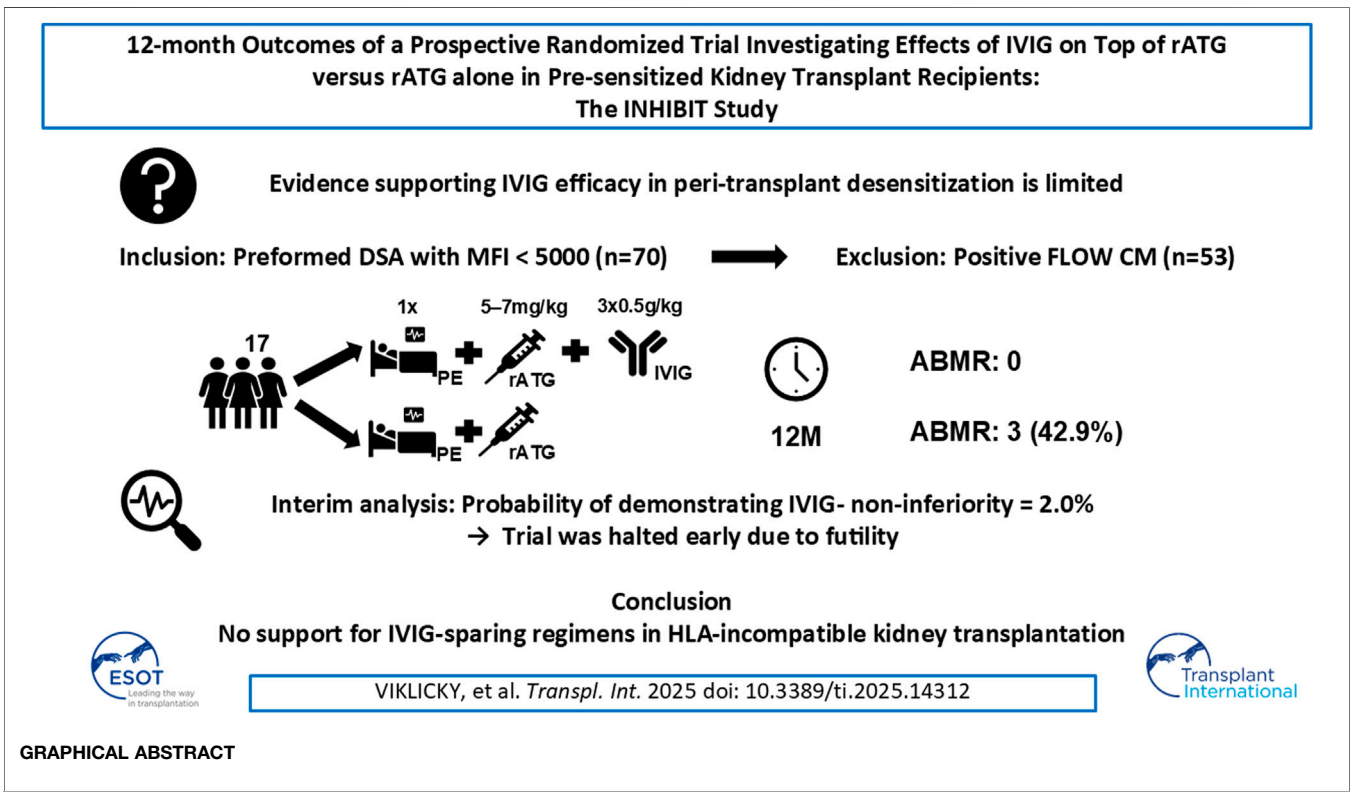
Desensitization and induction protocols in HLAi transplantation are based mainly on centre experiences rather than robust data-based evidence [5]. Among the desensitization armamentarium are intravenous immunoglobulin (IVIG) that have been widely used for decades [6, 7]. The mechanism of action is not well understood, but several have been proposed, including non-specific blockade of Fc receptor, expansion of regulatory T cells, inhibition of B-cell activation and proliferation, inhibition of antibody rebound or immunoregulatory functions of natural antibodies [8–14]. However, the use of IVIG for desensitization has not yet received FDA approval, and the evidence supporting this practice remains limited and weak [5]. In the sole randomized controlled trial performed to date, graft survival was comparable between IVIG and placebo groups, but higher rejection rate was observed in those treated with IVIG despite a reduction in panel-reactive antibodies (PRA) [8]. Observational studies have suggested potential benefits of IVIG, however, these studies should be interpreted with caution due to their retrospective and observational design [15].

Given the limited supporting data, high cost and limited availability due to the human origin of the products, further research into its role in peri-transplant desensitization is of clinical importance. Therefore, we conducted an investigator-initiated randomized trial to evaluate whether rabbit anti-thymocyte globulin (rATG) alone is as effective as rATG combined with IVIG, which is currently the standard of care in HLAi kidney transplantation.

MATERIALS AND METHODS

Study Design and Population

This is a prospective interventional randomized single-centre open-label two-arm Phase IIIb non-inferiority investigator-initiated pilot clinical trial. The aim was to determine whether the induction with rATG alone (intervention) is clinically non-



GRAPHICAL ABSTRACT

inferior to rATG combined with IVIG (centre standard of care) in preventing biopsy proven rejection within the first 12 months following HLAi transplantation. This study was approved by the Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer Hospital (No. A-19-13) and was conducted in accordance with the Helsinki Declaration and good clinical practice guidelines. The clinical trial is registered at ClinicalTrials.gov under Identifier NCT04302805.

The main inclusion criterion was the presence of low levels of preformed anti-HLA DSA, defined as mean fluorescence intensity (MFI) of <5,000 (class I and class II antibodies, except for DQ antibodies where higher MFI values might be accepted). The main exclusion criterion was flow-cytometry crossmatch (FCXM) and/or complement-dependent cytotoxic (CDC) crossmatch positivity prior to transplant surgery. Therefore, the study population consists of patients at category 3 risk of current recommendations of the ENGAGE working group [4]. All the inclusion and exclusion criteria are listed in **Supplementary Table S1**.

Participants were enrolled at the study centre by the study investigators. Participants were sequentially allocated a unique identification number that was generated electronically via an electronic case report form by the study investigators. Participants were randomized either into the IVIG- or the IVIG+ group prior to the transplantation by a stratified randomization algorithm and assigned to intervention by the study investigators. Random allocation was made in blocks of 4 in a 1:1 ratio and was stratified according to baseline characteristics: gender (male vs. female), donor type (deceased vs. living donor) and type of transplantation (first transplantation vs re-transplantation). The planned follow-up period was 12 months, with protocol biopsies scheduled at months 3 and 12. The study visit schedule and procedures are outlined in **Supplementary Table S2**.

Treatment Protocol

After obtaining written informed consent from participants, all patients underwent a single plasma exchange (1x total plasma volume) immediately before kidney transplantation. IVIG was administered at post-operative days (POD) 1, 3, and 5 at a dose of 0.5 g/kg [15]. Rabbit anti-thymocyte globulin (rATG; Thymoglobulin, Sanofi) was initially administered during transplant surgery (1.5 mg/kg), followed by daily doses until a cumulative dose of between 5 mg/kg and 7 mg/kg was achieved. Methylprednisolone 500 mg was given prior to reperfusion and on POD1. Maintenance immunosuppression consisted of once-daily extended-release tacrolimus formulation given pre-transplantation with target range of 8–12 ng/mL, mycophenolate mofetil (2000 mg tapered to 1,000 mg by month 3) and tapered prednisone, starting at 20 mg.

Infection prophylaxis consisted of valganciclovir for 100 days and trimethoprim/sulfamethoxazole 480 mg/day for 6 months. Further details regarding the study treatment protocol can be found in **Supplementary Table S3**.

Outcome Measures

The primary outcome measure was efficacy failure, defined as biopsy-proven ABMR and/or T-cell mediated rejection (TCMR) according to the Banff 2017 classification regardless of biopsy indication (for-cause or per-protocol) up to 12 months post-transplantation.

Secondary efficacy outcomes included the incidence of individual rejection types and biopsy findings (active ABMR, chronic active ABMR, acute TCMR, chronic TCMR) both in for cause and protocol biopsies, time to active ABMR, and incidence of delayed graft function (DGF). Molecular assessment of all available 12-month protocol biopsies was conducted using the Molecular Microscope Diagnostic System (MMDx) platform [16]. The dynamics of estimated glomerular filtration rate (eGFR), albuminuria (expressed as albumin/creatinine ratio; ACR) and donor-specific antibodies (DSA) were evaluated at regular time-points.

Secondary safety outcomes included the incidence of all-cause mortality, graft loss, leucopenia (requiring treatment of immunosuppression adjustment), post-transplant diabetes mellitus (PTDM), cardiovascular disease, malignancy and infectious complications, including bacterial infections and viral infections such as BK polyomavirus (BKV) and cytomegalovirus (CMV).

Anti-HLA Antibody Evaluation

Anti-HLA antibodies were analysed using single antigen bead (SAB) technology with LabScreen Mixed and LabScreen Single Antigen Luminex technique (both One Lambda, Inc.). Donor HLA typing was used for the assessment of donor specific antibodies (DSA). Organ donors were typed using polymerase chain reaction sequence specific primer (SSP) low-resolution kits (Olerup SSP, and Histo Type SSP, BAG). DSA assessment was performed with the HLA fusion software (One Lambda, Inc.). FCXM was performed according to previously described methodology [17].

Sample Size Calculation

The primary hypothesis of clinical non-inferiority of IVIG-as compared to IVIG+ at the non-inferiority margin of 20% (absolute incidence) of the primary endpoint was chosen to be tested by a one-sided 90% Wald confidence interval for difference in incidence rates. Based on the assumption of expected incidence rates of 45% in both study groups, the required sample size for 80% study power was calculated to be 138 patients total (69 per study group) while correcting for 20% drop out. The reduced level of statistical significance and the relatively large non-inferiority margin were chosen with regard to the limited number of potential participants available in the population and also to the fact that IVIG+ was the centres' standard of care. Therefore, it was considered more ethical to first conduct a study with smaller sample size to limit the exposition of a potentially inferior treatment to many patients, despite the limited conclusion that could have been made due to increased chance of the type I error.

TABLE 1 | Study population characteristics.

	IVIG+ (n = 10)	IVIG- (n = 7)
Sex (male), n (%)	5 (50%)	2 (28.6%)
Age (years), median (IQR)	58 (44.8–60.9)	53.4 (45.1–58.6)
CPRA (%), median (IQR)	96.4 (69.2–99.1)	66.9 (48.3–77.1)
PRA max (%), median (IQR)	14 (11.5–55)	22 (12–37)
HLA mismatch, median (IQR)	3 (2–5)	4 (4–6)
Re-transplantation, n (%)	5 (50%)	3 (42.9%)
CMV mismatch, n (%)	3 (30%)	1 (14.3%)
Donor age (years), median (IQR)	48 (39.5–56.8)	52 (42–57)
Dialysis vignette duration (years), median (IQR)	2.4 (0.6–3.8)	4.3 (1.1–4.6)
Deceased donor, n (%)	10 (100%)	7 (100%)

Abbreviations: CMV, cytomegalovirus; cPRA, calculated panel reactive antibodies; HLA, human leukocyte antigen; PRA, panel reactive antibodies; IQR, interquartile range.

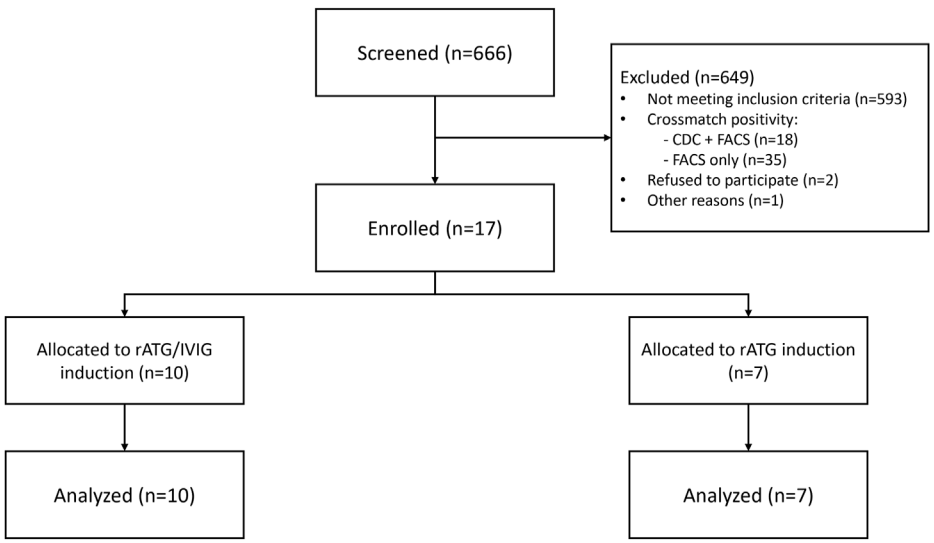


FIGURE 1 | Study flow chart. A total of 666 patients were screened during the study period; 593 did not meet the inclusion criteria, mainly by the absence of donor specific antibodies (DSA). From the 73 DSA positive patients who met the other inclusion criteria, 56 could not be enrolled due to CDC and/or FCXM crossmatch positivity. Finally, 17 patients undergoing HLA-incompatible transplantation were enrolled, 10 were randomized into the rATG/IVIG (IVIG+) group, and 7 were randomized into the rATG without IVIG (IVIG-) group.

Statistics

Statistical analysis was performed using R, version 4.3.2 (R Core Team 2023; R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL[1]).

Continuous variables are reported as medians with interquartile ranges (IQR), categorical as proportions (%). Barnard's test, an alternative to the Fisher's test that is suited for examining contingency tables with a single fixed marginal (as in this study), was used to compare categorical variables, including primary and secondary outcomes. The non-parametric Wilcoxon test was used to compare continuous variables. In the interim analysis, the conditional power was evaluated by performing random simulations of the trial with the expected event rates given as equally weighted averages of the observed rates and the originally assumed rates. Following the early termination of the study at the interim analysis (described below) due to futility in demonstrating the non-inferiority of IVIG-, a post-hoc test for differences in the main outcome between the study groups was conducted. Confidence intervals for the primary outcome were calculated using the Miettinen-Nurminen method. The alpha level for this post-hoc test and for all the other analyses performed was the common standard of 5%. The full statistical analysis plan is provided in **Supplementary Material S1**.

RESULTS

Patient Characteristics

A total of 17 patients were randomized between 18 September 2020, and 23 November 2022, with 10 assigned to the IVIG+

[1]https://www.r-project-org

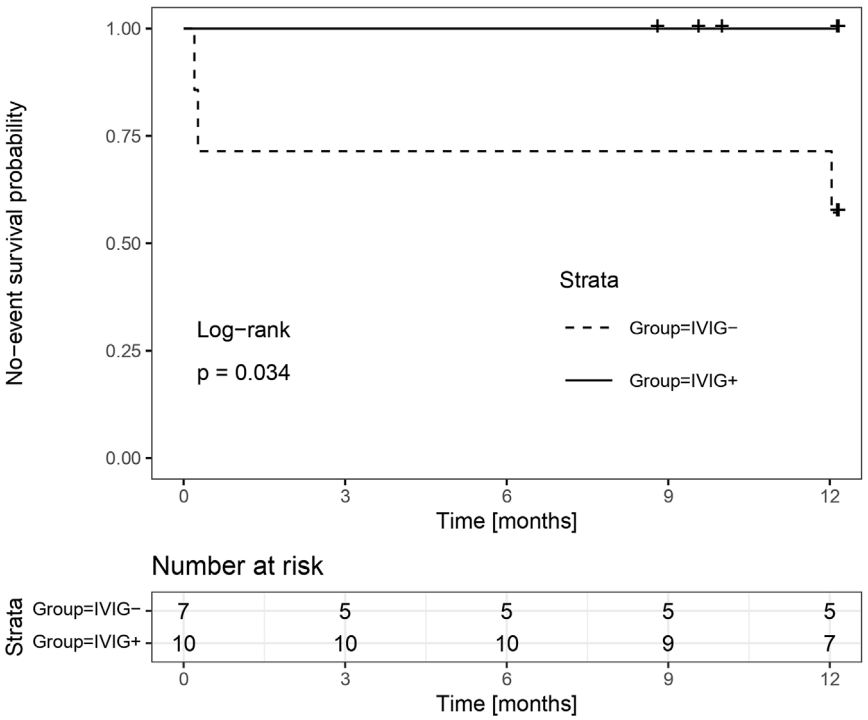


FIGURE 2 | Interim analysis. Rates of the primary outcome at the time of the interim analysis estimated with the use of the Kaplan-Meier method. The primary outcome measure was efficacy failure defined as a biopsy proven antibody mediated rejection and/or T-cell mediated rejection up to 12 months post-transplantation.

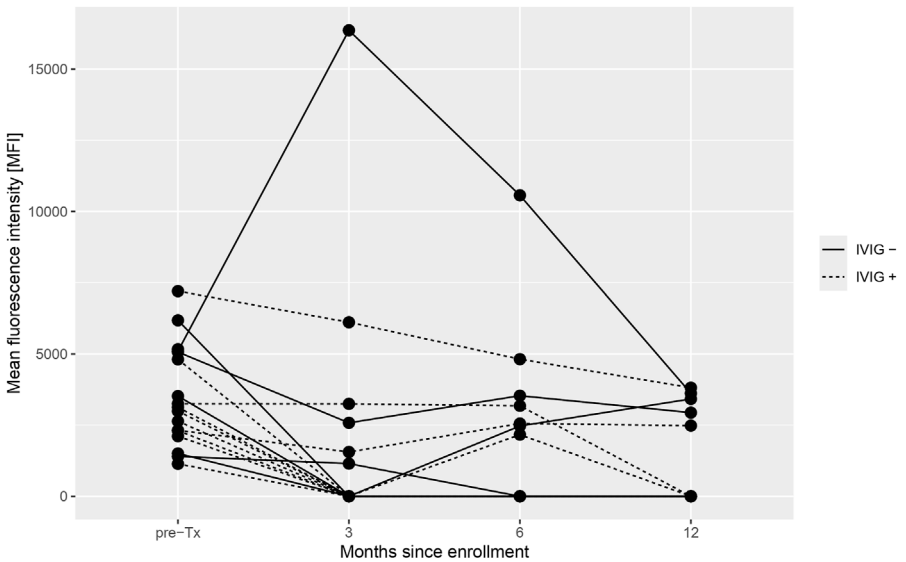


FIGURE 3 | Development of immunodominant donor-specific antibodies (DSA) of each enrolled patient during the 12-month study period DSA were measured during the predefined timepoints at month 3, 6, and 12. Immunodominant DSA was defined as the DSA with the highest mean fluorescent intensity prior to transplantation.

group and 7 to the IVIG-group. Baseline characteristics are shown in **Table 1**, and the study flowchart is presented in **Figure 1**.

Interim Analysis and Trial Termination

An interim analysis was conducted earlier than originally planned due to slower-than-expected enrolment. At this point, 17 patients

had been enrolled. It was revealed that at the time of the interim analysis there were 3 primary events in the IVIG-group, while no primary event occurred in the IVIG+ group (**Figure 2**). The one-sided 90% confidence interval (CI) for the difference in event rates based on these data was (-∞, +83%), far exceeding the non-inferiority margin of 20%. Moreover, the conditional power (the probability of demonstrating IVIG- non-inferiority if the study continued until the originally planned sample size after updating assumptions about the future based on the data that was already observed) was only 2.0%. Given these findings, the trial was terminated for futility.

Primary Endpoint and Incidence of Antibody-Mediated Rejection

After the 1-year follow-up, the primary endpoint occurred in 3 patients (42.9%) in the IVIG-arm and 0 patients (0%) in the IVIG+ arm ($p = 0.026$), with a one sided 90% CI of −100%–66.4%, which is not in favour of non-inferiority of IVIG-at the non-inferiority margin of 20% absolute difference. The corresponding two-sided 95% CI was 6.8%–75.6%, indicating the superiority of IVIG+ over IVIG-.

Of the three primary endpoint occurrences, there were two cases of active ABMR early after transplantation diagnosed at post-operative day 6 and 8, respectively, and one case of chronic active ABMR at a 12-month protocol biopsy. There were no instances of acute or chronic T-cell mediated rejection during the study period.

There was one case of graft loss in the IVIG-arm on day 8 post-transplantation. This was due to a case of ABMR which presented with TMA and extensive infarctions at the time of biopsy. Importantly, CDC crossmatch was performed on the day of biopsy, which was positive. Graftectomy was performed due to the serious histology finding and poor prognosis.

Protocol Biopsies at 3 and 12 Months

Protocol biopsies were performed in 14 out of 16 patients (87.5%) with functioning grafts at 3 months and 13 out of 16 patients (81.3%) at 12 months, with 2 and 3 patients, respectively, declining the biopsy and 1 experiencing graft loss prior to the 3-month mark.

Histological evaluation of the 3-month biopsies revealed no definitive rejections while the MMDx assessments were not performed at this time-point. The evaluation of the 12-month biopsies revealed one case of chronic active ABMR in the IVIG-arm, confirmed by biopsy-based transcripts assessment evaluated by the Molecular Microscope Diagnostic System (MMDx). MMDx also identified early-stage of molecular ABMR in 2 additional biopsies, one from each arm, that did not fully satisfy the Banff criteria for ABMR (**Supplementary Table S4**).

Molecular rejections were not counted towards the primary endpoint, as molecular assessment was not originally planned for in the study protocol as the MMDx platform was not available at the study's inception and it was not performed in all biopsies. However, a post-hoc analysis, incorporating both histological and molecular findings, identified 3 events (43%) in the IVIG-group and 1 event (10%) in the IVIG+ group ($p = 0.16$).

Evolution of Anti-HLA Donor-Specific Antibodies During the Study Period

In 11 patients (64.7%), pre-formed DSA either decreased or became undetectable during follow-up, with only one patient in the IVIG- group showing notable transient increase in preformed DSA. One case of *de-novo* DSA with low MFI was observed in the IVIG+ group. The dynamics of immunodominant DSA for each patient during follow-up are shown in **Figure 3** and detailed in **Supplementary Table S5**.

Safety Outcomes

No patients died during the study follow-up. The incidence of delayed graft function did not differ significantly between the IVIG+ and IVIG-groups (42.9% vs. 30%, respectively; $p = 0.62$). Similarly, there were no significant differences in the frequency of bacterial or viral infections between the groups. For a comprehensive overview of the secondary outcomes see **Table 2**. Details of therapeutic drug monitoring are provided in **Supplementary Table S6**.

DISCUSSION

The use of IVIG in peri-transplant desensitization protocols for HLA-incompatible kidney transplantation is widespread, despite the low quality of supporting evidence [5]. In this prospective, randomized trial, we hypothesized that an IVIG-free induction protocol would be as efficacious as a combined regime, provided the immunological risk was well characterized at the time of transplantation. However, the findings from the pilot INHIBIT study, although not definitive, do not support the use of IVIG-sparing regimens in HLA-incompatible kidney transplantation, even when pre-transplant FCXM is negative, and DSA levels are low.

Recently, several groups have studied outcomes of DSA positive kidney transplantation, demonstrating a high incidence of ABMR, including subclinical cases [3, 18–20]. Outcomes of HLAi transplantation depend on how pretransplant risks are defined. The definition of acceptable DSA levels for transplantation remains unclear and varies considerably across transplant centres. Some centres do not accept DSAs of any level, in other centres certain DSA levels are acceptable when CDC crossmatch is negative and peri-transplant desensitization is applied. Moreover, DSA MFI thresholds for organ acceptance also vary, influenced by centre practice, analytical platform, antigen type, and delisting strategies among others [4, 21–25]. One approach adopted by several centres, including ours, is to accept HLAi kidney offers if the FCXM is negative, as the risk of ABMR is arguably acceptable [17, 26, 27]. In our study, however, the inclusion of FCXM testing for all HLA-incompatible transplants was associated with a 76% FCXM positivity among "low" DSA positive patients who were invited to the centre for a physical crossmatch. While this approach likely improved the identification of patients at lower risk of ABMR, it also contributed to an unexpectedly high drop-out rate and delayed enrolment. However, the rates

TABLE 2 | Secondary and safety outcomes.

	IVIG+ (n = 10)	IVIG- (n = 7)	p-value
Secondary efficacy outcomes			
Incidence of active ABMR, n (%)	0 (0%)	2 (28.6%)	0.077
Incidence of chronic active ABMR, n (%)	0 (0%)	1 (14.3%)	0.26
Incidence of acute or chronic TCMR, n (%)	0 (0%)	0 (0%)	1
Delayed graft function, n (%)	3 (42.9%)	3 (30%)	0.62
eGFR at 12 months, mL/min/1.73 m^2 (IQR)	0.89 (0.62–1.05)	1.33 (0.72–1.41)	0.32
Albumin/creatinine ratio at 12 months, g/mol (IQR)	2.3 (1.9–3.5)	4.2 (0.6–18.2)	0.64
Secondary safety outcomes			
Mortality during the study period, n (%)	0 (0%)	0 (0%)	1
Graft loss during the study period, n (%)	0 (0%)	1 (14.3%)	0.26
Leucopenia requiring treatment or immunosuppression adjustment, n (%)	4 (40%)	3 (42.9%)	0.92
Incidence of cardiovascular disease, n (%)	3 (30%)	0 (0%)	0.16
Incidence of post-transplant diabetes mellitus, n (%)	1 (10%)	0 (0%)	0.77
Incidence of malignancy, n (%)	0 (0%)	1 (14.3%)	0.56
Bacterial infection requiring antibiotic therapy, n (%)	7 (70%)	4 (57.1%)	0.6
COVID-19, n (%)	2 (20%)	4 (57.1%)	0.13
CMV replication above 1,000 copies/mL or CMV disease, n (%)	2 (20%)	1 (14.3%)	0.9
EBV replication above 500 copies/mL, n (%)	1 (10%)	1 (14.3%)	0.98
BKV replication above 10,000 copies/mL or BKV nephropathy, n (%)	0 (0%)	0 (0%)	1
Permanent discontinuation of study treatment, n (%)	0 (0%)	0 (0%)	1

Abbreviations: ABMR, antibody mediated rejection; BKV, BK polyomavirus; CMV, cytomegalovirus; COVID-19, coronavirus disease 2019; eGFR, estimated glomerular filtration rate; EBV, Eppstein-Barr virus; IQR, interquartile range; TCMR, T-cell mediated rejection.

of ABMR were much higher when FCXM was not available for decision making prior to HLAi transplantations in our centre in the past [17], and similarly, high ABMR occurrences were observed in several previous observational studies where FCXM-positive patients were transplanted [28–30].

Importantly, our study used an approximate MFI threshold of <5,000 as one of the criteria for inclusion. While some studies indicate that patients with preformed DSA in the range of 5,000–10,000 MFI may have similar outcomes to those with MFI <5,000 [20], others suggest that MFI >5,000 is associated with a substantially increased risk of ABMR [31]. Given the intervention in our study was IVIG elimination, we opted for a more conservative DSA MFI threshold and implemented FCXM pretransplant as a go/no go rule. Therefore, our study population corresponds to category 3 of the recent ENGAGE recommendations, characterized by acceptable medium-term graft survival, but with recommended adaptation of immunosuppression [4]. IVIG is frequently employed in such cases, and our study provides further evidence supporting its utility.

Molecular diagnostic methods are currently being recommended to improve diagnostic precision in HLAi transplantation [32]. To better understand intragraft molecular processes, we retrospectively performed biopsy-based transcripts diagnostics using validated MMDx platform in all available 12-month protocol biopsies. Molecular rejection was identified in two cases in the IVIG-sparing arm and one case in the IVIG+ arm, with only one of these cases showing clear corresponding histological finings. These results suggest that molecular diagnostics may offer a sensitive tool to clarify ABMR cases in biopsies with histologic ambiguity [18, 33–36].

Encouragingly, no rejection episodes by histology occurred in the IVIG+ group during follow-up. The explanation why IVIG therapy given early after transplantation might be effective likely stems from its proposed desensitization mechanism of action. In most patients, DSA levels decreased and remained below the threshold of positivity during follow-up, while persistent or increasing DSA levels were observed in all ABMR cases, all of whom were in the IVIG-group. However, as not all patients with persistent DSA developed ABMR, be it histological or molecular, it is possible that other immune mechanisms, such as those involving plasma cells or natural killer cells, contribute to ABMR pathogenesis, as is recently discussed [33].

A major limitation of our study was the lower-than-expected sample size, which was due to the slow enrolment rate associated with frequent FCXM positivity among DSA-positive patients. Nonetheless, the study was ultimately terminated early for futility, as interim results indicated that proving non-inferiority of the IVIG-sparing regimen was highly unlikely, even if the planned number of participants had been reached. Contrary to our hypothesis, significantly higher rates of the primary endpoint were observed in the IVIG-sparing arm, and this important biological signal must be taken seriously. Furthermore, in theory, type I error could have been inflated if study results were tested multiple times during enrolment and the trial terminated whenever a significant result would have been reached. However, in the case of our study, the data were analysed only at a single time point during the interim analysis so the risk of type I error should not be increased.

In conclusion, the results of this pilot study, although not definitive, do not support the use of IVIG-sparing regimens in HLA-incompatible kidney transplantation, despite the low number of participants and premature study termination. IVIG, the current standard of care, should likely remain an integral part of induction protocols to achieve the best possible outcomes in patients undergoing HLA-incompatible transplantation.

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 Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors were responsible for the conceptualisation of the study. OV, IZ, JS, AP, KJ, LJ, PH, and KO took part in data curation, and JM, IZ, OV, MR, and PH were responsible for formal analysis. OV was responsible for funding acquisition. JM, IZ, and OV conducted the investigation, and IZ, JS, OV, and JM were responsible for the methodology. OV was responsible for project administration and supervision. All authors participated in the acquisition of resources. JM and VP were responsible for the software support. IZ, OV, VP, and PH validated the results. JM and VP had taken part in the visualisation. IZ and OV wrote the original draft. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

OV received speaker and/or consultancy honoraria from Astellas and Chiesi.

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

GENERATIVE AI STATEMENT

The authors 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.2025.14312/full#supplementary-material

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ORIGINAL RESEARCH
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The Association Between Early Graft Function, Donor Type and Long-Term Kidney Transplant Outcomes

Karthik Venkataraman[1,2,3†], *Georgina L. Irish*[1,2,3†], *Michael G. Collins*[1,2,3†] and *Philip A. Clayton*[1,2,3*†]

[1]Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, SA, Australia, [2]Central and Northern Adelaide Renal and Transplantation Service, Royal Adelaide Hospital, Adelaide, SA, Australia, [3]Transplant Epidemiology Group (TrEG), Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry, South Australian Health and Medical Research Institute (SAHMRI), Adelaide, SA, Australia

Delayed graft function (DGF), is associated with inferior graft outcomes. Whether poor graft function without dialysis, termed slow graft function (SGF), affects outcomes is unclear. We investigated associations between SGF (serum creatinine dropping by less than 30% between days 1 and 2), DGF and graft outcomes by donor type in a cohort of 17,579 Australian and New Zealand kidney transplant recipients from 2001–2021. The primary outcomes were graft survival and death-censored graft survival Compared with immediate graft function, both SGF (Adjusted hazard ratio [aHR] 1.48 (95% CI 1.14–1.91) and DGF [aHR 1.97 (1.42–2.73)] were associated with reduced graft survival in living donor and donation after brain death (DBD) recipients [SGF aHR 1.13 (1.01–1.27); DGF aHR 1.37 (1.24–1.51)]. In donation after circulatory death (DCD) recipients, DGF [(aHR 1.52 (1.13–2.04)] but not SGF [(aHR 1.55 (1.13–2.13)] was associated with reduced graft survival. Findings were similar for death-censored graft survival. In secondary analyses, SGFwas associated with reduced patient survival in living donor recipients. SGF and DGF were associated with lower 12-month eGFR for all donor types. DGF increased the odds of rejection for all donor types; for SGF this association was significant only for DBD recipients. SGF is associated with adverse outcomes in live donor and DBD kidney recipients.

Keywords: graft function, kidney transplant, ANZDATA, graft survival, delayed graft function (DGF)

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***Correspondence**
Philip A. Clayton,
phil@anzdata.org.au

†ORCID:
Karthik Venkataraman
orcid.org/0000-0001-8873-0734
Georgina L. Irish
orcid.org/0000-0003-0758-1867
Michael G. Collins
orcid.org/0000-0003-2169-9087
Philip A. Clayton
orcid.org/0000-0001-9190-6753

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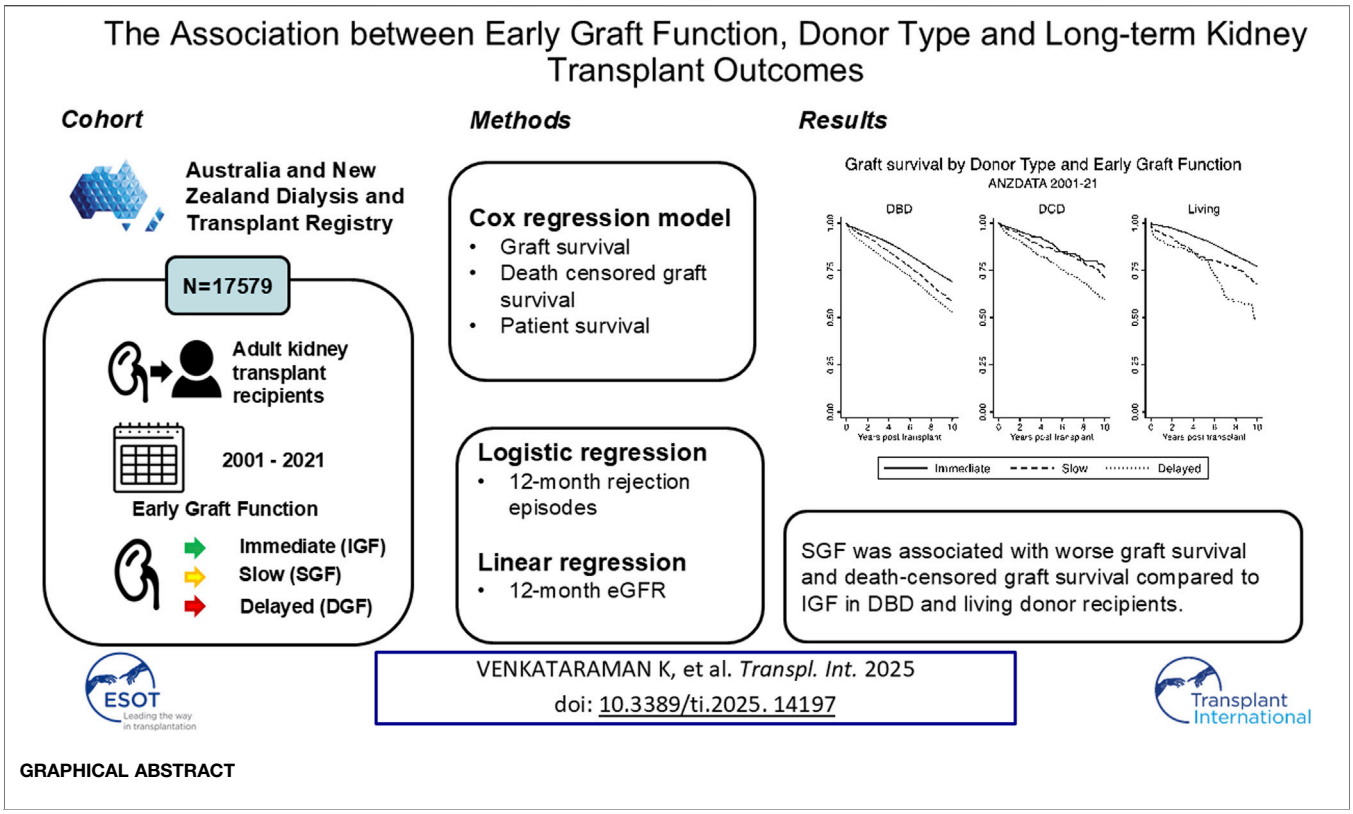
INTRODUCTION

Kidney transplantation provides improved quality of life and improved survival, at reduced cost, when compared to dialysis as a kidney failure treatment [1–3]. The function of the transplant graft in the days after kidney transplantation, termed early graft function, has important clinical implications. Poor EGF is associated with increased post-transplant dialysis sessions, increased days in hospital and increased resource utilisation [4, 5]. In addition, poor early graft function may influence clinical decision-making around calcineurin inhibitor dosing and result in

Abbreviations: ANZDATA, Australia and New Zealand Dialysis and Transplant Registry; BMI, body mass index; DBD, donation after brain death; DCD, donation after circulatory death; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; IGF, immediate graft function; LD, living donor; SGF, slow graft function.



GRAPHICAL ABSTRACT

interventions such as kidney biopsy, thus exposing patients to the complications associated with these interventions [6, 7].

Early graft function can be broadly categorised into immediate graft function (IGF), slow graft function (SGF) or delayed graft function (DGF) [8, 9]. DGF is widely defined as the requirement for dialysis within 1 week of transplantation [10–12]. SGF is characterised by slower-to-improve graft function, when compared to IGF, without the need for dialysis. In essence, SGF can be thought to exist on a spectrum between IGF and DGF [13]. In this, SGF is similar to the concept of functional DGF (fDGF) described in the literature [14, 15]. Both SGF and fDGF have had varying definitions in the literature.

DGF is linked to poorer graft survival and increased episodes of early rejection [10, 16, 17]. SGF has also been linked to poorer graft outcomes in some studies [7, 18–20] but not in others [21, 22] resulting in uncertainty regarding its clinical significance. This may be linked to the aforementioned variability in definition [21, 23, 24]. Outcomes after SGF may vary by donor type, with some studies showing it portends a poorer prognosis in LD transplants [25–27]. However, previous studies have been underpowered to assess the effect of donor type on the association between SGF and long term graft outcomes. Additionally, there is uncertainty on the magnitude of effect that DGF and SGF have on long-term patient survival [7, 10].

We hypothesized that, compared to IGF, both SGF and DGF are associated with reduced long term graft survival and death censored graft survival in recipients of a kidney transplant.

PATIENTS AND METHODS

Study Population

We included all adult (aged ≥18 years) recipients of kidney-alone transplants performed in Australia and New Zealand between 2001 and 2021 from the Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry. Transplants that occurred outside Australia and New Zealand, pathological donors (defined as kidneys transplanted after nephrectomy for tumour), patients that experienced primary graft failure (i.e., graft loss within 7 days) and multi-organ transplants were excluded.

Early Graft Function Definitions

The definitions of DGF and SGF used were as recorded by the ANZDATA registry. Prior to 2017, SGF was defined as the absence of a spontaneous fall in serum creatinine of >10% within 72 h of transplant, without the need for dialysis; DGF was the requirement for dialysis within 72 h post-transplantation. IGF in this era was defined as a spontaneous fall in serum creatinine by over 10% within 72 h of transplantation. From 2017 onwards, these definitions were updated to align with internationally accepted definitions: DGF was defined as requirement for dialysis within 7 days of transplant, and SGF by a reduction in serum creatinine of ≤30% between day one and day two post transplantation. IGF in this era was defined as a spontaneous fall in serum creatinine by over 30% by day 2 post transplantation. We included an adjustment for transplantation

era in our statistical analysis models to account for the change in definitions, and also assessed for interactions between era and early graft function in the different models.

Clinical Outcomes

The primary outcomes assessed were; a) graft survival, defined as time from transplantation until return to dialysis, repeat kidney transplantation or death with a functioning graft and b) death-censored graft survival (DCGS), defined as time from transplantation until return to dialysis or repeat kidney transplantation, censored for death with a functioning graft.

The secondary outcomes assessed were; a) patient survival, defined as the time from date of transplantation to patient death and not censored at graft failure, b) 12 months estimated glomerular filtration rate (eGFR), calculated using the original CKD Epidemiology Collaboration (CKD Epi) equation [28] and c) acute rejection at 12 months, defined as any episode of acute rejection (either biopsy proven or suspected) at 12 months respectively, as reported to the registry [16].

Data Variables

Baseline recipient characteristics obtained from the ANZDATA registry included age, gender, ethnicity, primary kidney disease, body mass index (BMI), time on dialysis, repeat transplantation, calculated panel reactive antibodies and comorbid conditions (smoking status, diabetes mellitus, ischaemic heart disease, peripheral vascular disease, cerebrovascular disease and chronic lung disease).

Baseline donor characteristics obtained from the ANZDATA and the Australia and New Zealand Organ Donor (ANZOD) and Australian and New Zealand Living Kidney Donor registries included age, sex, BMI and comorbid conditions (smoking status, hypertension and diabetes mellitus). Donors were classified as either living donor (LD), donation after brain death (DBD) or donation after circulatory death (DCD).

Transplant related characteristics obtained included total ischaemic time, ABO compatibility status and number of human leukocyte antigen (HLA) mismatches at the A, B and DR loci.

All comorbidities were from the ANZATA survey the year prior to transplantation.

Statistical Analysis

Continuous variables were reported as mean and standard deviation, or median and interquartile range, as appropriate. Categorical variables were reported as counts and percentages. We created Kaplan Meier Curves for all survival outcomes. We hypothesised that the association between early graft function would differ by different donor types. To account for this difference, *a priori* strata were assumed between donor type and early graft function (i.e., the baseline hazard will be constant only within the donor types). We used stratified Cox proportional hazard models for all survival outcomes. All survival times were censored at the end of follow-up on 31 December 2021. All variables were assessed for linearity through categorisation of continuous variables and Martingale Residuals. For graft survival and death censored graft survival, age was non-linear and transformed using fractional polynomials. We hypothesised that due to a change in how ANZDATA collected SGF over time there may be a difference in the association between early graft function and the different outcomes by era. To investigate this, we assessed for an interactions between early graft function subtype and era (years 2001–2016 vs. years 2017–2021) using forward elimination with a threshold p value of 0.1 (**Supplementary Figure S3**). The donor variables assessed for inclusion in the models were age, sex, BMI, hypertension, smoking, and diabetes mellitus. The recipient variables assessed for inclusion in the models were age at transplant, recipient sex, graft number, years on dialysis, ischaemic time, peak PRA, primary kidney disease, BMI, smoking, peripheral vascular disease, diabetes mellitus, ischaemic heart disease, cerebrovascular disease, chronic lung disease and number of HLA mismatches. No interactions were found. Non-significant variables were excluded from the model using backward elimination with a threshold p value of 0.157 [29]. The proportional hazard assumption was assessed using Schoenfeld residuals. During the creation of the models, implausible values for included variables, including donor body mass index (BMI) > 80 or <10 (8), ischaemic time >40 h (4), recipient BMI >50 kg/m^2 (11) and height under 100 cm (48) were considered missing. There were 71 (<0.5%) such implausible values that were considered missing. Given the low rate of missingness we did not perform additional analyses accounting for missingness using multiple imputation.

A fixed effects linear regression model, with fixed effects for donor type was created for the outcome of 12-month eGFR. Collinearity was assessed using the variance inflation factor. The linearity assumption for continuous variables was assessed using scatter plots of residual values.

A fixed effect logistic regression model, with fixed effects for donor type was created for the outcome of 12-month rejection. Collinearity was assessed with the variance inflation factor. The linearity assumption was assessed using categorisation of all continuous variables for covariates. For both logistic and linear regression models, interactions were assessed for using the forward elimination method. Backward elimination was used to remove non-significant variables with a threshold p value of 0.157 [29]. All models are available in the **Supplementary Material**. The analyses were conducted in Stata/IC 17.0 (Stata Corp, College Station TX).

RESULTS

Study Population

Between January 2001 and December 2021, a total of 20,520 transplants were performed in Australia and New Zealand and reported to the ANZDATA registry (**Figure 1**). 2941 transplant recipients were excluded: 260 recipients with missing early graft function data, 929 recipients aged <18 years, 420 transplants which occurred outside of Australia and New Zealand, 942 multi-organ transplants, 110 pathological donors, and 280 primary graft

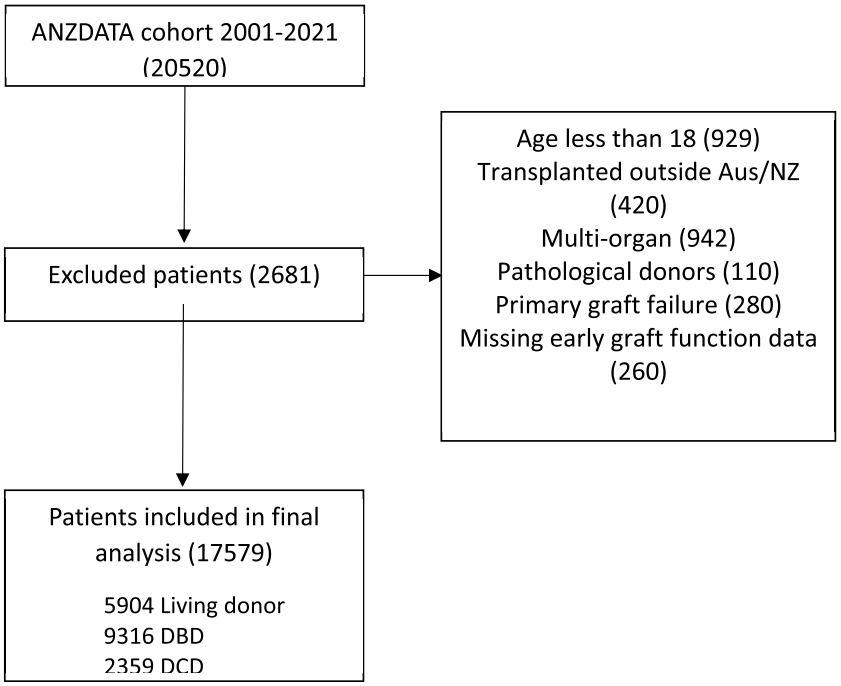


FIGURE 1 | Cohort diagram (DBD, donation after brain death; DCD, donation after circulatory death).

failures. A total of 17,579 transplants were included in this study, comprised of 2,359 (13.4%) donation after circulatory death (DCD) transplants, 9,316 (53.0%) donation after brain death (DBD) transplants and 5,904 (33.6%) living donor transplants. The baseline characteristics of the study population are described in **Table 1**. The median follow-up time was 6.8 (IQR 3.3–11.6) years. The proportion of recipients with DGF was 3,604/17,579 (20.5%), the proportion with SGF was 2277/17,579 (12.9%), and the proportion with IGF was 11,698/17,579 (66.6%). During the follow up period, there were 2,434 (13.9%) deaths and 2,575 (14.7%) experienced graft loss. 243 (1.38%) recipients were lost to follow up.

Primary Endpoints

Figure 2 shows the Kaplan-Meier curves, comparing graft survival by donor type, stratified by early graft function subtype. DGF and SGF in living donor recipients and recipients of DBD transplants, but not DCD transplants, were associated with a reduction in graft survival when compared to recipients with immediate graft function. **Table 2** shows the multivariable analyses of the primary endpoints, along with patient survival, stratified by donor type.

SGF [aHR 1.48 (95% CI 1.14, 1.91)] and DGF [aHR 1.97 (95% CI 1.42, 2.73)] were associated with increased graft loss in living donors (**Supplementary Figure S4**). Both SGF [aHR 1.13 (95% CI 1.01, 1.27)] and DGF [aHR 1.37 (95% CI 1.24, 1.51)] were associated with increased graft loss when compared to IGF in DBD transplant recipients. In DCD transplant recipients, DGF [aHR 1.52 (95% CI 1.13, 2.04)] was associated with increased graft loss. However, in DCD transplant recipients, there was no statistically significant difference between SGF [aHR 1.01 (95% CI 0.70, 1.44)] and IGF.

Similarly, when assessing death censored graft loss (**Supplementary Figure S5**), SGF [aHR 1.53 (95% CI 1.11, 2.11)] and DGF [aHR 1.93 (95% CI 1.27, 2.95)] were associated with increased death censored graft loss in living donors. Both SGF [aHR 1.33 (95% CI 1.15, 1.55)] and DGF [aHR 1.49 (95% CI 1.31, 1.70)] were associated with increased death censored graft loss when compared to IGF in DBD transplant recipients. In DCD transplants, DGF [aHR 1.80 (95% CI 1.19, 2.73)] was associated with increased death censored graft loss. SGF [aHR 1.23 (95% CI 0.74, 2.03)] was not significantly associated with death censored graft loss in DCD transplants.

Secondary Endpoints

Patient Survival

Both DGF [aHR 2.01 (95% CI 1.37, 2.94)] and SGF [aHR 1.55 (95% CI 1.13, 2.13)] were associated with decreased patient survival in living donor transplant recipients (**Supplementary Figure S6**). DGF was associated with decreased patient survival in both DBD [aHR 1.29 (95% CI 1.17, 1.44)] and DCD [aHR 1.47 (95% CI 1.06, 2.04)] transplant recipients. SGF was not associated with decreased survival in DBD [aHR 1.02 (95% CI 0.90, 1.16)] or DCD [aHR 1.03 (95% CI 0.69, 1.53)] transplant recipients.

Graft Function

For all donor types, SGF and DGF were associated with lower eGFR at 12-month post-transplant (**Figure 3**). In living donors,

TABLE 1 | Patient characteristics (IGF, Immediate Graft Function; SGF, Slow Graft Function, Delayed Graft Function; DCD, Donation after circulatory death; DBD, Donation after brain death; LD, living donor).

Characteristic	IGF	SGF	DGF	p-value
N	11,698	2,277	3,604	
Age at transplant, median (IQR)	49 (38, 59)	53 (43, 61)	54 (44, 62)	<0.001
Recipient Male	7,118 (60.8%)	1,518 (66.7%)	2,451 (68.0%)	<0.001
Recipient Ethnicity				<0.001
Unknown	584 (5.0%)	111 (4.9%)	185 (5.1%)	
White/European	8,513 (72.8%)	1,600 (70.3%)	2,381 (66.1%)	
Aboriginal/Torres Strait Islander	280 (2.4%)	92 (4.0%)	218 (6.0%)	
Maori	334 (2.9%)	67 (2.9%)	108 (3.0%)	
Pacific	365 (3.1%)	86 (3.8%)	150 (4.2%)	
Asian	1,342 (11.5%)	257 (11.3%)	441 (12.2%)	
Other	280 (2.4%)	64 (2.8%)	121 (3.4%)	
Primary Renal Disease				<0.001
GN	5,190 (44.8%)	917 (40.6%)	1,440 (40.1%)	
Polycystic	1,665 (14.4%)	321 (14.2%)	429 (12.0%)	
Reflux	1,036 (8.9%)	165 (7.3%)	251 (7.0%)	
Hypertension	674 (5.8%)	147 (6.5%)	248 (6.9%)	
Diabetes	1,223 (10.6%)	339 (15.0%)	665 (18.5%)	
Other	1790 (15.5%)	369 (16.3%)	556 (15.5%)	
Recipient Smoker	4,566 (39.8%)	1,028 (45.8%)	1,662 (46.8%)	<0.001
Recipient Diabetes Meillitus	1828 (15.7%)	497 (21.9%)	994 (27.6%)	<0.001
Recipient Ischaemic heart disease	1,680 (14.4%)	488 (21.5%)	877 (24.4%)	<0.001
Recipient Peripheral vascular disease	840 (7.2%)	231 (10.2%)	486 (13.5%)	<0.001
Recipient Cerebrovascular disease	566 (4.8%)	152 (6.7%)	248 (6.9%)	<0.001
Recipient Chronic lung disease	772 (6.6%)	217 (9.6%)	384 (10.7%)	<0.001
Recipient Body Mass Index (BMI) kg/m^2, median (IQR)	25.9 (22.8, 29.4)	27.1 (24.0, 30.5)	27.8 (24.4, 31.5)	<0.001
Time on dialysis (years), median (IQR)	1.6 (0.5, 3.5)	2.6 (1.2, 4.8)	3.4 (2.0, 5.4)	<0.001
Total ischaemia (to nearest hour), median (IQR)	6 (3, 12)	11 (7, 15)	12 (8, 15)	<0.001
ABO incompatible transplant	552 (4.7%)	31 (1.4%)	33 (0.9%)	<0.001
Graft number >1	1,337 (11.4%)	261 (11.5%)	533 (14.8%)	<0.001
HLA-A mismatch				<0.001
0	2,615 (22.7%)	437 (19.3%)	633 (17.6%)	
1	5,680 (49.2%)	1,098 (48.4%)	1,627 (45.2%)	
2	3,248 (28.1%)	734 (32.3%)	1,339 (37.2%)	
HLA-B mismatch				<0.001
0	1819 (15.8%)	320 (14.1%)	469 (13.0%)	
1	5,176 (44.8%)	890 (39.2%)	1,263 (35.1%)	
2	4,547 (39.4%)	1,059 (46.7%)	1867 (51.9%)	
HLA-DR mismatch				<0.001
0	3,452 (30.0%)	707 (31.2%)	1,008 (28.0%)	
1	4,972 (43.1%)	843 (37.3%)	1,301 (36.2%)	
2	3,101 (26.9%)	713 (31.5%)	1,286 (35.8%)	
Any induction therapy	9,541 (%)	1965 (%)	3,139 (%)	<0.001
Donor type				<0.001
DCD	543 (23.0%)	518 (22.0%)	1,298 (55.0%)	
DBD	5,672 (60.9%)	1,493 (16.0%)	2,151 (23.1%)	
Living	5,483 (92.9%)	266 (4.5%)	155 (2.6%)	
Paired kidney Exchange	400 (89.3%)	27 (6.0%)	21 (4.7%)	
Donor age, median (IQR)	48 (36, 57)	52 (40, 61)	51 (40, 60)	<0.001
Donor Male	5,481 (49.2%)	1,206 (53.5%)	2,118 (59.1%)	<0.001
Donor Body Mass Index (BMI) kg/m2, median (IQR)	26.1 (23.6, 29.3)	26.7 (24.0, 30.0)	27.4 (24.4, 31.1)	<0.001
Donor Smoker	5,597 (51.0%)	1,353 (60.3%)	2,245 (62.7%)	<0.001
Donor Hypertension	1,650 (15.1%)	581 (26.1%)	1,022 (28.9%)	<0.001
Donor Diabetes Meillitus	283 (2.6%)	118 (5.3%)	238 (6.7%)	<0.001

SGF was associated a reduction in eGFR at 12 months of 5.2 mL/min (95% CI 2.6–7.8) and DGF was associated with a reduction in eGFR at 12 months of 10.1 mL/min (95% CI 6.3–13.8). In DBD recipients, SGF was associated a reduction in eGFR at 12 months of 4.6 mL/min (95% CI 3.4–5.8) and DGF was associated with a reduction in eGFR at 12 months of 6.1 mL/min (95% CI 5.1–7.2). In DCD recipients, SGF was associated a reduction in eGFR at 12 months of 3.1 mL/min (95% CI 0.5–5.8) and DGF was associated with a reduction in eGFR at 12 months of 6.3 mL/min (95% CI 4.0–8.5).

Rejection

Figure 4 shows the association between early graft function and episodes of rejection at 12 months. In DBD recipients, both SGF

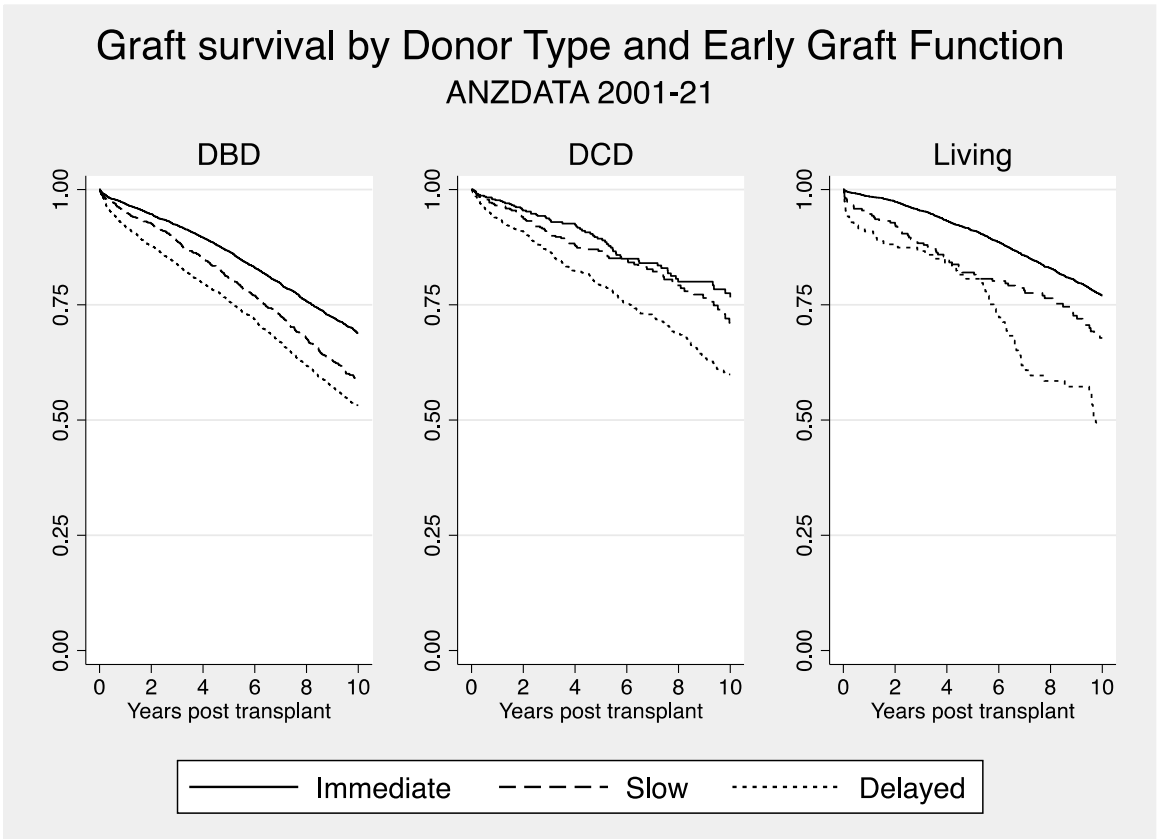


FIGURE 2 | Kaplan Meier Graft Survival curves by donor type, stratified by early graft function subtypes (DCD, Donation after circulatory death; DBD, Donation after brain death; Living, living donor.

TABLE 2 | Adjusted associations between SGF and DGF and Graft Survival, Death Censored Graft Survival (DCGS) and Patient Survival (SGF, slow graft function; DGF, delayed graft function; DBD, donation after brain death; DCD, donation after circulatory death; aHR, adjusted hazard radio.

	Graft Survival			DCGS			Patient surivival		
	aHR	95% CI	p value	aHR	95% CI	p value	aHR	95% CI	p value
LD									
SGF	1.47	1.14, 1.91	<0.001	1.53	1.11, 2.11	0.011	1.55	1.13, 2.13	0.007
DGF	1.97	1.42, 2.73	<0.001	1.93	1.27, 2.94	0.003	2.01	1.37, 2.94	<0.001
DBD									
SGF	1.13	1.01, 1.27	0.008	1.33	1.15, 1.55	<0.001	1.02	0.90, 1.16	0.694
DGF	1.37	1.24, 1.51	<0.001	1.49	1.31, 1.70	<0.001	1.29	1.16, 1.44	<0.001
DCD									
SGF	1.01	0.70, 1.44	0.596	1.23	0.74, 2.03	0.44	1.03	0.69, 1.53	0.869
DGF	1.52	1.14, 2.04	<0.001	1.80	1.19, 2.73	0.006	1.47	1.06, 2.04	0.017

[OR 1.28 (95% CI 1.08–1.52) and DGF [OR 1.74 (95% CI 1.51–2.02) were associated with an increased odds of rejection at 12 months. For DCD recipients, DGF was associated with increased odds of rejection at 12 months [OR 1.50 (95% CI 1.20–1.88)]. However, SGF was not associated with a statistically significant increase in the odds of rejection [OR 1.32 (95% CI 0.98–1.80)]. In recipients of living donors, DGF was associated with an increased odds of rejection [OR 2.15 (95% CI 1.39– 3.34). However, SGF was not significantly associated with rejection at 12 months [OR 1.13 (95% CI 0.79–1.61)].

DISCUSSION

In this study, involving 17,579 kidney transplant recipients, we showed that both DGF and SGF are associated with poorer graft

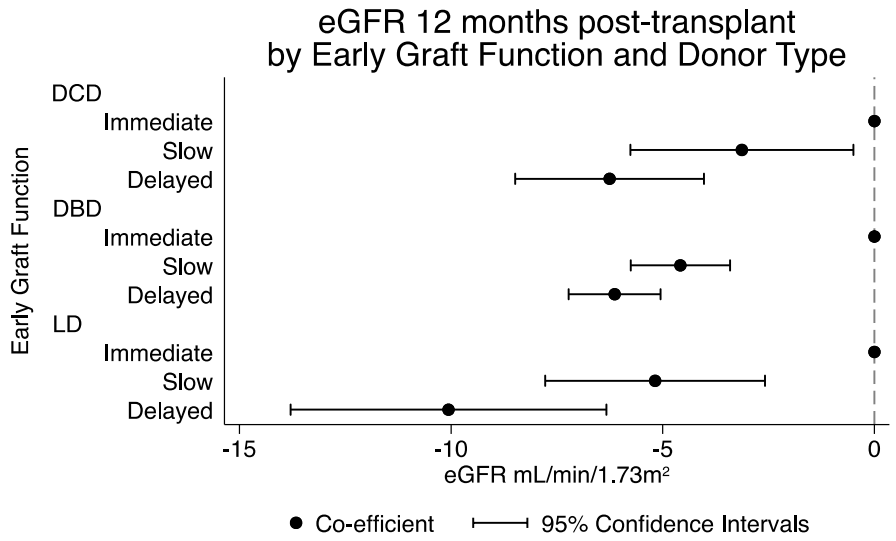


FIGURE 3 | Association between early graft function stratified by donor type donor type on eGFR at 12-month post-transplant adjusted model.

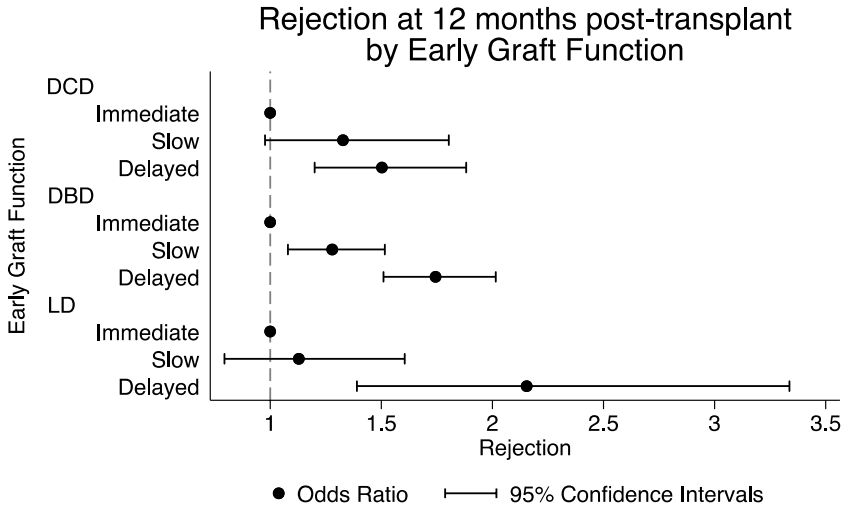


FIGURE 4 | Episodes of rejection at 12 months, stratified by donor type and early graft function adjusted model.

outcomes after kidney transplantation. SGF is associated with worse graft survival and death censored graft survival in living and DBD, but not DCD, recipients. This study also demonstrates that SGF is associated with worse patient survival in live donor recipients when compared to IGF. Additionally, we demonstrated that both SGF and DGF are associated with increased risk of early rejection and worse eGFR at 12 months post transplantation.

Importantly, this study demonstrates that the associations between early graft function and long-term outcomes differ by different donor types, and shows evidence for the consequences of SGF in DCD, DBD and living donor kidney transplants. In the DBD cohort, SGF was associated with reduced graft survival and increased rejection, but was not associated with worse patient survival. This is consistent with previous findings in deceased donor transplantation [7, 20, 30]. We did not find an association between SGF and adverse graft survival in DCD transplants. The point estimate of hazard ratio for DCGS (1.22) does not exclude an adverse association that this study was underpowered to find. Additionally, the reduction in 12-month eGFR and increased episodes of rejection suggest some clinically meaningful associations of SGF in DCD transplants.

The association of SGF with poor long term graft outcomes in living donors is consistent with findings in smaller, singe-center studies [25, 26, 31]. Our study confirms and expands on this prior literature using data from a large multi-centre registry analysis. Kinoshita et. al. assessed 10-year graft survival in 272 living donor

transplant recipients with and without SGF, defined as a CRR on day 2 of less than 30% [25]. They reported decreased graft survival at 5 and 10 years, however, did not find a difference in rejection rates or eGFR at 12 months or a statistically significant change in eGFR at 12 months. We also did not find an increased odds of rejection in living donor recipients with SGF when compared to living donor recipients with IGF, but found a decrease in eGFR at 12 months. Lee et al. reported 10-year graft outcomes in 310 living donor transplants and found that the decreased graft survival seen in living donor transplant recipients with SGF appeared to be associated with an increased incidence of acute rejection [32]. In contrast, our findings suggest that the reduction in graft survival is not mediated through rejection, as we did not find an increase in odds of rejection in living donors with SGF but did find significant reduction in graft survival.

Our finding of a reduction in patient survival in living donors with SGF has not been reported previously. Live donor surgery is undertaken in a planned, elective fashion, and typically involves a short cold ischaemic time. As such, perioperative and recipient factors may be more significant factors in the development of SGF and DGF in these transplants in comparison to the deceased donor setting, where donor characteristics and organ storage play a highly significant role. This may allude to perioperative morbidity impacting both early graft function and patient survival. It is also possible that there are other yet to be identified factors that play a role. Further studies in different populations and settings should be performed to confirm and validate our findings.

Consistent with the findings of prior studies using ANZDATA, and conducted in other countries and settings, we found that DGF is associated with poor graft outcomes and reduced patient survival across donor types [10, 17, 33, 34]. While the adverse consequences of DGF have been well established, the consequences of SGF remain less clearly elucidated. The binary nature of the accepted definition of DGF, which is defined by the requirement for dialysis within the first week in most studies, makes DGF an easily identifiable entity in clinical settings [35]. SGF, which is characterised clinically by poor kidney function measured biochemically without the need for dialysis, has been more variably defined in the literature [8]. The heterogeneity of reported outcomes associated with SGF may reflect the heterogeneity in definitions of SGF. A study by Hall et. al. correlated the various definitions of SGF in the literature and found that a creatinine over 2.5 mg/dL (221 μmol/L) at day 7 post-transplant or a creatinine reduction ratio (CRR) between days 1 and 2 of <25% had the best correlation to the eGFR at 12 months [8]. While eGFR at 12 months is a surrogate endpoint and definitions of SGF have not been validated against harder clinical endpoints such as graft survival, this study provides support in the use of the CRR between days 1 and 2 by 30% as a definition for SGF. It is the nature of these definitions of early graft function to take the continuum of graft function between IGF and DGF and create categorically definable entities. While these distinctions are artificial, as long as they represent clinically distinct phenotypes, these definitions are important.

Previous studies suggesting a link between SGF and long-term graft outcomes have largely consisted of single centre, or small multi-centre observational studies that each included fewer than 1,500 patients [9, 18–20, 22, 30, 31, 36–38]. While several of these studies have shown an association between SGF and graft survival at 5–10 years or eGFR at 12 months, most have been underpowered to evaluate these associations in subgroups of donor types. The results of our study support the findings from existing larger cohort studies. Wang et al assessed the association between SGF and long-term graft survival and death censored survival, as well as all-cause mortality in 1,222 recipients of both living and deceased donor kidney transplants, using two different definitions of SGF. This study suggested that both definitions of SGF were associated with worse graft survival and DCGS, but not worse mortality.

Our findings demonstrate that SGF has important implications for clinical practice. Simply dichotomising early graft function into DGF or IGF is an oversimplification, which results in inattention to the clinically significant adverse effects of SGF. Recognising SGF as a distinct clinical entity with associated poor outcomes is an important step towards improving long term graft outcomes. Recent interventions have been shown to reduced DGF, such as balanced crystalloids [39] and machine perfusion [40]. Similarly, there may be interventions that reduce SGF. The magnitude of impact of SGF appears to vary across donor type, with the data demonstrating that SGF appears particularly significant in living donor transplant recipients.

In addition to these clinical implications, this evidence for the importance of SGF has important implications for clinical research. SGF may be an important intermediate end point that has the potential to be used in clinical trials, in addition to DGF, as a surrogate for long-term graft outcomes. Future work is needed to assess the impact of interventions that reduce rates of SGF on long term graft outcomes.

Our study has several strengths. It includes data on DGF and SGF from the largest cohort of transplant recipients to date and provides robust evidence for the association between early graft function and long-term graft outcomes. This study reports key patient-centred outcomes including survival and graft loss, as well as frequently reported surrogate measures such as 12-month graft function [41, 42]. The results increase the certainty of evidence for the observation that SGF represents a clinically significant intermediate phenotype between immediate graft function and DGF. Our findings also highlights the implications that SGF has for different donor types, with increasing clinical relevance in the DBD and living donor transplant recipient cohorts, compared to DCD transplant recipients. This is also the first such study that has been adequately powered to detect clinical differences in outcomes between donor types.

Several limitations must be noted. The data are retrospective and observational, and thus there is the potential for residual confounding. As a registry study, it is reliant on accurate data capture, and there is evidence that registry recorded data on co-morbidities vary from those recorded in hospital administrative datasets [43]. However, despite this, the predictive power of registry-recorded co-

morbidity data for mortality and other outcomes has been demonstrated to be robust [43]. The definition of SGF recorded in the ANZDATA registry was changed in 2017, and this may have affected our analysis. While our modelling controlled for the effects of transplantation era, this change in definition might have resulted in some misclassification.

In conclusion, both SGF and DGF represent meaningful clinical entities with significant implications for patient outcomes. SGF is associated with poorer long-term graft outcomes in DBD and living donor kidney transplant recipients, as well as reduced patient survival in living donor recipients. Further research is needed to assess if interventions that improve early graft function and avoid SGF could lead to better graft survival, improved patient survival in recipients of living donor transplants, and better healthcare resource utilisation.

DATA AVAILABILITY STATEMENT

Data may be available on request, subject to ANZDATA policies. Request to be made to corresponding author.

ETHICS STATEMENT

The studies involving humans were approved by Central Adelaide Local Health Network Human Research Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

KV: Conception and design of the study, as well as drafting the manuscript. GI: Performed the analysis and was involved interpretation of the data, as well as drafting the manuscript. MC: Involved in design of the study, as well as interpretation of the data and revision the manuscript. PC: Conception and design of the study, involved with the analysis and interpretation of the data. Revised the manuscript ahead of submission. All authors contributed to the article and approved the submitted version.

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AUTHOR DISCLAIMER

The interpretation and reporting of these data are the responsibility of the authors and in no way should be seen as an official policy or interpretation of the Registry.

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.2025.14197/full#supplementary-material

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Proenkephalin A 119-159 in Kidney Transplantation: A Novel Biomarker for Superior Tracking of Graft Function Trajectories

*Louise Benning[1], Marvin Reineke[1], Camila Eleuterio Rodrigues[2,3], Florian Kälble[1], Claudius Speer[1], Claudia Sommerer[1], Christoph F. Mahler[1], Felix C. F. Schmitt[4], Markus Mieth[5], Martin Zeier[1], Christoph Michalski[5], Arianeb Mehrabi[5], Oliver Hartmann[6], Markus Zorn[7], Sophie C. Anker[7], David Czock[8], Markus A. Weigand[4], Zoltan Endre[2], Christian Morath[1] and Christian Nusshag[1]**

[1]Department of Nephrology, Heidelberg University Hospital, Medical Faculty, Heidelberg University, Heidelberg, Germany, [2]Department of Nephrology, Prince of Wales Hospital, Sydney, NSW, Australia, [3]Department of Nephrology, Hospital das Clínicas - University of São Paulo School of Medicine, São Paulo, Brazil, [4]Department of Anaesthesiology, Heidelberg University Hospital, Medical Faculty, Heidelberg University, Heidelberg, Germany, [5]Department of General, Visceral, and Transplantation Surgery, Heidelberg University Hospital, Medical Faculty, Heidelberg University, Heidelberg, Germany, [6]SphingoTec GmbH, Berlin, Germany, [7]Central Laboratory of University Hospital Heidelberg, Department of Endocrinology and Metabolism, Heidelberg University Hospital, Medical Faculty, Heidelberg University, Heidelberg, Germany, [8]Department of Clinical Pharmacology and Pharmacoepidemiology, Heidelberg University Hospital, Medical Faculty, Heidelberg University, Heidelberg, Germany

Accurate assessment of graft function trajectories after kidney transplantation is essential for optimizing patient management. Slow graft function (SGF) and delayed graft function (DGF) are associated with impaired recovery, yet current diagnostic tools lack granularity for timely risk stratification. Proenkephalin A 119-159 (penKid) may improve graft function assessment, enhancing risk stratification for SGF, DGF, and associated outcomes. This prospective study evaluated 159 kidney transplant recipients at Heidelberg University Hospital to compare plasma penKid levels with current risk-indicators for poor (functional) graft trajectories. Validation was conducted using an independent transplant cohort from Sydney. Clinical relevance of biomarker-indicated changes in graft function was assessed using multivariable regression models and AUROC analyses. From day one post-transplant, penKid outperformed serum creatinine (SCr) in identifying functional trajectories associated with DGF (AUROC penKid: 0.87 vs. SCr: 0.56) and differentiated SGF from DGF (AUROC penKid: 0.79 vs. SCr: 0.33) up to eight days earlier. PenKid further demonstrated superior granularity in assessing DGF severity and 30-day outcomes. After adjustment for common risk factors, penKid remained the

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***Correspondence**
Christian Nusshag,
christian.nusshag@med.uni-heidelberg.de

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Abbreviations: AKI, acute kidney injury; AUROC, area under the receiver operating characteristic curve; BMI, body mass index; CI, confidence interval; CIT, cold ischemia time; CKD, chronic kidney disease; DCD, donation after circulatory death; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; mGFR, measured glomerular filtration rate; IGF, immediate graft function; IQR, interquartile range; IRI, ischemia reperfusion injury; KRT, kidney replacement therapy; NGAL, neutrophil gelatinase-associated lipocalin; NPV, negative predictive value; penKid, Proenkephalin A 119-159; PPV, positive predictive value; SCr, Serum Creatinine; SGF, slow graft function.

strongest risk stratifier for all tested outcomes. PenKid is a superior biomarker for earlier assessment of graft function trajectories, offering potential to enhance personalized care and clinical trial designs in kidney transplantation.

Keywords: delayed graft function, proenkephalin A, risk stratification, graft function trajectory, study enrichment, slow graft function, kidney graft recovery

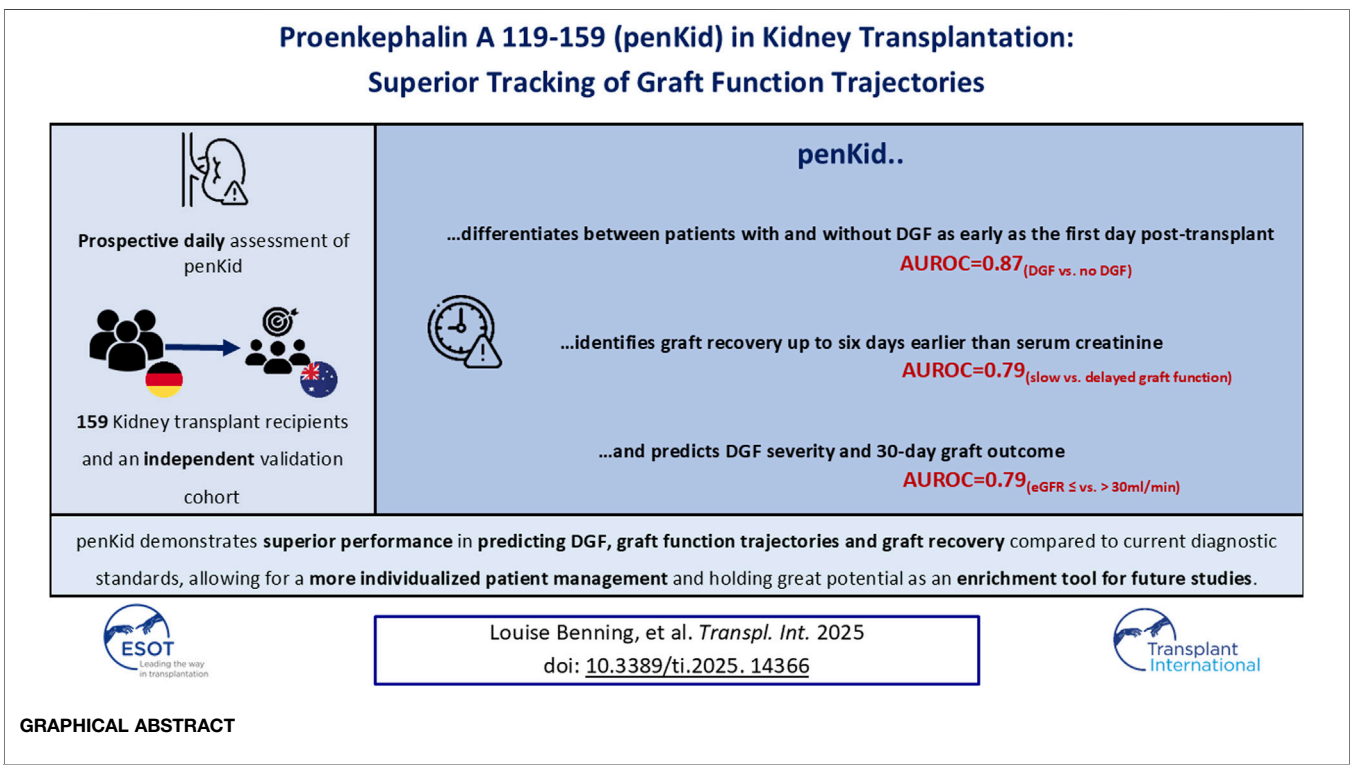
INTRODUCTION

Early and accurate discrimination between diverse graft function trajectories following kidney transplantation is essential for individualized patient management. Utilizing appropriate diagnostic tools can enable timely risk assessment for adverse outcomes, such as delayed graft function (DGF) and its severity, thereby supporting informed clinical decision-making. DGF is a common complication after kidney transplantation, with reported incidences ranging from 5% to 50% [1–4]. Early identification of functional trajectories at risk for DGF is therefore of critical importance. Especially, prolonged DGF has been shown to negatively impact one-year graft function and long-term graft survival [5–14].

DGF is typically defined as the requirement for kidney replacement therapy (KRT) within the first week post-transplantation [5, 9]. However, this definition is inherently limited due to its dependence on subjective clinical judgment and variability in institutional protocols regarding the initiation of KRT apart from emergency criteria. Moreover, it lacks granularity, as it encompasses a wide range of indications for KRT, from transient issues such as hyperkalemia to more severe conditions like critical hypervolemia, vascular complications, metabolic disturbances, and rejection episodes [5, 9, 15]. As of today, the severity of DGF and its complications can only be retrospectively defined.

The optimal clinical management, such as the start of KRT (in the absence of emergency criteria) or the timing of kidney biopsies is particularly hindered by the absence of timely and accurate tools for assessing critical graft function trajectories at risk using current diagnostic standards. Especially in patients without immediate graft function (IGF), evaluating graft function trajectories remains largely speculative and is typically based on clinical experience, incorporating donor criteria and postoperative trends in serum creatinine (SCr) or urine output. However, the slow and insensitive kinetics of SCr, the weak correlation between urine output and kidney function, and the influence of non-renal factors on SCr levels - such as KRT, muscle mass, and medication - further complicate the assessment [16–18].

These limitations likewise impede the development of new therapeutic strategies and the establishment of appropriate inclusion criteria for interventional trials. Consequently, there is a pressing need for more reliable biomarkers to enable early and accurate assessment of graft function trajectories, thereby



GRAPHICAL ABSTRACT

optimizing patient care and therapeutic approaches in high-risk populations.

Recently, proenkephalin A 119-159 (penKid) has emerged as a novel biomarker that may more adequately reflect kidney function, particularly in critically ill patients with acute kidney injury (AKI) and under non-steady state conditions [19, 20]. penKid is a byproduct derived from the breakdown of the same precursor molecule as endogenous opioids, called enkephalins [21]. With its small molecular mass (4.5 kDa), penKid appears to be freely filtered through the glomerulus with no evidence of protein binding [19], rendering it a biomarker for assessing kidney functional integrity.

Given the pathophysiological similarities between cold ischemia (CIT)-induced injury in transplanted kidneys and ischemia-reperfusion injury (IRI) in native kidneys [3, 22], we hypothesize that penKid could enable earlier and more robust differentiation of individual graft function trajectories and their associated outcomes. Such capabilities could significantly enhance risk stratification and clinical decision-making in the post-transplant setting, paving the way for improved patient outcomes and interventional trials aimed at mitigating DGF in the future.

MATERIALS AND METHODS

Study Design

Between November 2021 and July 2023, this prospective, single-center, real-world study at Heidelberg University Hospital quantified daily plasma penKid levels on weekdays in 159 consecutive kidney transplant recipients, from admission to discharge (Heidelberg study). The study was part of the PARTICIPATE study, evaluating the diagnostic utility of penKid in routine clinical practice across various settings. It was approved by the University of Heidelberg ethics committee and registered in the German Clinical Trials Register (DRKS00026776). Patient consent was waived as penKid assessment was integrated into routine diagnostics, imposing no additional burden. The reliability of penKid kinetics and diagnostic performance was validated in an independent Sydney cohort, with pre- and first post-transplant day data analyzed. This study was approved by the South Eastern Sydney Local Health District Human Research Ethics Committee (2021/ETH11450) and registered in the Australian New Zealand Clinical Trials Registry. Both studies adhered to the Principles of the Declaration of Istanbul on Organ Trafficking and Transplant Tourism and the Declaration of Helsinki.

Quantification of Proenkephalin A 119-159

PenKid was quantified in EDTA plasma using the sphingotest® penKid® immunoassay from SphingoTec GmbH (Hennigsdorf, Berlin), as described previously [23].

Definition of Transplant-Related Outcomes

In alignment with previous DGF biomarker studies [10, 24], recovery of graft function in patients without DGF was additionally classified in slow graft function (SGF) and immediate graft function (IGF). SGF and IGF were distinguished using a SCr reduction ratio (difference between the initial SCr collected within an hour of transplantation and the SCr on day 7 divided by the initial SCr) of <0.7 and ≥0.7, respectively [10]. DGF was primarily defined as the necessity for KRT within the initial 7 days post-transplant, aligning with the widely adopted definition for DGF. The KRT indication was made by the respective treating physician. Considering the significant duration-dependent negative impact of prolonged DGF [8, 9, 11–13], we categorized the severity of DGF for further analysis as follows: (1) No DGF (primary graft function); (2) KRT only within the first 24 h (mild DGF); (3) KRT up to Day 7 post-transplant (moderate DGF); and (4) KRT required beyond Day 7 post-transplant (severe DGF). Poor 30-day graft outcome was defined as eGFR ≤30 mL/min/1.73 m^2 using the CKD-EPI equation.

Statistics

Quantitative data are reported as median with interquartile range (IQR). Group comparisons for continuous variables used the Kruskal-Wallis test, while categorical data were analyzed with Pearson's Chi-squared Test. Biomarker data were log-transformed. Receiver-operating-characteristic (ROC) curves assessed sensitivity and specificity, with the area under the ROC (AUROC) used to compare predictive accuracy. To assess penKid's independence from other variables (e.g., cold ischemia time, recipient KRT vintage, transplant modality, donor age, and donor SCr), likelihood ratio chi-square tests were applied to nested multivariable logistic regression models for DGF, comparison of SGF versus DGF and 30-day graft outcomes. To determine which factors influence absolute penKid concentrations in patients prior to transplantation (pre-Tx) or changes in penKid levels after transplantation (d0/d1), two linear regression models were performed for pre-transplant penKid levels (including the variables KRT duration pre-transplant, age, diabetes, body mass index, congestive heart failure, sex, adipositas, hypertension and peripheral artery disease) and penKid changes from pre-transplant to first post-transplant day (including the variables donor modality, donor age, donor SCr, CIT, and KRT duration pre transplant). For continuous variables, odds ratios (OR) were standardized to describe the OR for a change of one IQR. Cases missing penKid or SCr data were excluded. All statistical tests were two-tailed, with significance set at $P < 0.05$. Analyses were conducted using R version 4.2.2 (libraries: rms, Hmisc, ROCR) and SPSS version 22.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Study Cohort

Between November 2021 and July 2023, a total of 159 kidney transplant recipients were consecutively enrolled in the Heidelberg study. Baseline characteristics and outcomes for patients with and without DGF are summarized in **Table 1**. Recipients with DGF were generally older, male, had higher body mass index (BMI), and a longer KRT vintage. They were also

TABLE 1 | Baseline characteristics.

Variable	All N = 159	No DGF N = 106	DGF N = 53	*P*-value
Recipient				
Age (years), median (IQR)	49 (39–60)	47 (36–58)	53 (46–61)	0.02
Sex (female), N (%)	72 (45.3)	55 (51.9)	17 (32.1)	0.03
BMI (kg/m^2), median (IQR)	25.0 (22.5–29.0)	24.0 (21.7–26.8)	26.9 (24.4–30.9)	<0.001
Dialysis Vintage (years), median (IQR)	6.5 (2.2–9.0)	4.8 (1.1–8.3)	8.0 (5.0–9.5)	<0.001
Donor				
Age (years), median [IQR]	55 (46–62)	55 (45–62)	56 (48–61)	0.90
Sex (female), N (%)	74 (46.5)	58 (54.7)	18 (34.0)	0.01
Hypertension, N (%)	44 (28.8)	22 (21.6)	22 (43.1)	0.01
Diabetes, N (%)	6 (3.9)	2 (2.0)	4 (7.8)	0.19
S-Creatinine (mg/dL), median (IQR)	0.8 (0.7–1.0)	0.8 (0.7–0.9)	0.9 (0.7–1.3)	0.09
Transplant-Related				
Transplant Modality				
Living, N (%)	50 (31.4)	48 (45.3)	2 (3.8)	<0.001
Deceased, N (%)	109 (68.6)	58 (54.7)	51 (96.2)	
Number of Transplants				
First, N (%)	140 (88)	97 (92)	43 (95)	0.07
Retransplants, N (%)	19 (12)	9 (8)	10 (19)	
Cold Ischemia Time (hours), median (IQR)	10.0 (2.5–14.1)	7.7 (2.0–13.3)	12.7 (9.9–16.2)	<0.001
Median HLA (A, B, DR) Mismatches (IQR)	3 (2–4)	3 (2–4)	3 (2–4)	0.49
Complement-dependent cytotoxicity (panel reactivity of >30%), N (%)	28 (17.6)	16 (15.1)	12 (22.6)	0.34
Induction Therapy				
Rituximab, N (%)	8 (5.0)	6 (5.7)	2 (3.8)	0.90
Anti-thymocyte globulin, N (%)	34 (21.4)	18 (17.0)	16 (30.2)	0.09
Interleukin-2 receptor antagonist, N (%) Other, N (%)	116 (73.0)	80 (75.5)	36 (67.9)	0.41
	8 (5.0)	8 (7.5)	0 (0)	0.10
Short-Term Outcomes				
Length of Stay (days), median (IQR)	16.0 (12.0–21.5)	13.5 (12.0–17.0)	23.0 (18.0–31.0)	<0.001
S-Creatinine at Discharge (mg/dL), median (IQR)	1.7 (1.3–2.4)	1.4 (1.2–1.8)	2.5 (1.8–3.7)	<0.001
penKid at Discharge (pmol/L), median (IQR)	117.1 (87.2–149.5)	109.1 (80.9–133.8)	146.7 (115.0–242.1)	<0.001

BMI, body mass index; DGF, delayed graft function; HLA, human leucocyte antigen; IQR, interquartile range; N, number; penKid, Proenkephalin A 119-159.

more likely to have received organs from male donors or donors with a history of arterial hypertension. In addition, recipients with DGF more frequently received transplants from deceased donors and experienced longer CIT compared to those without DGF. Length of hospital stay post-transplantation was longer for DGF patients. At discharge, both SCr and penKid levels were significantly higher in patients with DGF compared to patients without DGF. No significant differences were observed between groups regarding donor age, donor SCr, history of diabetes mellitus, number of previous transplants, type of induction therapy, or complement-dependent cytotoxicity analysis.

Assessment of Graft Function Trajectory

Considering the variation in the scenarios of graft function trajectory beyond DGF, a SCr reduction ratio, calculated between the SCr pre-transplant and the SCr on day 7 post-transplant, was additionally employed to differentiate between slow (SGF), immediate (IGF) graft function and DGF. As shown in **Figure 1**, pre-transplant penKid levels did not differ in relation to the graft function trajectory, whereas SCr showed significant differences; but this rather determined by the timing of last KRT rather than true differences in kidney function pre-transplant. Absolute penKid levels and particularly changes from baseline provided robust diagnostic performance from day 1 post-transplant, distinguishing IGF, SGF, and DGF. In contrast, SCr only began to differentiate between SGF and DGF on days 6–8 (**Figures 1A–D**).

Individual patient trajectories (**Figures 2A–D**) further highlighted penKid's superiority and time advantage over SCr. Based on longitudinal data, four outcome scenarios were identified: primary graft function (immediate decline in both biomarkers, **Figure 2A**), SGF (no KRT, immediate decline in penKid but not SCr, **Figure 2B**), moderate DGF severity with favorable outcomes (KRT, elevated SCr, earlier penKid decline, **Figure 2C**), and severe DGF severity with poor outcomes (KRT, persistent elevation of both markers, **Figure 2D**). Notably, unlike SCr, penKid levels were unaffected by KRT, as shown in **Figures 2C,D**.

Assessing the Severity of Delayed Graft Function

As the conventional definition of DGF does not allow to differentiate early from late recovery of graft function after the first KRT was initiated and thus does not reflect the different severity levels of DGF, penKid and SCr levels were also assessed in relation to varying degrees of DGF severity, namely mild DGF,

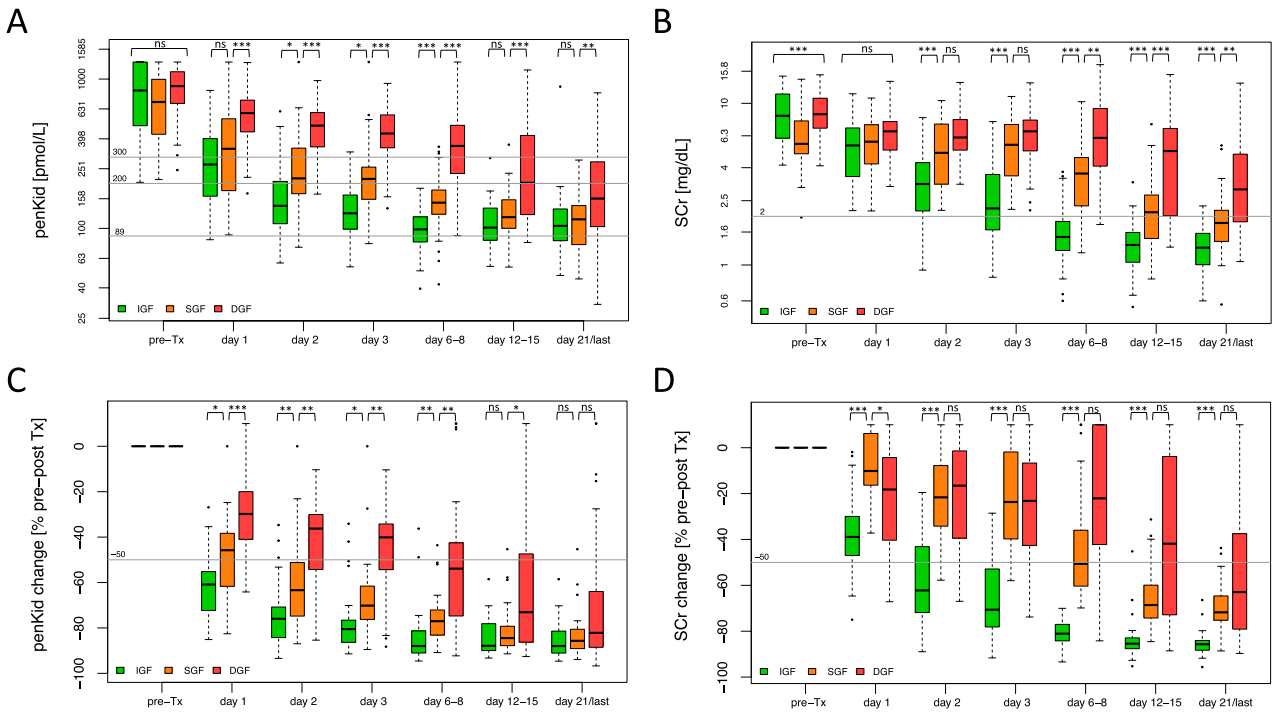


FIGURE 1 | Biomarker trajectory of proenkephalin A 119-159 and serum creatinine to discriminate critical scenarios of graft function recovery. **(A, B)** Absolute biomarker trajectories until patient discharge stratified by recovery of graft function. **(C, D)** Relative biomarker changes until patient discharge comparing pre-transplant biomarker levels to the respective post-transplant days stratified by recovery of graft function. IGF (green): N = 61, SGF (orange): N = 45, DGF (red): N = 53. Data are reported as box-and-whisker plots (interquartile range, minimum to maximum). The grey lines indicate penKid cut-offs at 300 pmol/L, 200 pmol/L and 89 pmol/L (the last being the upper reference limit for healthy individuals) **(A, B)**, or a 50% decrease cut-off compared to pre-transplant biomarker levels **(C, D)**. For SCr, the grey line signifies an SCr of 2 mg/dL for orientation. Both y-axes are log-transformed. d, days; DGF, delayed graft function; IGF, immediate graft function; penKid, Proenkephalin A 119-159; SCr, serum creatinine; SGF, slow graft function; Tx, transplant. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$. NS, $P > 0.05$.

moderate DGF, and severe DGF (**Figure 3**). In mild DGF, functional improvement was evident by days 6–8 post-transplant, with lower absolute penKid levels and more pronounced changes from baseline compared to moderate DGF (**Figures 3A,C**). Absolute SCr levels and changes, however, failed to differentiate severity during this timeframe but reflected improvement later, with declines apparent at days 12–15 for mild DGF (**Figures 3A–D**). Similarly, penKid levels decreased in moderate DGF starting on days 12–15, while SCr showed comparable trends only by day 21.

Graft Function Trajectory and Its Association to Critical Outcomes

To demonstrate the clinical relevance of early identification of distinct graft function trajectories for critical outcomes, we performed AUROC analyses and multivariate logistic regression models, incorporating established risk factors for poor graft outcomes across various outcome scenarios.

For discriminating SGF from DGF, the AUROC for penKid on day 1 was 0.79 (95% CI 0.68–0.90, $P < 0.001$), while SCr changes never provided significant discrimination ability at that time (change was higher in the SGF group compared to the DGF group) (**Figure 4A**). PenKid changes continued to outperform SCr changes through days 2 and 3 with an AUROC of 0.76 (95% CI 0.64–0.89, $P < 0.001$) and 0.81 (95% CI 0.70–0.92, $P < 0.001$), respectively. Corresponding AUROCs for SCr were 0.51 (95% CI 0.36–0.66, $P = 0.539$) and 0.52 (95% CI 0.38–0.66, $P = 0.644$) on days 2 and 3, respectively.

Similar patterns were observed for identifying patients with DGF or poor 30-day graft outcome (**Figures 4C–F**). As early as the first post-transplant day, penKid changes distinguished between patients with and without DGF with an AUROC of 0.87 (95% CI 0.81–0.94, $P < 0.001$), outperforming SCr (AUROC 0.56, 95% CI 0.45–0.68, $P = 0.332$). Comparable performance for penKid was observed on days 2 and 3, with AUROCs of 0.86 (95% CI 0.78–0.94, $P < 0.001$), compared to SCr's lower AUROCs of 0.73 (95% CI 0.63–0.83, $P < 0.001$) and 0.74 (95% CI 0.64–0.83, $P < 0.001$) (**Figure 4C**). Even in predictive performance analysis across subpopulations (deceased vs. living, male vs. female, etc.), penKid was a consistent risk stratifier for predicting DGF (**Supplementary Figure S1**).

Stratifying 30-day graft outcomes by eGFR ≤30 mL/min/1.73 m^2, penKid changes from pre-transplant to day 1 yielded an AUROC of 0.79 (95% CI 0.69–0.90, $P < 0.001$) for predicting 30-day eGFR ≤30 mL/min/1.73 m^2, compared to SCr changes

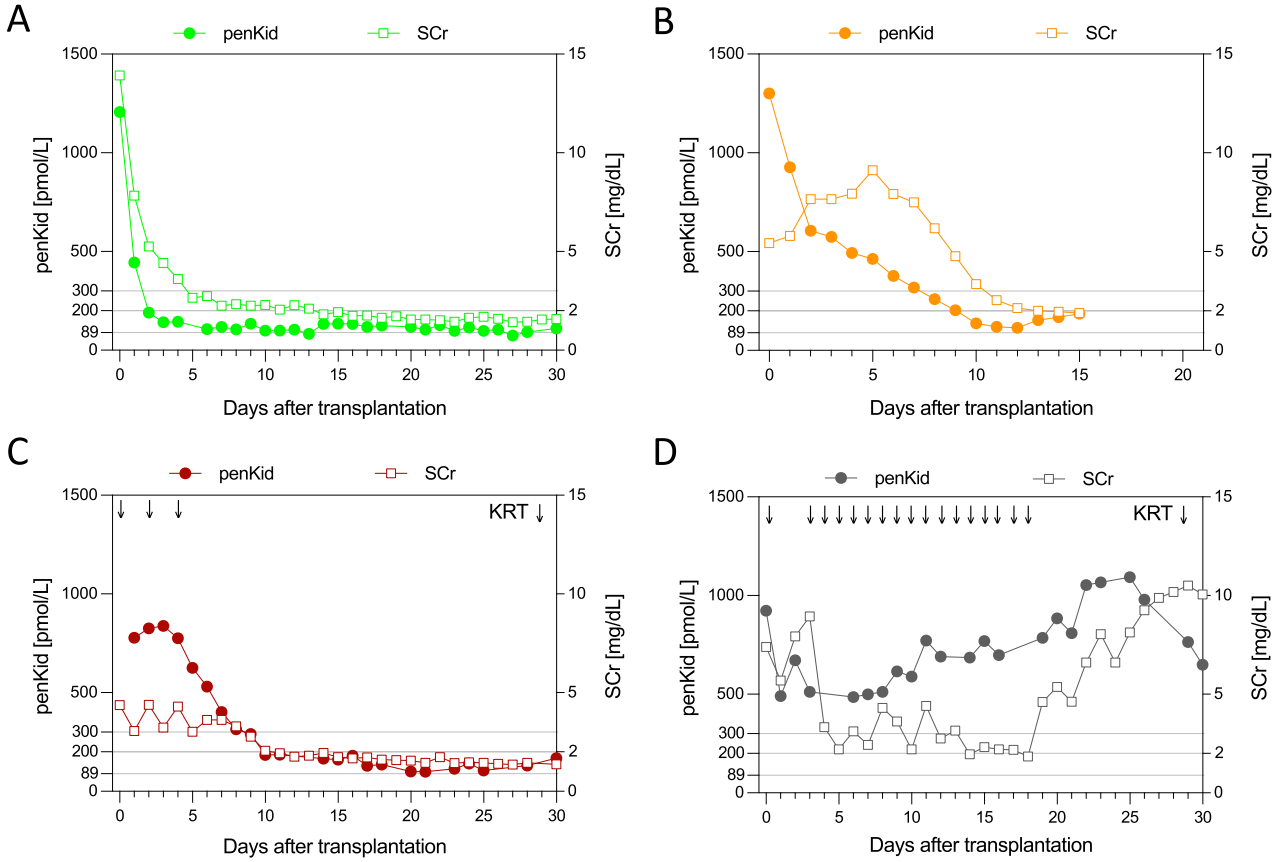


FIGURE 2 | Individual biomarker trajectories of proenkephalin A 119-159 and serum creatinine identify four outcome scenarios. Patient with primary/immediate graft function **(A)**, patient with slow graft function **(B)**, patient with DGF and favorable 30d-graft outcome **(C)**, and a patient with DGF and poor 30-d graft outcome **(D)**. The grey lines indicate penKid cut-offs at 300 pmol/L, 200 pmol/L, and 89 pmol/L (the last being the upper reference limit for healthy individuals). For SCr, the grey line signifies an SCr of 2 mg/dL for orientation. Both y-axes are log-transformed. KRT, kidney replacement therapy; penKid, Proenkephalin A 119-159; SCr, serum creatinine.

with an AUROC of 0.63 (95% CI 0.51–0.76, P = 0.080) (**Figure 4E**).

Multivariate logistic regression analysis revealed that graft function changes indicated by penKid changes were the strongest discriminator for SGF versus DGF (**Figure 4F**) and strongest predictor for the tested outcomes DGF and eGFR ≤30 mL/min/1.73 m^2 (**Figures 4D,F**). Likewise, after adjustment, changes in penKid effectively identified patients with higher risk profiles across different outcome scenarios. Specifically, the OR (per IQR of penKid) for SGF versus DGF was 5.2 (95% CI: 1.8–15.2), for DGF versus no DGF it was 17.3 (95% CI: 5.0–60.6), and for eGFR ≤30 mL/min/1.73 m^2 it was 4.6 (95% CI: 1.8–11.8). In contrast, when penKid was replaced by SCr in the multivariate model, the ability to stratify risk via a functional biomarker was significantly diminished. The OR (per IQR SCr) for SGF versus DGF, DGF versus no DGF and for eGFR ≤30 mL/min/1.73 m^2, dropped to 0.3 (95% CI: 0.1–0.8), 1.0 (95% CI: 0.5–2.1) and 1.6 (95% CI: 0.7–3.7), respectively.

Further, linear regression analysis was used to assess the association between penKid levels and recipient- and donor-related factors relevant to transplant outcomes (**Supplementary Figure S2**). Absolute penKid levels pre-transplant were mainly related to the duration of KRT prior transplantation, but also to recipient age, whereas changes in penKid levels were exclusively associated with donor modality (living vs. deceased donation).

Cut-Offs to Identify Graft Function Trajectories at Risk for Delayed Graft Function or Poor 30-Day Graft Outcome

To develop a "rule out" test for DGF with >95% sensitivity, a penKid cut-off of >300 pmol/L on day 1 post-transplant achieved 95.1% sensitivity (95% CI 83.9–98.7) and 56.5% specificity (95% CI 46.3–66.2), with an OR of 25.4 (95% CI 5.8–111.3), a PPV of 49.4% and an NPV of 96.3%. For SCr, a cut off of 3.5 mg/dL selected to achieve a comparable sensitivity of 95%, achieved a sensitivity of 95.1% (95% CI 83.9–98.7) and 20.7% specificity (95% CI 13.6–30.0), with an OR of 5.1 (95% CI 1.1–22.9), a PPV of 35.0% and an NPV of 90.5%.

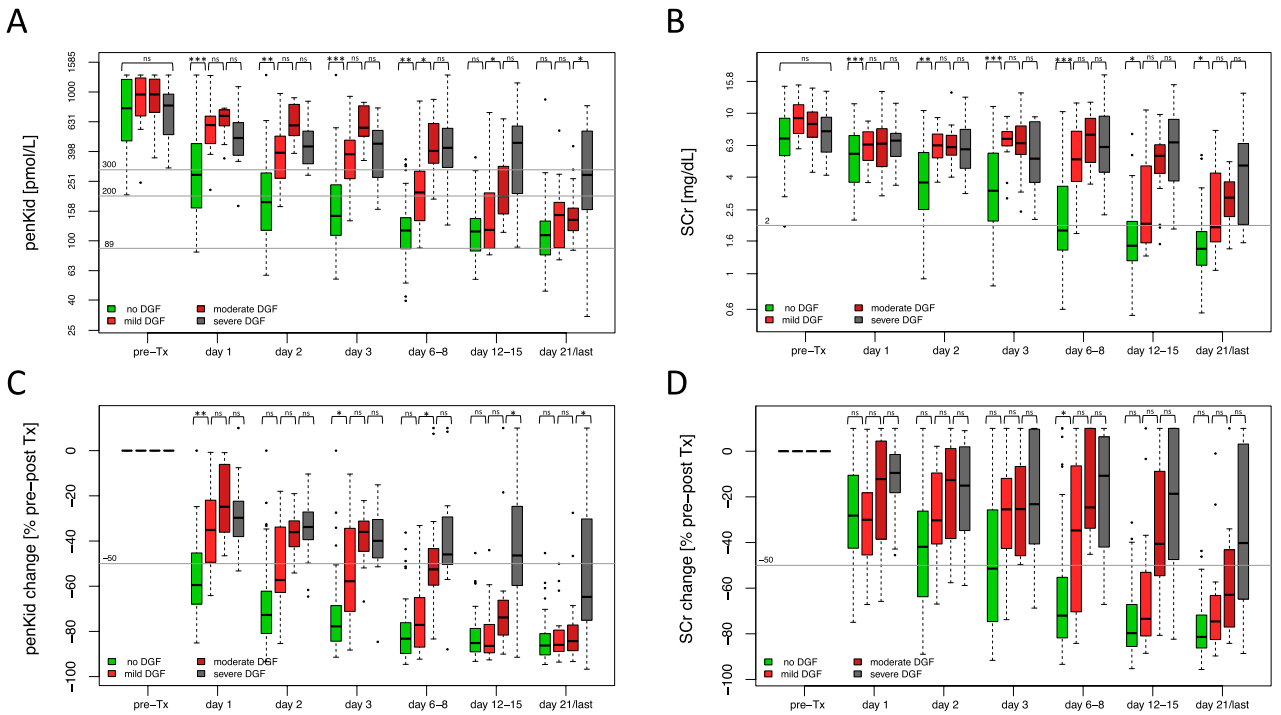


FIGURE 3 | Overall biomarker trajectories of proenkephalin A 119-159 and serum creatinine in relation to severity of delayed graft function. **(A, B)** Absolute biomarker trajectories until discharge stratified by DGF severity. **(C, D)** Relative biomarker changes until patient discharge comparing pre-transplant biomarker levels to the respective post-transplant days stratified by DGF severity. No DGF (green): N = 106, mild DGF (red): N = 19, moderate DGF (dark red): N = 17, severe DGF (grey): N = 17. Data are reported as box-and-whisker plots (interquartile range, minimum to maximum). The grey lines indicate penKid cut-offs at 300 pmol/L, 200 pmol/L, and 89 pmol/L (the last being the upper reference limit for healthy individuals) **(A, B)**, or a 50% decrease cut-off compared to pre-transplant biomarker levels **(C, D)**. For SCr, the grey line signifies an SCr of 2 mg/dL for orientation. Both y-axes are log-transformed. d, days; DGF, delayed graft function; KRT, kidney replacement therapy; penKid, Proenkephalin A 119-159; SCr, serum creatinine; Tx, transplant. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$. NS, $P > 0.05$.

A ≤50% reduction in penKid from pre-transplant to day 1 yielded 89.2% sensitivity (95% CI 75.3–95.7) and 66.7% specificity (95% CI 55.2–76.5) with an OR of 16.5 (95% CI 5.2–52.0). For a ≤50% reduction in SCr, the OR was 1.6 (95% CI 0.4–6.4, p = 0.711).

For predicting 30-day eGFR ≤30 mL/min/1.73 m^2, a penKid cut-off of >300 pmol/L on day 1 showed 84% sensitivity (95% CI 65.4–93.6) and 45.8% specificity (95% CI 36.7–55.2), with an OR of 4.4 (95% CI 1.4–13.8), a PPV of 26.5% and an NPV of 92.5%. For SCr, a cut off of 3.5 mg/dL achieved 96.0% sensitivity (95% CI 80.5–99.3) and 18.7% specificity (95% CI 12.4–27.1), with an OR of 5.5 (95% CI 0.7–43.2), a PPV of 21.6% and an NPV of 95.2%. A ≤50% reduction in penKid yielded 81.8% sensitivity (95% CI 61.5–92.7) and 55.8% specificity (95% CI 45.3–65.8), with an OR of 5.7 (95% CI 1.8–18.2), a PPV of 32.1% and an NPV of 92.3%. For a ≤50% reduction in SCr, the OR is 3.1 (95% CI 0.4–25.2, p = 0.473).

Validation in an Independent Transplant Cohort

In the Sydney study, 60 patients were recruited from September 2022 to June 2024. Patient characteristics and biomarker trajectories for penKid and SCr closely resembled those of the Heidelberg study (**Supplementary Table S1**; **Supplementary Figures S3–S5**). Extent of penKid changes (d0 vs. d1) correlated with DGF development and 30-day eGFR ≤30 mL/min/1.73 m^2, achieving an AUROC of 0.88 (95% CI 0.75–1.0, P < 0.001) and 0.82 (95% CI 0.64–0.99, P = 0.007), respectively (**Supplementary Figures S3E, S5E**). Similar trends in biomarker differentiation for IGF, SGF, and DGF, and comparable diagnostic performance using a penKid cut-off of 300 pmol/L or a 50% reduction rate, were confirmed (**Supplementary Figures S3–S5**; **Supplementary Table S2**).

DISCUSSION

The increasing use of marginal kidneys and implementation of DCD programs to address organ shortages has elevated the incidence of organs without IGF and/or DGF, presenting a significant clinical challenge [1, 3, 25]. Although no effective treatment strategies currently exist, early diagnosis and risk stratification of graft function trajectory are essential for improving individualized care and developing future therapeutic approaches.

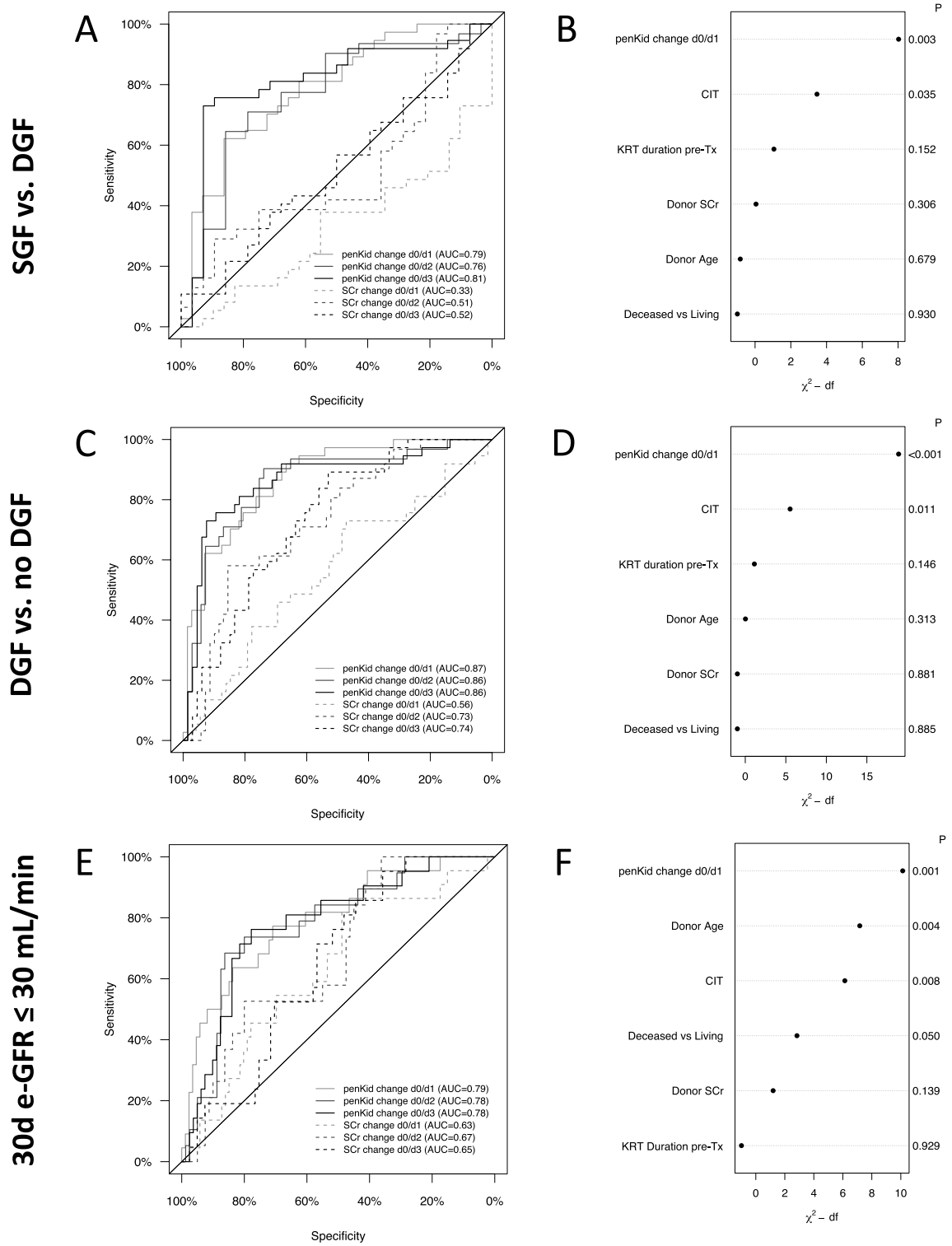


FIGURE 4 | Longitudinal changes of proenkephalin A 119-159 or serum creatinine and their association with critical graft outcomes. Receiver-operating characteristics analysis for relative biomarker change from pre-transplant to first three post-transplant days to distinguish between SGF and DGF **(A)**, DGF and no DGF **(C)** and 30-day graft outcome **(E)**. Multivariate logistic regression model to analyze the value of penKid changes to distinguish SGF from DGF **(B)**, DGF from no DGF **(D)** and 30-day graft outcome **(F)**. IGF: N = 61, SGF: N = 45, DGF: N = 53, no DGF = 106, 30d-eGFR≤30 mL/min: N = 35, 30d-eGFR>30 mL/min: N = 124. AUC, area under the curve; CIT, cold ischemia time; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; IGF, immediate graft function; P, p-value; penKid, Proenkephalin A 119-159; SCr, serum creatinine; SGF, slow graft function; Tx, transplant.

This monocentric, prospective study is the first to evaluate the diagnostic value of penKid for assessing and risk stratifying graft function trajectories and outcomes in the immediate postoperative phase following kidney transplantation. penKid demonstrated the ability to provide significantly earlier insights in patients without immediate graft function by differentiating SGF from DGF, up to 6 days prior to detectable improvements in SCr. This early discrimination may facilitate more informed clinical decision-making through a personalized, penKid-guided approach. Specifically, early identification of SGF could allow for the avoidance of unnecessary interventions such as KRT or kidney biopsy, whereas in cases of DGF, earlier initiation of these measures may be justified. The same applies to distinguishing DGF from no DGF, where penKid outperformed SCr and donor criteria as early as the first post-transplant day in total cohort as well as in subgroup analyses, offering a diagnostic time advantage of several days for individual patients.

On the other hand, penKid's superior granularity in identifying graft function recovery in patients with DGF allows for nuanced sub-classification of DGF severity and associated outcomes, overcoming the limitations of the previously binary DGF definition (DGF versus no DGF) and acknowledging the severity-dependent impact of DGF on long-term outcomes [11–13]. Further, multivariate models incorporating established poor graft outcome risk factors confirmed penKid as the strongest independent risk discriminator of SGF versus DGF, DGF versus no DGF and poor 30-day outcomes, and underlined the independent role of penKid as a marker of kidney integrity by showing a strong association of baseline penKid levels and penKid changes with KRT duration prior transplantation and donor modality, respectively.

Interestingly, our data indicated that, unlike SCr, penKid levels remained remarkably unaffected by KRT, suggesting that penKid may be a more reliable marker of kidney function integrity than SCr during KRT. While we have validated these very unique characteristics in other AKI contexts among critically ill patients [26], further *in-vivo* studies utilizing various KRT techniques are necessary to better understand and confirm these findings. A possible explanation could be a high turnover rate of penKid, characterized by rapid production and metabolism, as it is a small protein with no evidence of protein binding.

Recently, studies have highlighted the considerable potential of penKid in predicting AKI and related outcomes, particularly in critically ill, non-transplanted patients. PenKid has been identified as an early predictor of AKI, an indicator of subclinical AKI [20, 27], and a correlate of GFR and AKI severity. It has also shown promise as a potential risk stratifier for death or the requirement of KRT in clinical contexts such as sepsis and cardiac surgery [27–30]. Consistent with these observations, Beunders et al. demonstrated in a cohort of patients with septic shock that penKid concentrations more accurately reflected measured GFR than traditional estimates of kidney function, such as from endogenous creatinine clearance [31]. The authors further validated a novel penKid-SCr-based GFR equation, showing that this outperformed most creatinine-based equations [32]. Beyond the critical care setting, Schulz et al. found that higher penKid levels were associated with faster kidney function decline and an increased risk of new-onset chronic kidney disease (CKD) over a 16.6-year follow-up in a cohort of 2,568 participants without baseline CKD [33]. This again suggests that penKid may be a more sensitive diagnostic marker for changes in kidney function than SCr.

In the single published study of penKid in kidney transplant recipients, Kieneker et al. found that higher penKid levels were significantly associated with poor long-term outcomes and graft failure in a cohort of 664 recipients, measured, however, at least 1 year post-transplant [34].

In contrast, our present study is the first to investigate penKid as a longitudinal biomarker in the immediate post-transplant period, aiming to predict and stratify different graft function trajectories, and their outcomes. This is particularly important given that despite advances in identifying potential biomarkers for DGF, such as Neutrophil Gelatinase-Associated Lipocalin (NGAL), Kidney Injury Molecule-1 (KIM-1), and plasma cell-free DNA, none have yet been incorporated into clinical practice [21, 35–39]. Further, a key limitation of such damage-based biomarkers is their limited specificity for functional changes or DGF in general, as IRI during kidney retrieval inevitably releases damage-associated molecules [35]. In addition, unlike functional biomarkers such as penKid, damage biomarkers do neither correlate linearly with kidney function impairment nor with recovery or residual kidney function capacity after reperfusion.

Despite these encouraging findings, several limitations need to be addressed. First, although validation was performed using an independent transplant cohort, larger multicenter studies across diverse healthcare systems and organ donation settings are necessary to confirm these results and ensure broader generalizability. Second, as the study design was observational, it remains speculative whether real-time clinical decision-making based on penKid levels could directly improve patient management and outcomes. Nevertheless, penKid-guided risk stratification and diagnostic enrichment could play a pivotal role in optimizing future clinical trial designs, individualized patient management and therapeutic interventions. Lastly, this study did not include a direct comparison of penKid with other established or emerging kidney biomarkers, such as Cystatin C, NGAL, KIM-1 and others. The real-world clinical setting of our study limited the feasibility of incorporating these additional biomarkers. Future research should prioritize head-to-head comparisons between penKid and other kidney biomarkers to clarify their relative accuracy and clinical utility in assessing graft function trajectories and transplant outcomes.

In conclusion, our findings contribute to the growing body of evidence supporting penKid as a superior biomarker reflecting kidney function and integrity, extending clinical utility beyond an established role in predicting (subclinical) AKI and outcomes in critically ill patients. In this real-world post-transplantation setting, penKid demonstrated for the first time robust reliability as a biomarker for distinguishing IGF, SGF, and DGF, assessing DGF severity, and predicting associated 30-day graft outcomes earlier than current clinical standards across two independent transplant cohorts.

Given its high discriminatory power in detecting and sub-characterizing changes in graft function, penKid holds great potential for use in future studies investigating DGF incidence

in transplant programs utilizing DCD, in evaluating machine perfusion techniques, or as an enrichment tool for studies evaluating potential therapeutic interventions to mitigate SGF or DGF in the future.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author. Requests to access the datasets should be directed to christian.nusshag@med.uni-heidelberg.de.

ETHICS STATEMENT

The study was approved by the University of Heidelberg ethics committee and registered in the German Clinical Trials Register (DRKS00026776). Patient consent was waived as penKid assessment was integrated into routine diagnostics, imposing no additional burden. The reliability of penKid kinetics and diagnostic performance was validated in an independent Sydney cohort, with pre- and first post-transplant day data analyzed. This study was approved by the South Eastern Sydney Local Health District Human Research Ethics Committee (2021/ETH11450) and registered in the Australian New Zealand Clinical Trials Registry. Both studies adhered to the Principles of the Declaration of Istanbul on Organ Trafficking and Transplant Tourism and the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

LB and CN designed the study. LB and CN analyzed and interpreted the data and drafted the manuscript. LB, MR, and CN enrolled patients and collected the data. LB, FK, CSp, CSo, CFM, FS, MM, MZ, AM, MW, CMo, and CN were responsible for the clinical management of patients, assisted in data acquisition and revised the manuscript critically. OH assisted in data management, graphical design and statistical analyses. MZo and SA were involved in the study design, established the biomarker measurements and logistics in the central laboratory and revised the manuscript. CMo, MZ, AM, MW, DC and CN supervised the project and revised the manuscript. ZE and CR were responsible for patient enrollment, data collection, and data analysis of the Sydney study, and assisted with data analysis of the overall study and revision of the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

CN, MW and OH are inventors on a filed patent application related to the use of the penKid assay for early diagnosis/early risk stratification of delayed graft function in kidney transplant recipients. All potential, future patent rights have been transferred to SphingoTec GmbH prior to this submission. CN and DC received travel expense reimbursements from SphingoTec. Assays for quantifying Proenkephalin A 119-159 in the Heidelberg and Sydney study were funded by SphingoTec. SphingoTec was not involved in study design, collection of data, nor in the decision to submit the paper for publication.

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

GENERATIVE AI STATEMENT

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.14366/full#supplementary-material

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ORIGINAL RESEARCH
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Factors Influencing the Information Support Provided by Health Care Professionals to Patients in a Dialysis Center Regarding Kidney Transplantation: A Nationwide Study

Paulina Kurleto[1], Lucyna Tomaszek[1], Irena Milaniak[1], Grażyna Dębska[1], Edyta Turkanik[1], Barbara Siekierska[1], Roman Danielewicz[2] and Alicja Dębska-Ślizień[3]*

[1]Faculty of Health Sciences, Andrzej Frycz Modrzewski Krakow University, Krakow, Poland, [2]Department of Surgical and Transplant Nursing, Medical University of Warsaw, Warsaw, Poland, [3]Department of Nephrology, Transplantology and Internal Diseases, Faculty of Medicine, Medical University of Gdansk, Gdansk, Poland

For patients undergoing renal replacement therapy, kidney transplantation (KTx) is the preferred therapeutic method. The aim of this study was to investigate selected factors affecting the information support provided by healthcare professional to patients in dialysis center regarding KTx. A multiple logistic regression was carried out to assess the relationship between information support, socio-demographic factors, life satisfaction (Satisfaction with Life Scale), self-esteem (Self-Esteem Scale), perceived self-efficacy (General Self-Efficacy), attitude, knowledge about organ transplantation. Of the 1,093 respondents aged 22–72 years, 501 respondents (45.8%) always informed patients about the possibility of treatment with KTx. Physicians vs. nurses (OR = 1.79; CI 95%: 1.48–2.16), and those who supported legalization of unspecified living kidney donation in Poland (OR = 1.30; CI 95%: 1.07–1.59) and believed that blood donation is safe (OR = 1.29; CI 95%: 1.12–1.47) were more likely to provide informational support. Knowledge level (OR = 1.32; CI 95%: 1.18–1.47) and self-esteem (OR = 1.06; CI95%: 1.03–1.10) correlated positively with information support. Male participants were less likely to provide informational support than females (OR = 0.78; CI 95%: 0.62–0.99). The results reveal inadequate information provided by healthcare professional to patients about KTx. This highlights the urgent need for comprehensive educational programs.

Keywords: kidney transplant, education and training, healthcare professionals, dialysis, information support

***Correspondence**
Irena Milaniak,
imilaniak@uafm.edu.pl

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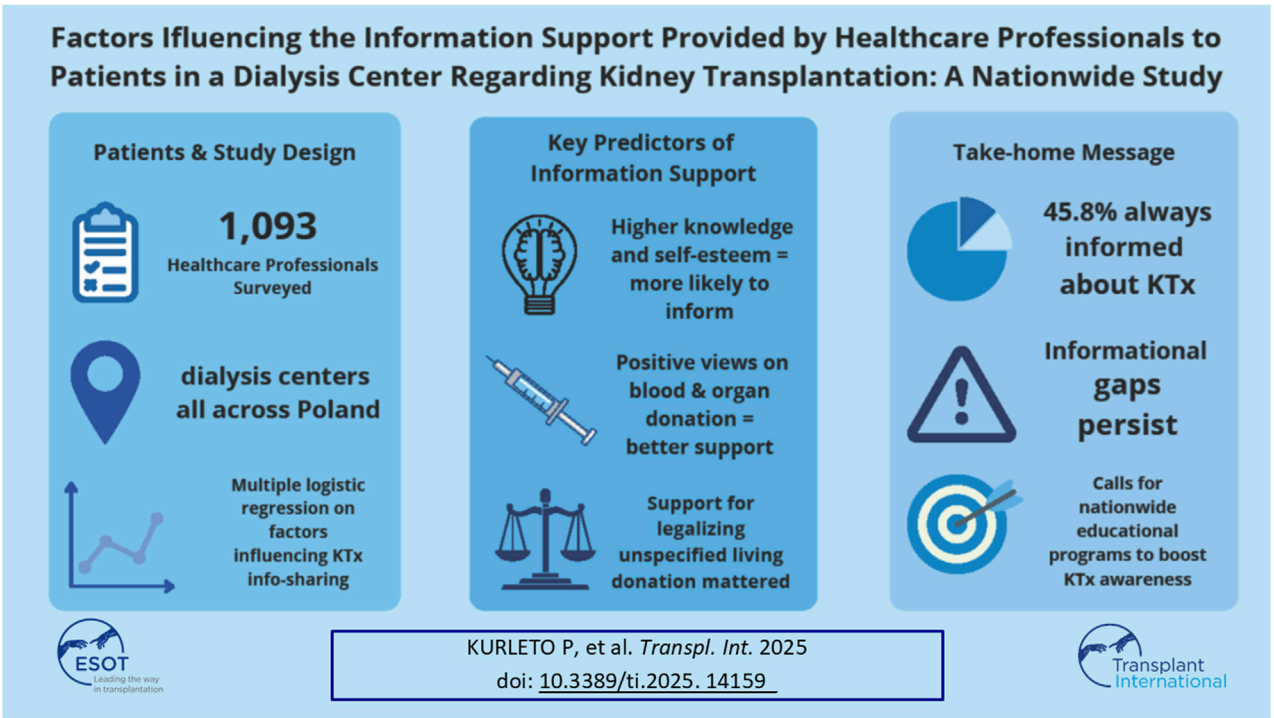
INTRODUCTION

For 20,536 patients undergoing renal replacement therapy in the form of dialysis in Poland, kidney transplantation is the preferred therapeutic method offering improved survival and quality of life [1–3]. With the constant increase in the number of patients suffering from chronic kidney disease (CKD) and waiting for a kidney transplant, living donation has become the most important alternative in many countries [4–6]. In Poland, in 2023, kidney transplantation was performed in 3.4% of hemodialysis (HD) patients and in 13.4% of peritoneal dialysis patients (PD) [1], of which 963 (24.27 pmp) organs came from deceased and 78 (1.9 pmp) from living donors (7% of all kidney



GRAPHICAL ABSTRACT

transplants, which is a record rate since the beginning of the living donation program) [7]. For comparison, other European countries, such as the Netherlands or the United Kingdom, had a living donor donation rate of 49.5% and 28% respectively [8]. Data on the number of dialysis patients and people registered on the national transplant waiting list show, that about 6% of patients undergoing renal replacement therapy are on the transplant waiting list [7].

Given these data, every effort should be made to continue to increase the rate of kidney transplantation, with particular emphasis on living donors. However, each patient and potential donor must weigh the benefits of transplantation against the potential risks of the procedure in order to choose a treatment method. Scientific studies have shown, that patient education is an important component of informed decision-making regarding the treatment of ESRD (9); however, there is evidence that patients do not have adequate knowledge about kidney transplantation [9–11]. Therefore, the tasks of medical personnel in the era when kidney transplantation is considered the best therapeutic method include, among others, presenting possible therapeutic options, including the option of kidney transplantation. In practice, nephrologists are often the first to inform patients about the possibility of treatment with a kidney transplant from a living or deceased donor. Based on the circumstances, the qualification process may begin in a nephrology department or in a dialysis center. A conversation with the patient and family about a potential living donation is obligatory in Poland. The reporting physician must note this fact when entering the patient onto the National Waiting List. After qualifying for a kidney transplant from a deceased or living donor, the potential kidney recipient is placed on the National Waiting List [12].

According to a study by Kucirka et al. from 2012, as many as 30.1% of patients were not informed by nephrologists about the possibility of kidney transplantation in the initial phase of end-stage renal failure [13]. Additionally, studies conducted by Waterman et al. in 2012 show that dialysis center staff were only able to correctly answer questions regarding knowledge about kidney transplantation in 50% of cases, and some employees admitted that they still have very limited time to educate dialysis patients and their families. Moreover, almost one third (30%) declare, that they do not have sufficient knowledge to conduct such education [14]. There are reports in the scientific literature identifying many barriers that hinder effective education about transplantation, including time constraints and poor access to educational materials, as well as barriers that make it difficult for patients to learn, such as fear or lack of trust in medical personnel [9].

The Acts on the Medical and Nursing Professions define their competencies in providing patient care. The doctor explains the patient's health condition and discusses the therapeutic process in detail. The nurse provides information about the patient's health condition to the extent necessary to provide nursing care, health education, and health promotion [15, 16]. According to the educational model proposed by the University Clinical Centre in Gdańsk, educational training is offered to all patients with

CKD at any stage of the disease (and their families), considering renal replacement therapy, including kidney transplantation. An interdisciplinary educational team comprises of four nurses, four doctors, and a dietician [17]. The primary members of the educational team are a nurse and a nephrologist. However, the nurse is usually the patient's primary contact person and is coordinating patient care [18]. In Poland, organized education on renal replacement therapy is conducted mainly by dialysis centers or clinical nephrology centers with dialysis therapy [17, 19].

Due to the increasing number of dialysis patients and the possibility of becoming a potential kidney recipient those awaiting kidney transplantation a study was designed and conducted to show the attitudes and knowledge on kidney transplantation among dialysis center staff and to identify educational methods used in dialysis centers. A more comprehensive understanding of the attitudes of medical personnel towards transplantation, including living donation, will allow for the preparation of public health and educational programs to support living kidney donation. It is worth emphasizing the scientific value of the study, as it is the first one conducted on such scale, gathering data from all Polish voivodeships and in regards of the number of studied personnel.

MATERIAL AND METHODS

Study Desing, Setting

A cross-sectional study was conducted between February 2023 and June 2024 after obtaining the consent of the Bioethics Committee of the Andrzej Frycz Modrzewski Krakow University (decision no. KBKA/3/O/2023). The study included a group of 1,093 employees (physicians and nurses) from public and private dialysis centers across Poland. The guidelines of the Helsinki Declaration (World Medical Association, 2013) and STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) [20], as well as The General Data Protection Regulation [21] were followed. The study was registered at ClinicalTrials.gov (ID: NCT05797337).

Participants

We conducted a cross-sectional study among specialists working in dialysis centers throughout Poland. The study involved employees who voluntarily agreed to participate in the study, had a communicative knowledge of Polish, and had the right to practice medicine or nursing. We assumed that education is delivered in a dialysis centre by a multidisciplinary team including, a nephrologist, and a nurse [17–19]. Before starting the study, each participant received comprehensive information about the purpose and course of the study.

Instruments

The study used a diagnostic survey with a questionnaire technique. The questionnaires were distributed in paper and online form. The researchers sent 1,451 paper surveys to 74 dialysis centers across the country, where consent was obtained from the facility director and the head of the dialysis center. The online surveys were obtained in cooperation with the industry publishing house Practical Medicine (Medycyna Praktyczna). Additionally, dialysis center employees were encouraged to use the snowball method. The study used a self-assessment questionnaire, which included, among others, a socio-demographic data sheet and questions regarding the respondents' attitudes towards kidney transplantation, knowledge in this area and educational methods used in the facilities where they provide care for dialysis patients. Overall knowledge scores being a sum of correct answers ("definitely yes") to 5 questions from a given area (questions 27–31) ranged from 0 to 5 pts. The higher the score, the better the knowledge. For knowledge evaluation, the following statements were presented: 1) Kidney transplantation contributes to the quality of life of patients with chronic kidney disease; 2) Kidney transplantation is a better therapeutic method than dialysis therapy; 3) Kidney transplantation from a living donor is more beneficial for recipients than transplantation from a deceased donor; 4) Kidney transplantation from a living donor can pose a major threat for the donor's health and life; 5) Kidney transplantation from a living donor will significantly deteriorate the donor's quality of life.

Standardized tools were the Polish version [22, 23] of the Satisfaction with Life Scale [24]. The Satisfaction with Life Scale contained five statements in which the respondent assessed the extent to which each of them referred to his or her life so far. The responses were measured on a 7-point Likert scale: 7 – strongly agree, 6 – agree, 5 – somewhat agree, 4 – neither agree nor disagree, 3 – somewhat disagree, 2 – disagree, 1 – I definitely disagree. The measurement result was a general indicator of the sense of satisfaction with life ranging from 5 to 35 points (a score of 20 is considered neutral). The instrument is characterized by good psychometric properties. Internal consistency measured by Cronbach's alpha was 0.86. The test-retest stability of the results was satisfactory (0.85–0.93 in three-week intervals, 0.87–0.88 in six-week intervals and 0.86 in nine-week intervals). The higher the score, the higher the life satisfaction.

Self-esteem was measured using the Rosenberg Self-Esteem Scale (RSES) [25] RSES is a 10-item scale that measures global self-worth by evaluating positive and negative feelings about one's self. The responses were measured on a 4-item Likert scale: 0 (strongly agree), 1 (agree), 2 (disagree), and 3 (strongly disagree). Five of the items are positively worded (items 1, 2, 4, 6, and 7) whereas the remaining five are negatively worded (3, 5, 8, 9, and 10). The maximum score is 30, where higher scores indicate higher self-esteem. The range of possible results is from 0 to 30 points. Raw results were converted into standard units on the sten scale. The SES scale has good psychometric properties, with Cronbach's alpha ranging from 0.81 to 0.83. The Rosenberg self-assessment scale was used in the Polish adaptation of Dzwonkowska et al. [26].

The Generalized Self-Efficacy Scale (GSES) by Schwarzer and Jerusalem (Polish adaptation: Juczyński) was also used as a standardized tool to measure generalized self-efficacy; the scale consists of 10 statements that form one factor, and the results are calculated according to a key that should be interpreted in

relation to sten norms; the Polish version of the scale has good psychometric properties, Cronbach's alpha coefficient = 0.85 [24].

Statistical Analysis

Qualitative variables were presented as the frequency of a given category and its corresponding percentage, while quantitative variables were showed as medians (upper and lower quartiles) and means (standard deviations). Intergroup differences for qualitative data were assessed using the Chi-square test, while for quantitative variables using Mann–Whitney test. Spearman correlation analysis was used to assess the relationship between two quantitative variables. The correlation coefficient (R) was interpreted as: negligible (<0.1), weak (0.1–0.39), moderate (0.4–0.69), strong 0.7–0.89 and very strong (0.9–1.0). The Shapiro–Wilk test was used to examine the normality of the distribution of variables. A multiple logistic regression was carried out to assess the relationship between dependent variable "information support" (always/ not always) and independent variables such as: gender; profession; place of residence; blood donation is safe; bone-marrow transplant is safe; support for legalization of unspecified living kidney donation in Poland; consent to donate organs after death; consent to donate organs after death of a family member; acceptance for family member's decision to donate after death; knowledge level; life satisfaction; self-esteem; perceived self-efficacy.

First, a simple logistic analysis was performed to select predictors–a variable which had a p-value <0.1 was then entered into the multiple regression model. To ensure the model's effectiveness, backward elimination technique was utilized. The Hosmer-Lemeshow test suggested, that the model is a good fit to the data as $p > 0.05$. Nagelkerke's R^2 describes the proportion of variance in the outcome that the model successfully explains. To test the significance of individual coefficients in the model, the Wald statistics were used. The odds ratio with 95% confidence interval was also calculated. The variance inflation factor (VIF) was used to detect multicollinearity in all final regression models (VIF <5 was assumed as acceptable) [27].

Internal consistency rate of the Satisfaction with Life Scale (Cronbach's alpha: 0.86), the Rosenberg Self-Esteem Scale (Cronbach's alpha: 0.83), and the Generalized Self-Efficacy Scale (Cronbach's alpha: 0.86) was estimated; a scale is considered reliable if its Cronbach's alpha is equal to 7 or higher [23, 24]. Statistical analysis was carried out with Statistica 13.3 (®1984–2017 TIBCO Software Inc, Stat Soft Poland, Krakow) and Set Plus (Stat Soft Polska Sp. z o. o. 2024, Set Plus version 5.1.0.[1]). The threshold of statistical significance for all tests was set at $p = 0.05$.

Outcomes

The primary outcomes described the percentage of health care professionals who always informed patients about the possibility of treatment with a kidney transplant (information formulated on the basis of the statement: information regarding kidney transplantation is provided at least once to all patients eligible for transplantation, regardless of whether they have expressed an interest in transplantation or not). The secondary outcomes included: socio-demographic factors, life satisfaction, self-esteem, perceived self-efficacy and attitude and knowledge about organ transplantation.

RESULTS

Of the 1,451 nurses and physicians who were approached to participate in the study, 1,093 responses were received that met all inclusion criteria. The overall response rate was 68%. Respondents were divided into two groups: those who always do (45.8%) and those who do not always provide information support (54.2%); **Figure 1**.

Participant Characteristics

The analysis included survey data of 1,093 health care professionals working in dialysis centers. The number of the nurses and physicians were 850 (77.8%) and 243 (22.2%), respectively. The female-to-male ratio was 963: 130. Median age of respondents in the whole sample was 50 [43; 55] and ranged from 22 to 72 years. The vast majority of them were married or in a committed relationship (75.1%; n = 821), had children (78.4%; n = 857) and siblings (87.2%; n = 953). They were mostly urban residents (74.6%; n = 815). Median job seniority was 20 [7; 27] and the dialysis center was the main workplace for 71.4% of respondents (n = 780). Fifty-two percent (n = 568) of health care professionals worked no more than 160 h per month. **Table 1** presents the socio-demographic characteristics of health care professionals who always informed patients about the possibility of treatment with a kidney transplant and those who did not. The study groups differed significantly in terms of profession, academic degrees and titles, gender, qualification training program, monthly working time, and place of residence.

Life Satisfaction, Self-Esteem, and Perceived Self-Efficacy

The median total scores of The Satisfaction with Life Scale (24 [20; 27] vs. 23 [20; 26]; $Z = -3.88$; $p = 0.0001$), Self-Esteem Scale (21 [18; 25] vs. 20 [18; 22]; $Z = -5.78$; $p < 0.0001$), and Perceived self-efficacy (30 [28; 33] vs. 29 [27; 31]; $Z = -3.51$; $p = 0.004$) among health care professionals was significantly higher in the group of health care professionals who always informed patients about the possibility of treatment with a kidney transplant than those who did not.

Attitude Towards Organ, Blood and Bone Marrow Donation

If necessary, 85% of respondents would donate a kidney to their child, 53% to a parent, 48% to a sibling and spouse, and

[1]http://www.statsoft.pl

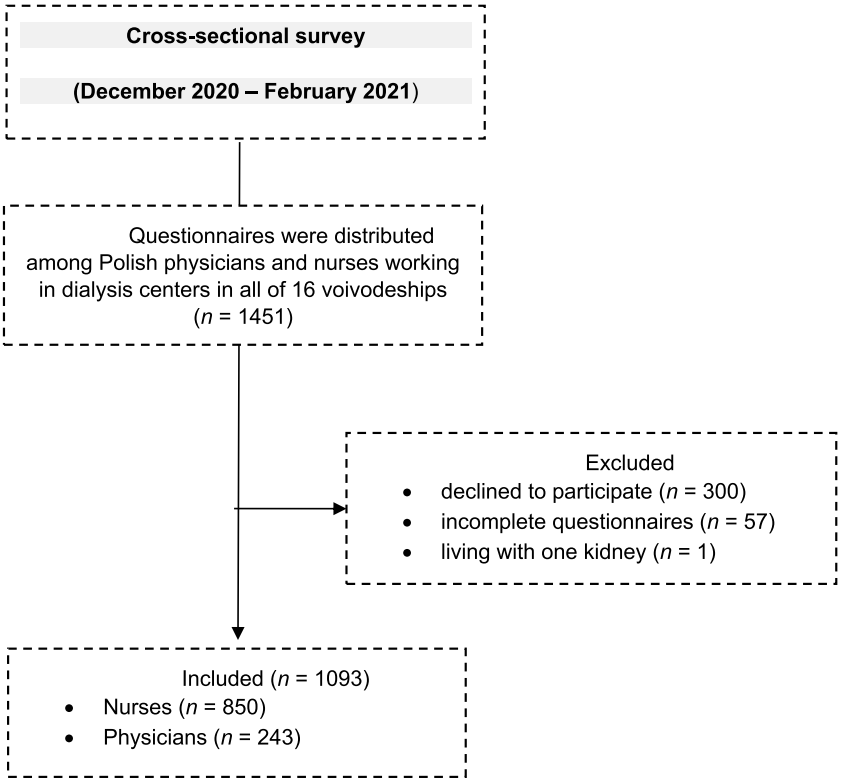


FIGURE 1 | Flow diagram–participants.

6% to a stranger. The respondents most often declared that they would accept a kidney from a dead donor (50%). In case of a living transplant, 40% would accept a kidney from a spouse, 32% from a sibling, 31% from a parent, 27% from a stranger, 12% from a child, 17% were not sure whether they would accept a kidney from anyone, and 4.5% would not agree to a transplant. This question was a multiple-choice question. Almost a quarter of the whole sample (24.4%; n = 267) declared themselves a blood donor at least once in their life and 19% (n = 208) of persons registered with the bone marrow donor bank. Health care professionals who always informed patients about the possibility of treatment with a kidney transplant, were more likely to believe blood donation (69.6% vs. 48.3%; $p < 0.0001$) and bone-marrow transplant (56.7% vs. 36.7%; $p < 0.0001$) are safe than those who did not declare it. They were also determined to donate their own organs for transplantation after death (72.3% vs. 58.4%; $p < 0.0001$) and expressed their acceptance of organ donation from close relatives after their death (62.1% vs. 44.4%; $p < 0.0001$). They were also more likely to believe that - despite the principle of presumed consent - asking the family whether the deceased expressed their objection to organ donation after death during their lifetime and in the presence of two witnesses (39.5% vs. 29.2%) is necessary and should not change. They would also be more willing to support the legalization of kidney donation to a stranger in Poland (18.4% vs. 9.1%; $p = 0.00001$) (**Table 2**).

Knowledge About Kidney Transplantation

Over 80% of all respondents believe that kidney transplantation definitely contributes to improving the quality of life of patients with chronic kidney disease (n = 913) and is a better therapeutic method than dialysis therapy (n = 882). Over 53% (n = 585) of respondents have doubts whether kidney transplantation from a living donor is more beneficial for the recipient than transplantation from a deceased donor. According to only 17.2% (n = 189) of respondents, kidney transplantation from a living donor definitely does not pose a significant threat and in the opinion of 23% (n = 253), kidney transplantation from a living donor will definitely not affect the deterioration of his quality of life. The knowledge of the respondents about kidney transplantation in the group that always provided information support was significantly higher than in the group that did not always give such support (median 3 [2; 4] vs. 2 [2; 3]; $Z = -8.53$; $p < 0.0001$).

Weak positive correlations were noted between variables: knowledge and job seniority ($R = 0.08$; $t = 2.70$; $p = 0.01$), knowledge and self-esteem ($R = 0.18$; $t = 6.01$; $p < 0.0001$), and knowledge and perceived self-efficacy ($R = 0.14$; $t = 4.56$; $p < 0.0001$).

Transplant Education Practices

According to the vast majority of health care professionals working in dialysis centers (90.6%, n = 990), patients with end-stage renal disease are interested in kidney transplantation as a one of the treatment options. However, in the process of

TABLE 1 | Sociodemographic characteristics of healthcare professionals who always informed patients about the possibility of treatment with a kidney transplant and those who did not.

Variables	Information support			
	Always n = 501	Not always n = 592	Statistics values	*P*-values
Age (years)				
• Me, Q_{25}; Q_{75}	50 [43; 55]	50 [41; 55]	Z = −0.84	0.40
• M ± SD	48.3 ± 10.5	47.6 ± 10.7		
Job seniority (years)				
• Me, Q_{25}; Q_{75}	20 [7; 27]	19 [7; 27]	Z = −0.76	0.45
• M ± SD	18.1 ± 11.6	17.6 ± 11.3		
Gender				
• Female	427 (85.2)	536 (90.5)	χ^2 = 7.30	0.007
• Male	74 (14.8)	56 (9.5)		
Place of residence				
• City	393 (78.4)	422 (71.3)	χ^2 = 7.33	0.007
• Village	108 (21.6)	170 (28.7)		
Married or in a committed relationship	377 (75.2)	444 (75.0)	χ^2 = 0.009	0.92
Having children	390 (77.8)	467 (78.9)	χ^2 = 0.17	0.67
Having siblings	438 (87.4)	515 (86.9)	χ^2 = 0.04	0.83
The dialysis center as the main workplace	359 (71.7)	421 (71.1)	χ^2 = 0.04	0.84
Monthly working time (hours)				
• ≤160	232 (46.3)	336 (56.8)	χ^2 = 11.87	0.0006
• ≥161	269 (53.7)	256 (43.2)		
Profession				
• Nurses	332 (66.3)	518 (87.5)	χ^2 = 70.75	<0.0001
• Physicians	169 (33.7)	74 (12.5)		
The specialization program completed	270 (53.9)	226 (38.2)	χ^2 = 27.04	<0.0001
Academic degrees and titles				
• Doctor (degree)	48 (9.6)	15 (2.5)	χ^2 = 30.44	<0.0001
• Doctor habilitated (degree)	6 (0.5)	3 (1.2)		
• Professor (title)	8 (1.6)	3 (0.5)		
Nurses				
Education (n = 850; 100%)				
• Master of Science in Nursing	115 (34.6)	160 (30.9)	χ^2 = 2.67	0.26
• Bachelor in Nursing	111 (33.4)	165 (31.8)		
• Registered Nurse	106 (31.9)	193 (37.3)		
Specialization program (*n* = 320; 100%):				
• Nephrology nursing	63 (44.1)	78 (44.1)	χ^2 = 0.00	0.99
• Internal nursing	80 (55.9)	99 (55.9)		
Qualification training program (*n* = 490; 100%)				
• Internal nursing	8 (4.0)	14 (4.8)	χ^2 = 7.51	0.02
• Nephrology nursing with dialysis	186 (93.5)	277 (95.2)		
• Transplant nursing	5 (2.5)	0 (0.0)		
Physicians				
Specialization program (*n* = 176; 100%)				
• Nephrology	111 (87.4)	41 (83.7)	χ^2 = 2.31	0.31
• Clinical transplantology	1 (0.8)	2 (4.1)		
• Nephrology and clinical transplantology	15 (11.8)	6 (12.2)		

Age and job seniority were presented as median [upper and lower quartile] and mean (± standard deviation). Categorical variables were presented as absolute numbers and percentages.

TABLE 2 | Attitude towards organ, blood and bone marrow donation.

Variables	Information support			
	Always n = 501	not always n = 592	Statistics values	*P*-values
Blood donation is safe	348 (69.6)	286 (48.3)	χ^2 = 50.45	<0.0001
Bone-marrow donation is safe	284 (56.7)	217 (36.7)	χ^2 = 43.85	<0.0001
Consent to donate organs after death	362 (72.3)	346 (58.4)	χ^2 = 22.68	<0.0001
Consent to donate organs after death of a family member	311 (62.1)	263 (44.4)	χ^2 = 33.88	<0.0001
Accepted the family members will donate an organ	198 (39.5)	173 (29.2)	χ^2 = 12.83	0.0003
Support for legalization of unspecified living kidney donation in Poland	92 (18.4)	54 (9.1)	χ^2 = 20.02	0.00001
Organ trafficking risk	434 (86.6)	524 (88.5)	χ^2 = 0.89	0.34

Categorical variables were presented as absolute numbers and percentages.

TABLE 3 | Factors influencing the information support provided by healthcare professionals to patients in a dialysis center regarding kidney transplantation.

Variables	B	SE (B)	Wald test	p	OR (CI 95%)
Simple logistic regression					
Male [Reference: Female]	0.25	0.09	7.19	0.01	1.28 (1.07–1.55)
City [Reference: Village]	0.19	0.07	7.29	0.01	1.21 (1.05–1.39)
Physician [Reference: Nurse]	0.63	0.08	66.24	0.00	1.89 (1.62–2.20)
Blood donation is safe	0.45	0.06	49.49	0.00	1.56 (1.38–1.77)
Bone-marrow donation is safe	0.25	0.06	16.88	0.00	1.29 (1.14–1.45)
Support for legalization of unspecified living kidney donation in Poland	0.40	0.09	19.33	0.00	1.50 (1.25–1.79)
Consent to donate organs after death	0.31	0.06	22.44	0.00	1.36 (1.20–1.55)
Acceptance of organ donation following the death of a family member	0.36	0.06	33.52	0.00	1.43 (1.27–1.61)
Asking the family whether the deceased expressed their objection to organ donation after death during their lifetime and in the presence of two witnesses is necessary and should not change	0.23	0.06	12.76	0.00	1.26 (1.11–1.43)
Knowledge level	0.43	0.05	69.76	0.00	1.53 (1.39–1.70)
Life satisfaction	0.05	0.01	14.01	0.00	1.05 (1.02–1.07)
Self-esteem	0.09	0.02	30.58	0.00	1.09 (1.06–1.12)
Perceived self-efficacy	0.05	0.01	11.39	0.00	1.05 (1.02–1.08)
Multiple logistic regression model _ physicians					
R^2 Nagelkerke = 0.11; Hosmer Lemeshow = 8.43; p = 0.39					
Knowledge level	0.42	0.11	13.97	0.00	1.53 (1.22–1.91)
Perceived self-efficacy	0.08	0.03	5.09	0.02	1.08 (1.01–1.15)
Multiple logistic regression model _ nurses					
R^2 Nagelkerke = 0.11; Hosmer Lemeshow = 13.43; p = 0.10					
Knowledge level	0.25	0.07	15.49	0.00	1.28 (1.13–1.45)
Support for legalization of unspecified living kidney donation in Poland	0.32	0.11	8.09	0.004	1.37 (1.10–1.70)
Blood donation is safe	0.26	0.08	11.49	0.001	1.29 (1.12–1.51)
Self-esteem	0.06	0.02	9.88	0.002	1.06 (1.03–1.11)
Multiple logistic regression model_ the whole group					
R^2 Nagelkerke = 0.19; Hosmer Lemeshow = 11.16; p = 0.19					
Male [Reference: Female]	−0.24	0.12	3.98	0.046	0.78 (0.62–0.99)
Physician [Reference: Nurse]	0.58	0.10	36.63	0.00	1.79 (1.48–2.16)
Knowledge level	0.28	0.06	25.08	0.00	1.32 (1.18–1.47)
Support for legalization of unspecified living kidney donation in Poland	0.27	0.10	7.07	0.008	1.30 (1.07–1.59)
Blood donation is safe	0.25	0.07	13.14	0.00	1.29 (1.12–1.47)
Self-esteem	0.06	0.02	13.33	0.00	1.06 (1.03–1.10)

B, Regression coefficient; SE, Standard error; OR, Odds ratio; and CI, Confidence interval.

qualifying for kidney transplantation, the percentage of physicians and nurses declaring that they had not always talked to the recipient/family about a potential live donation was 30.4% and 60.9%, respectively.

In addition to the oral form, educational practices such as providing handouts/brochures about transplant (58.4%; n = 638), displaying transplant posters (25.3%; n = 277), providing list of transplant websites (15.3%; n = 167), organizing meetings between patients and a living kidney donor (5%; n = 55) or educational meetings about living donation for family members of patients (4.7%; n = 52) were also used. Only 16.8% (n = 184) of respondents indicated that a formal transplant education program existed in their dialysis unit.

It should be noted that most of health care professionals (71.6%; n = 783) spend very little time providing transplant education to patients (from a few minutes to half an hour). Only 39.1% (n = 95) of physicians and 9.1% (n = 77) of nurses declared sufficient knowledge of kidney transplantation and were able to answer most of the patients' questions. It should also be noted that physicians devote more time to self-education per month (several days or more than several days) compared to nurses (31.3%, n = 76 vs. 16.9%; n = 144; $\chi 2 = 36.06$; $p < 0.0001$). The sources of knowledge on this subject are: scientific journals (66.3%; n = 725), textbooks (64%; n = 700), specialist/further training courses (55.5%; n = 607), personnel (47.6%, n = 520), scientific conferences (41.3%; 452), Internet portals (39.1%; n = 427), websites of scientific societies (34%; n = 374). Physicians are twice as likely as nurses to participate in scientific conferences and use websites of scientific societies.

Factors Associated With Information Support

Table 3 shows the three multiple logistic regression models for information support. All presented models are statistically significant ($p < 0.05$). In all obtained regression models VIF ranged between 1.0 and 2.1, indicating that multicollinearity did not influence the regression results.

In the case of *physicians* only knowledge level and perceived self-efficacy were statistically significant in the regression model. The contribution to the *nurse's* model comes from knowledge level, support for legalization of kidney donation to a stranger from living donors in Poland, safety of blood donation, and self-esteem. The all parameters in above-mentioned two models are positively associated with information support.

The multiple logistic regression model developed for the *whole group* reviled that physician (vs. nurses), and those who supported legalization of unspecified living kidney donation in Poland and believed that blood donation is safe were more likely to provide informational support. Knowledge level and self-esteem correlated positively with information support. Male participants were less likely provide informational support than female.

DISCUSSION

The results of this study showed that physicians were more likely to provide informational support to dialysis patients than nurses. Gender differences in giving information support were recorded. Knowledge level and self-esteem correlated positively with informational support. Additionally, such support was provided by people who would support legalization of unspecified kidney donation from living donors in Poland, and believed that blood donation is safe.

The first factor "physicians were more likely to provide informational support to dialysis patients than nurses" is connected with the facts that physicians are responsible for the treatment plan and qualification and inclusion on the transplant waiting list [28, 29]. Trachtman H. et al. in their study found physicians' support for living kidney donation as a viable medical option [30].

Oriol-Vila et al. [31], based on a review of 12 studies on the process of deceased donor transplantation showed, that after nurse educational interventions, dialysis patients and kidney transplant recipients had better health outcomes. It is therefore alarming that 30.4% of Polish physicians and 60.9% of nurses caring for patients in the dialysis center declared that they did not always inform patients about kidney transplantation as the best therapeutic option. These national data are similar to the report by Kucirka et al. [13], which showed that 30.1% of American patients with ESRD did not have information from their nephrologists in dialysis centers about the possibility of transplantation. Educational neglect is one of the main barriers to access transplantation treatment [32], because uninformed patients have limited access to the transplant waiting list and transplantation [33]. Lack of education may contribute to poorer quality of life for dialysis patients, as dialysis is not an ideal long-term solution and transplantation offers a better perspective. Furthermore, dialysis is more expensive than kidney transplantation in the long term, leading to increased treatment costs [34].

The results of the study suggest that men–both doctors and nurses–are less likely to provide informational support to patients, than women. This may be due to differences in communication style, approach to patients, social and cultural conditions. The study by Roter and Hall [35] shows that female doctors were more likely to engage in conversations with patients, show more empathy and spend more time on health education than male doctors. Street et al. [36] found that regardless of gender, doctors showed more patient-centered communication, but only with patients they perceived as better communicators, more satisfied and more likely to follow recommendations. In contrast, Younas and Sundus [37] reported that patients perceived nurses as supportive and comforting and provided them with necessary information, but many of them did not answer their questions in a timely and sufficient manner.

Transplant programs worldwide are regulated by law; however, the knowledge and attitude of professionals and general society is important to increase the number of transplants. Our study showed that knowledge level of the professionals correlated positively with informational support for the patients. On the one hand patient education requires significant resources and in addition, some studies also show that nephrologists do not consistently discuss mortality risks with patients, both in the case of dialysis patients and during the

kidney transplant evaluation education process [38]. Available studies showed, that having good knowledge and good attitudes may lead to better practice in patient information about treatment options [39–41].

The another factor "support legalization of unspecified kidney donation from living donors in Poland" is important from the perspective of living donation. The rates of transplants from living donors in Poland are very low. In 2023 there were 78 kidney transplants from living donors, 5 more than in 2022 [7]. Anonymous live organ donors or unspecified donors are individuals willing to be organ donors for any transplant recipient especially kidney donor with whom they have no biological or antecedent emotional relationship [42]. Donation to a stranger is legal in numerous countries, including the USA, Canada, Australia, and Israel and European countries like: Great Britain, Sweden, or the Netherlands [43]. Unspecified living donations can help bridge transplant disparities, help mitigate the shortage of kidney grafts globally and improve organ allocation [44]. In our previous study we found that in Poland, there is a strong support for legalization of unspecified living kidney donation (60% of respondents) [45].

In our study, the positive attitude towards blood donation, especially nurses', is the factor that affects the informational support for dialysis patients. This association can be explained by several psychological and behavioral factors. It is likely, that these individuals tend to have a greater sense of social responsibility and are more involved in promoting health literacy, including organ donation and transplantation.

Our study revealed, based on logistic regression model constructed separately for nurses and the entire group, that self-esteem correlated positively with informational support. Self-esteem is considered an important factor in human behavior and plays a significant role in the professional functioning of medical personnel, especially nurses, by influencing their interpersonal skills and the way they communicate with patients. People with low self-esteem are characterized by a lack of self-confidence, and as a result, they are unable or reluctant to communicate effectively with patients or use inappropriate communication methods [46]. People with high self-esteem believe in their own competences, which may translate into a greater willingness to provide health education in the field of kidney transplantation and thus contribute to an increase in the number of transplants and improvement in the quality of life of patients. It is worth noting that in the logistic regression model developed only for doctors, a significant factor related to informing the patient was perceived self-efficacy, defined as an individual's belief in coping with difficult situations and obstacles [18]. A higher sense of self-efficacy increases motivation to act [47] and this probably explains the fact that doctors with a higher sense of efficacy are more likely to undertake patient education.

Implications for Clinical Practice

In the last year, an increase in kidney transplants from a living donor has been observed in Poland. For the first time in several decades of the existence of the living donation program, a rate of 7% was achieved; previously, it was a maximum of 5% of all kidney transplants [7]. Nevertheless, this is still a low rate compared to many Western European countries [8]. The results of our study reveal inadequate information provided by healthcare professionals to patients about kidney transplantation. This highlights the urgent need for comprehensive educational programs for both healthcare professionals and patients, with a focus on the benefits of kidney transplant programs and lifetime indefinite kidney donation. To assure these programs' effectiveness, the Polish transplant society should play the key role in developing the frameworks for such programs.

Future educational research should determine which techniques work best and how effective strategies can be made available to the entire population of patients with CKD and ESRD and their family members. Research studies confirm, that female healthcare professionals are more likely to provide informational support to patients than males. This disparity can be reduced through communication training, standardization of patient information procedures, and promotion of greater involvement of all healthcare professionals in patient education.

Research suggests that positive attitudes toward blood donation among healthcare professionals are associated with a greater likelihood of providing informational support to dialysis patients regarding kidney transplantation. Fostering a culture of blood donation awareness within healthcare teams can lead to better patient education and improved transplant outcomes.

Strengths and Limitations

The strengths of the study is the large sample sizes and use of standardized tools. Additionally, our study is the first nationwide study on this matter. The limitation of this study is: the self-assessment questionnaire used within this study was not validated, and therefore the results must be interpreted with this in mind. In addition, the sample structure was not calculated due to the lack of detailed data on the number of nephrologists and nurses in the country working in the dialysis center. The total number of nephrologists in 2022 was 1,386 (F: 846; M: 538) and 121 (F: 99; M: 22) for pediatric nephrologists [48]. There's however no data on if they work in dialysis centers, Nephrology Departments, or both. There is no exact information on the number of nurses working in dialysis centers, it is estimated that about 4,300 nephrology nurses work in Polish nephrology and transplant centers [49]. It is also worth noting, that many of dialysis center personnel work in more than one facility, thus it is hard to differentiate whether working in a public or private dialysis center has or has not an impact on the studied sample's views and practices. We also are aware of the fact, that our studied group are dialysis centers only–we have not targeted the Nephrology Departments personnel–again we have not asked about working elsewhere so there is a possibility of some personnel having their answers effected by this fact.

CONCLUSION

Summarizing, physicians were more likely to provide informational support to dialysis patients than nurses. Additionally, such support was provided by people who would support legalization of unspecified kidney donation from living

donors in Poland, believed that blood donation is safe and would also accept their family members decision to donate an organ after death. Knowledge level and self-esteem correlated positively with informational support.

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 Andrzej Frycz Modrzewski Krakow University (decision no. KBKA /3/O/2023). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PK Study Design, Data Collection, Statistical Analysis, Data Interpretation, Manuscript Preparation, Literature Search. LT Study Design, Statistical Analysis, Data Interpretation, Manuscript Preparation, Literature Search. IM Study Design, Data Interpretation, Manuscript Preparation, Literature Search. GD Data Interpretation, Literature Search. ET Data Collection. BS Data Collection. RD Data Interpretation, Literature Search. AD-S Data Interpretation, Literature Search. 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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ORIGINAL RESEARCH
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Urinary NGAL Outperforms ^{99m}Tc-MAG3 Renography in Predicting DCD Kidney Graft Function

Esther N. M. de Rooij[1,2†], Tirsa T. van Duijl[3†], Ellen K. Hoogeveen[1,2,4†], Fred P. H. T. M. Romijn[3†], Friedo W. Dekker[2†], Cees van Kooten[1†], Christa M. Cobbaert[3†] and Johan W. de Fijter[1,5,6†]*

[1]Department of Nephrology, Leiden University Medical Center, Leiden, Netherlands, [2]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands, [3]Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, Netherlands, [4]Department of Nephrology, Jeroen Bosch Hospital, Den Bosch, Netherlands, [5]Department of Nephrology and Hypertension, Antwerp University Hospital, Antwerp, Belgium, [6]Laboratory of Experimental Medicine and Paediatrics (LEMP), University of Antwerp, Antwerp, Belgium

Recipients of donation after circulatory death (DCD) kidneys are at high risk for delayed graft function (DGF) due to severe ischemia-reperfusion injury. We compared urinary biomarkers in predicting the duration of DGF with the tubular function slope (TFS) as the gold standard. In 89 DCD kidney transplant recipients, urinary TIMP-2, IGFBP7, B2M, NGAL, KIM1, CXCL9, and UMOD were quantified by LC-MS/MS analysis on postoperative days (PODs) 1, 4 and 10. Interstitial fibrosis and tubular atrophy (IF/TA) were assessed with protocol biopsies at POD 10. TFS was calculated with ^{99m}Tc-MAG3 renography. Predictive performance was compared with AUCs from ROC analyses. Of all 89 recipients, 22% experienced no (<7), 22% mild (≥7–14), 29% moderate (≥14-<21) and 26% severe (≥21 days) fDGF. The OR for the presence of IF/TA was 1.9 (95% CI:0.4; 10.0) for mild to moderate and 15.0 (95% CI:2.7; 84.8) for severe compared to no fDGF. At POD 4, urinary NGAL and fractional NGAL excretion (FE-NGAL) outperformed TFS and other biomarkers in predicting fDGF with AUCs of 0.97, 0.98 and 0.92, respectively. At POD10, FE-NGAL and PCR best predicted severe vs. mild to moderate fDGF, with AUCs of 0.74 and 0.76 versus 0.65 for TFS. Therefore, urinary NGAL and FE-NGAL may provide a viable alternative to ^{99m}TcMAG3 renography for monitoring fDGF clearance or guiding kidney transplant biopsy to exclude additional acute rejection.

Keywords: DCD donation, NGAL, urinary biomarkers, delayed graft function (DGF), kidney transplantation

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***Correspondence**
Esther N. M. de Rooij,
e.n.m.rooij@lumc.nl

†ORCID:
Esther N. M. de Rooij
orcid.org/0000-0003-3017-2885
Tirsa T. van Duijl
orcid.org/0000-0001-8069-6631
Ellen K. Hoogeveen
orcid.org/0000-0002-5482-2013
Fred P.H.T.M. Romijn
orcid.org/0000-0003-4470-7959
Friedo W. Dekker
orcid.org/0000-0002-2433-2494
Cees van Kooten
orcid.org/0000-0002-6257-0899
Christa M. Cobbaert
orcid.org/0000-0003-3565-1404
Johan W. de Fijter
orcid.org/0000-0003-3076-5584

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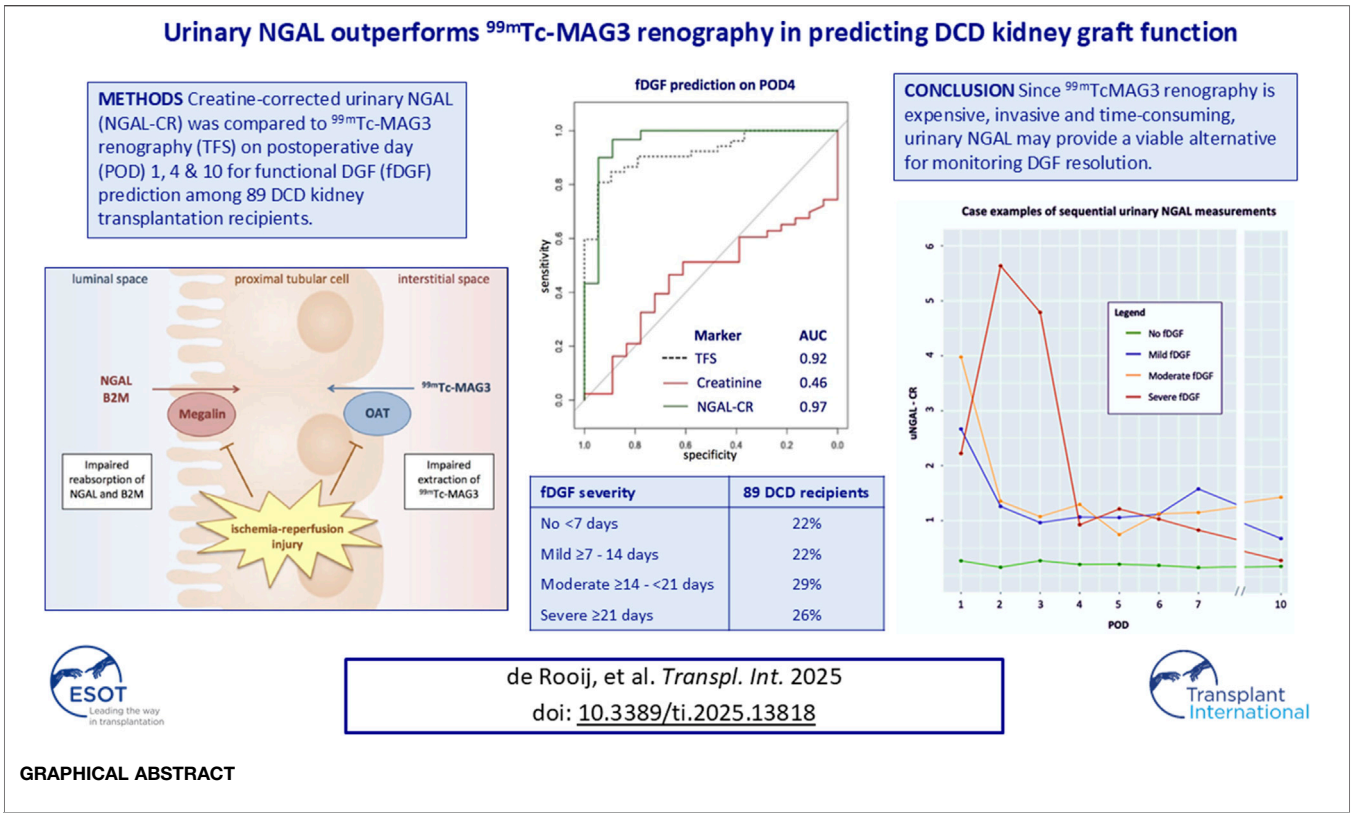
INTRODUCTION

For the majority of patients with end-stage kidney disease (ESKD), kidney transplantation is the preferred modality of renal replacement therapy (RRT), but with the ongoing gap between supply and demand, the waiting time while on dialysis is increasing [1]. Owing to the shortage of kidneys available for transplantation, many countries use donation after circulatory death (DCD) kidneys to expand the potential donor pool [2]. In the Netherlands, from 2017 to 2021, the relative contribution of DCD increased from 56% to 66% of all deceased kidney transplants [3].

Kidneys from DCD donors have a higher risk of severe ischemia-reperfusion injury (IRI) compared to kidneys from donation after brain death (DBD) or those from living donors. The



GRAPHICAL ABSTRACT

longer initial warm ischemia time (WIT) to which DCD kidneys are exposed increases the risk of primary nonfunction (PNF) and delayed graft function (DGF), with the latter estimated to be up to 50% [1, 2]. DGF is a manifestation of ischemia-reperfusion or acute kidney injury (AKI), most commonly due to acute tubular necrosis (ATN), which causes post-transplantation oliguria or anuria, increased allograft immunogenicity and may increase the risk of early acute rejection [3–6].

Traditionally, DGF has been defined as the need for dialysis in the first week after kidney transplantation [7]. However, since the indication for dialysis is clinically determined by nephrologists on an individual basis, this dialysis-based definition is subjective and does not always reflect the lack of adequate glomerular filtration. Therefore, the function-based definition of DGF (fDGF) has been proposed as an alternative for retrospective evaluation. fDGF is established when serum creatinine fails to decrease by at least 10% per day for 3 consecutive days within the first week after kidney transplantation [8].

Predicting the duration of fDGF and monitoring for the occurrence of a concomitant early acute rejection episode remains a major challenge in the first weeks after kidney transplantation [9]. Sequential ^{99m}Technetium-mercaptoacetyltriglycine (^{99m}Tc-MAG3) renography can be used to identify the evolution of tubular function in the case of DGF. ^{99m}Tc-MAG3 renography allows for the calculation of a standardized tubular function slope (TFS), which reflects the ^{99m}Tc-MAG3 uptake by renal tubular cells during the first minutes after ^{99m}Tc-MAG3 injection [10, 11]. The TFS has previously been shown to be a sensitive biomarker of functioning proximal tubular epithelial cells (PTECs) and has been associated with fDGF and long-term graft function [10]. However, ^{99m}Tc-MAG3 renography is an expensive, invasive and time-consuming investigation.

^{99m}Tc-MAG3 is transported by organic anion transporters (OAT) expressed on the basolateral side of PTECs. Urinary biomarkers that identify tubular damage may offer a safer, quicker, and cheaper alternative to ^{99m}Tc-MAG3 renography. Several novel markers of urinary kidney injury, such as tissue inhibitor of metalloproteinases-2 (TIMP-2), insulin-like growth factor–binding protein 7 (IGFBP7), kidney injury molecule 1 (KIM-1), CXC Motif Chemokine Ligand 9 (CXCL9), uromodulin (UMOD), neutrophil gelatinase–associated lipocalin (NGAL) and beta-2 microglobulin (B2M) have been investigated. In particular, urinary markers of PTEC dysfunction may potentially be of interest to monitor the return of PTEC functionality. Both NGAL and B2M are freely filtered and almost completely reabsorbed via the luminal side of the PTECs (**Figure 1**). However, their exact pathophysiological role and diagnostic value in different etiologies of kidney injury remain unclear. Here, we investigated the relation between kidney transplant tissue quality and the duration of fDGF. Subsequently, we assessed the change in these novel urinary biomarkers of kidney injury in DCD kidney transplantation recipients stratified by fDGF duration as a measure of IRI severity. Finally, we compared the performance of these markers in predicting fDGF duration to that of TFS

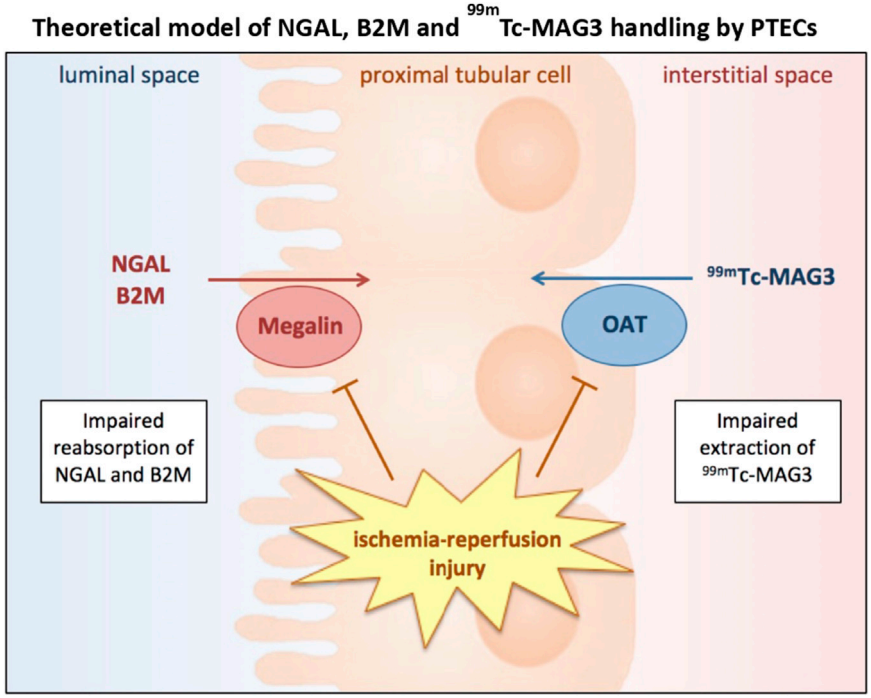


FIGURE 1 | Ischemic and/or reperfusion injury of PTECs resulting in impaired ^{99m}Tc-MAG3 extraction via OATs on basolateral membranes as well as impaired NGAL and B2M reabsorption via megalin on apical membranes. Abbreviations: B2M, beta-2 microglobulin; NGAL, neutrophil gelatinase-associated lipocalin; PTEC, proximal tubular epithelial cell; OAT, organic anion transporter, ^{99m}Tc-MAG3, ^{99m}Technetium-mercaptoacetyltriglycine.

(chosen as the gold standard), in order to identify markers that can be used to easily monitor PTEC functionality.

MATERIALS AND METHODS

Study Design and Population

We included 89 out of 92 DCD kidney transplant recipients who participated in the Prospective Trial on Erythropoietin in Clinical Transplantation (PROTECT) [12]. Three PROTECT participants were excluded from the current project as they experienced PNF due to early graft thrombosis and urine samples were therefore not available. Briefly, PROTECT was a randomized, double-blind study comparing high-dose recombinant human erythropoietin (EPO)-β to placebo for the combined primary endpoint of primary nonfunction and DGF. All consecutive patients scheduled to receive a DCD kidney transplant at Leiden University Medical Center between August 2005 and December 2009 were approached to participate. High-dose EPO was administered to the recipient as an intravenous bolus on 3 consecutive days (total dose 100.000 IE) starting 3–4 h before the transplantation. All donors were controlled DCD cases (Maastricht Category III). Kidneys were allocated according to the allocation algorithm and matching criteria of both the standard Eurotransplant Kidney Allocation System (ETKAS; n = 73) and the Eurotransplant Senior Program (ESP; n = 16). At that time, Super Rapid (SR) procurement with cold preservation perfusion or Normothermic Regional Perfusion (NRP) technique was not yet available. There was no donor age limit for acceptance of DCD kidneys. The median age (46 years) of the DCD cases in the Netherlands was previously found to be significantly lower than that of heart-beating donors (48.5 years) in the period before the PROTECT study commenced [13]. All consecutive patients scheduled to receive a DCD kidney transplant were approached to participate in the PROTECT study. Exclusion criteria included panel-reactive antibodies (PRAs) > 60% at the time of transplantation, donor serum creatinine >150 μmol/L, first warm ischemic time (WIT) ≥ 45 min or cold ischemic time (CIT) > 24 h. The immunosuppressive regimen consisted of induction therapy with anti-CD25 antibody (daclizumab; intravenous bolus 100 mg preoperatively and on postoperative day (POD) 10) and triple maintenance therapy with mycophenolate mofetil, corticosteroids and delayed introduction of cyclosporine A (CsA) microemulsion. CsA (initial dose 3 mg/kg twice daily) was introduced on POD 4, with subsequent dosing according to the 12-hour area under the curve (AUC) targets of 5,400 ng/mL/h for the first 6 weeks after transplantation and 3,250 ng/mL/h thereafter. All patients received prophylactic therapy with trimethoprim/sulfamethoxazole 480 mg/day for 6 months against pneumocystis jiroveci pneumonia. The study was approved by the Ethical Committee of the Leiden University Medical Center (NCT00157300). At 1 year, there was no difference in the incidence or duration of DGF and/or primary nonfunction in patients treated with high-dose EPO versus placebo. Further details and results have been described previously [12].

Data Collection

Collected information included recipient age, sex, primary kidney disease, previous kidney transplantations, time on dialysis, donor age, sex, cause of death, hypertension, serum creatinine, and transplant characteristics including HLA mismatch, PRA, CIT and WIT. Additionally the kidney donor risk index (KDRI), a widely used tool to predict the risk of graft failure based on deceased donor characteristics, was calculated [14].

Definition of Functional DGF (fDGF)

DGF was defined according to the functional definition (fDGF); a failure of serum creatinine to decrease spontaneously by ≥ 10% daily on three successive days during the week after transplantation or dialysis requirement [8]. The second of three consecutive days was used as the index day to group patients by duration of fDGF, resulting in 4 groups of <7 days, ≥7–14 days, ≥14–21 days and ≥21 days. fDGF ≥7 or ≥21 days can be considered as having either fDGF or severe fDGF, respectively.

Laboratory Measures

At the time of the study, urine and serum samples were collected at PODs 1-7, POD 10, at 6 weeks and 6 months after transplantation. For the current project, we only used urine data on PODs 1, 4 and 10, at 6 weeks and 6 months after transplantation since TFS and serum analyses for creatinine, B2M and NGAL were performed only on those days. Urine osmolality was determined by freezing point depression using an Osmo-Station (Auto & Stat model OM-6060, Arkray Inc., Kyoto, Japan). Urinary total protein (TP) was determined by turbidimetry (Cat. No. 05171954190), B2M by immunoturbidimetry (Cat. No. 08047430190), and creatinine by an enzymatic method (Cat. No. 3263991190), all using a Cobas C8000 c702 (Roche Diagnostics, Mannheim, Germany).

Urinary NGAL, IGFBP7, KIM-1, TIMP2, CXCL9, UMOD, SLC22A2 and nephrin were quantified in 36 batches between January 2021 and November 2021, using an in-house developed multiplex liquid chromatography tandem mass spectrometry (LC-MS/MS) test. The preanalytical and analytical phases of this LC-MS/MS test followed the standard operating procedure described elsewhere [15, 16]. To ensure LC-MS/MS performance, a system suitability test was carried out prior to each analysis batch of study samples (a maximum of 81 samples per batch). To monitor LC-MS/MS performance over time, two urine-based internal quality control (IQC) samples were prepared and analyzed with the study samples. The IQC results were monitored in Levey-Jennings charts and the test performance was considered stable over 1 year [17, 18]. All urine samples were stored for 10–15 years and underwent one to two freeze-thaw cycles. However, it is important to note that LC-MS/MS tests are relatively insensitive to freezing and thawing of samples.

With the serum and urinary biomarker and creatinine values, fractional excretion (FE) of B2M and NGAL were calculated, analogous to the FE of sodium. In analogy to the protein-to-creatinine ratio (PCR), we calculated the ratios of B2M/(TIMP2) and NGAL/(TIMP2). Theoretically, TIMP2 or IGFBP7 could substitute creatinine as a glomerular filtration marker, whereas B2M and NGAL are actively reabsorbed in proximal epithelial cells.

Protocol Kidney Biopsy

Per the protocol, all recipients underwent a kidney transplant biopsy on POD 10. Adequate biopsy samples were available for 64 recipients. Biopsies were unavailable (n = 25) due to: withdrawal of consent (n = 7), insufficient tissue (n = 11) or staining issues (n = 7). An experienced pathologist scored all biopsies according to the semi-quantitative ATN score and assessed interstitial fibrosis and tubular atrophy (IF/TA) according to the Banff 2009 classification, as a proxy for donor-derived fibrosis [19, 20]. Biopsy results have been published previously [21].

^{99m}Tc-MAG3 Renography

^{99m}Tc-MAG3 renography was performed on PODs 1, 4 and 10 to calculate the tubular function slope (TFS) [10]. Briefly, a bolus of 100 MBq of ^{99m}Tc-MAG3 was injected and frames were recorded with a large-field-of-view gamma camera (Toshiba GCA501S), at 1-second intervals for 120 frames, then at 20-second intervals for 90 frames. The ^{99m}Tc-MAG3 dose was corrected for extravasation. TFS was calculated by analyzing radiopharmaceutical uptake by renal tubular cells using a nuclear medicine computer (MAPS 10000 Web Link Medical). Two regions of interest were drawn semi-automatically; one around the graft and one representing the background. Subsequently, a background-subtracted graft and dose-adjusted ^{99m}Tc-MAG3 curve were generated. During the first two minutes of the renography two phases can be recognized in the graft: a rapidly ascending phase, representing the perfusion of the kidney, followed by a second phase of tubular extraction. Using a linear fit (least-squares error estimate), the slope of the second phase of this curve was determined and defined as TFS.

Statistical Analysis

First, baseline recipient, donor and transplant characteristics are presented here as mean (±SD) or number (proportion) for all recipients and stratified by fDGF duration. Second, we studied the relation between baseline KDRI, fDGF duration and IF/TA presence in the kidney biopsy on POD 10, using logistic regression analysis to investigate the clinical relevance of severe fDGF. Third, we studied the relation between fDGF duration and endogenous creatinine clearance (ECC) at 6 weeks and 12 months, using logistic regression analysis for the outcome ECC ≥40 vs. < 40 mL/min, and linear regression analysis for the change in ECC in mL/min.

Fourth, we calculated the median (interquartile range [IQR]) values of TFS, urine volume, creatinine, PCR, and creatinine-corrected NGAL, B2M, TIMP2, IGFBP7, KIM-1, CXCL9, UMOD, and FE-NGAL and FE-B2M on PODs 1, 4 and 10, for all recipients and stratified by fDGF duration.

Fifth, we calculated Pearson correlation coefficients to study the association between TFS and urinary creatinine, PCR, standardized creatinine-corrected NGAL, B2M and TIMP2, IGFBP7, KIM-1, CXCL9, UMOD, and FE-NGAL and FE-B2M, at PODs 1, 4 and 10. A p-value < 0.05 was considered statistically significant.

Sixth, we calculated AUCs with receiver operating characteristic (ROC) analyses for fDGF presence at PODs 1 and 4, and fDGF severity at POD 10 as predicted by standardized TFS, urinary creatinine, PCR and standardized creatinine-corrected urinary NGAL, B2M, TIMP2, IGFBP7,

TABLE 1 | Recipient, donor, and transplant characteristics of 89 donation after circulatory death kidney transplantations.

Characteristic	All	Functional delayed graft function[a]			
		No	Mild	Moderate	Severe
n	89	20	20	26	23
Recipients					
Age, years	54 (±13)	50 (±13)	53 (±12)	52 (±13)	59 (±12)
Male patients, *n* (%)	62 (70)	16 (80)	13 (65)	18 (69)	15 (65)
Primary kidney disease, *n* (%)					
Diabetes, hypertension or nephrosclerosis	29 (32)	6 (30)	7 (35)	8 (31)	8 (35)
Primary or systemic glomerular disease	28 (32)	7 (35)	3 (15)	11 (42)	7 (30)
Polycystic kidney disease	16 (18)	2 (10)	6 (30)	4 (15)	4 (17)
Other or unknown	16 (18)	5 (25)	4 (20)	3 (12)	4 (17)
PRA >5%, %	9 (10)	4 (20)	0 (0)	3 (12)	2 (9)
Repeat transplant, *n* (%)	3 (3)	0 (0)	2 (10)	1 (4)	0 (0)
Pre-emptive transplant, *n* (%)	1 (1)	1 (5)	0 (0)	0 (0)	0 (0)
Dialysis vintage, y	4.4 (±2.5)	4.9 (±4.3)	3.8 (±1.7)	4.5 (±1.9)	4.4 (±1.5)
Donor					
Age, years	46 (±15)	35 (±13)	48 (±13)	48 (±13)	52 (±18)
Male, *n* (%)	49 (55)	10 (50)	8 (40)	13 (50)	18 (78)
Cause of death: CVA, *n* (%)	36 (40)	6 (30)	6 (30)	12 (46)	12 (52)
Hypertension, *n* (%)	18 (20)	1 (5)	5 (25)	6 (23)	6 (26)
Serum creatinine, μmol/L	79 (±51)	76 (±27)	74 (±29)	93 (±85)	71 (±21)
Transplant					
KDRI score, *n* (%)					
<1	34 (38)	14 (70)	7 (35)	9 (35)	4 (17)
≥1 to 1.5	36 (40)	5 (25)	10 (50)	11 (42)	10 (44)
≥1.5 to 2	15 (17)	1 (5)	2 (10)	5 (19)	7 (30)
≥2	4 (5)	0 (0)	1 (5)	1 (4)	2 (9)
CIT, h	17 (±4)	16 (±4)	16 (±4)	17 (±4)	18 (±4)
WIT I, min	18 (±6)	16 (±5)	16 (±5)	19 (±6)	20 (±7)
WIT II, min	30 (±7)	32 (±7)	30 (±8)	28 (±6)	31 (±8)
Post-transplant day 10					
IF/TA present, *n* (%)	26 (33)	2 (13)	5 (26)	4 (17)	15 (68)
TFS	1.5 (±1.1)	2.5 (±1.2)	1.9 (±1.0)	1.0 (±0.8)	0.9 (±0.8)
Serum creatinine, μmol/L	520 (±273)	195 (±90)	416 (±152)	750 (±225)	632 (±182)
Endogenous creatinine clearance, mL/min	16 (±23)	50 (±16)	22 (±16)	8 (±5)	4 (±5)
24-h urine volume, L	1.6 (±1.3)	2.7 (±0.8)	2.4 (±1.4)	1.1 (±0.8)	0.7 (±0.8)
Proteinuria, g/24u	0.8 (±1.5)	0.7 (±0.5)	0.8 (±0.4)	0.7 (±0.4)	0.5 (±0.4)

Continuous variables are expressed as mean (± standard deviation).

[a]Defined on the basis of fDGF duration as <7, ≥7 to <14, ≥14 to <21, and≥21 for no, mild, moderate, and severe fDGF, respectively.

Abbreviations: CIT, cold ischemia time; CsA, cyclosporine A; CVA, cerebrovascular accident; DCD, donation after circulatory death; DGF, delayed graft function; fDGF, functional delayed graft function; KDRI, kidney donor risk index; PRA, panel reactive antibody; TFS, tubular function slope; WIT I, first warm ischemia time (time between clamping the aorta of the donor and cooling of the organ to 4°C); WIT II, second warm ischemia time (time during construction of vascular anastomoses and gradual heating of the organ, until removal of the aortic clamp and revascularization).

KIM-1, CXCL9, UMOD, FE-NGAL and FE-B2M. In all regression and ROC analyses, markers were divided by their SD to normalize their distributions.

Missing urine samples and measurements are reported in **Supplementary Table S3**. At PODs 4 and 10, 32 DCD kidney transplant recipients had complete data for ROC analyses. We conducted a complete case analysis to compare results with our main analyses. All analyses were performed using R version 4.0.3 (R Core Team, Vienna, Austria).

RESULTS

Cohort Characteristics

Urine and serum samples were available for 89 DCD recipients. The mean age of the recipients was 54 (±13) years and 62 (70%) were men. For 86 (96%) recipients, this was their first kidney transplant, with a mean dialysis vintage of 4.4 (±2.5) years. Only one recipient (1%) received a preemptive transplant. Of the 89 recipients, 20 (22%) had no fDGF (<7 days), 20 (22%) had mild fDGF (≥7–14 days), 26 (29%) had moderate fDGF (≥14 to <21 days), and 23 (26%) had severe fDGF (≥21 days). The mean age of donors was 46 (±15) years and 55% were men. Donors for recipients with fDGF (≥7 days) were older, more often men and had higher KDRI scores than those without fDGF. At POD 10, IF/TA was more often present, TFS and ECC were lower in those with fDGF, especially severe fDGF, compared to those without fDGF. Further donor, recipient and transplant characteristics are summarized in **Table 1**. Detailed causes of primary kidney disease are shown in **Supplementary Table S1**.

TABLE 2 | Risk of IF/TA according to KDRI or fDGF scores compared to the reference category in 89 DCD recipients.

Risk factor	IF/TA present at POD 10
	OR (95% CI)
KDRI score	
<1.0 (reference)	1
≥1.0 to 1.5	8.3 (1.7–40.9)
≥1.5	15.6 (2.8–86.8)
fDGF	
No (reference)	1
Yes	4.2 (0.9–20.1)
Mild and moderate	1.9 (0.4–10.0)
Severe	15.0 (2.7–84.8)

Abbreviations: DCD, donation after circulatory death; fDGF, functional delayed graft function; IF/TA, interstitial fibrosis/tubular atrophy; KDRI, kidney donor risk index; OR, odds ratio; POD, postoperative day; 95% CI, 95% confidence interval.

fDGF Duration: KDRI, Donor-Derived Fibrosis and 1-Year Renal Function

Using a KDRI of <1.0 as reference, scores of ≥1.0 to 1.5 and ≥1.5 were associated with an OR (95% CI) for POD 10 kidney biopsy IF/TA presence of 8.3 (1.7; 40.9) and 15.6 (2.8; 86.8), respectively. Compared to no fDGF, fDGF was associated with an OR of 4.2 (0.9; 20.1) for IF/TA presence at POD 10. For mild and moderate compared to no fDGF, the OR for IF/TA presence was 1.9 (0.4; 10.0), whereas this was 15.0 (2.7; 84.8) for severe fDGF (**Table 2**).

Longer duration of fDGF was clearly associated with a lower eGFR or ECC. The OR (95% CI) for ECC <40 vs. ≥ 40 mL/min at 6 weeks and 12 months for mild, moderate fDGF or severe fDGF compared to no fDGF were 5.2 (1.4; 19.3), 2.0 (0.5; 8.1) and 113.3 (10.8; 1,192.8), and 4.9 (0.6; 40.4), 3.5 (0.4; 30.5), and 9.5 (1.0; 89.0), respectively. For mild, moderate or severe compared to no fDGF, the RR (95% CI) for decrease in ECC in mL/min was −13.7 (−22.8; −4.7), −6.2 (−14.1; 1.8) at 6 weeks, and −30.4 (−39.6; −21.1), and −12.0 (−23.9; −0.1), −7.1 (−19.1; 4.9), and −24.2 (−38.7;−9.7) at 12 months, respectively (**Table 3**).

Kidney Injury Markers, Conventional Markers and TFS Over Time

Figure 2 shows median (IQR) levels at PODs 1, 4 and 10 of TFS, conventional markers (urinary creatinine and PCR), and the urinary creatinine-standardized kidney injury markers B2M and NGAL across all recipients and stratified by fDGF duration. Detailed levels of these markers along with urine volume, TIMP2, IGFBP7, KIM1, CXCL9, UMOD and FE-B2M and FE-NGAL are shown in **Supplementary Table S2** and **Supplementary Figure S1**. Pearson's correlation coefficients between standardized TFS and creatinine-corrected urinary markers at PODs 1, 4 and 10 are shown in **Supplementary Table S3** and **Supplementary Figure S2**. Correlations with TFS were generally poor or modest (as expected), with the strongest at POD 10 for urinary B2M and FE-B2M with coefficients of −0.53 (p = 0.00) and −0.56 (p = 0.00), respectively.

TABLE 3 | Among 89 recipients, the odds and risk ratios (95% CI) for the relation between duration of fDGF and endogenous creatinine clearance at 6 weeks and 12 months, calculated using logistic and linear regression analysis.

fDGF	OR (95% CI)
	ECC <40 vs. ≥ 40 mL/min at week 6
No (*reference*)	1
Yes	5 (1–19)
Mild and moderate	2 (1–8)
Severe	113 (11–1,193)
	ECC <40 vs. ≥ 40 mL/min at month 12
No (*reference*)	1
Yes	5 (1–40)
Mild and moderate	4 (0–31)
Severe	10 (1–90)
	RR (95% CI)
	ECC in mL/min at week 6
No (*reference*)	1
Yes	−14 (−24 to −5)
Mild and moderate	−6 (−14 to 2)
Severe	−30 (−40 to −21)
	ECC in mL/min at month 12
fDGF	
No (*reference*)	1
Yes	−12 (−24 to −0)
Mild and moderate	−7 (−19 to 5)
Severe	−24 (−39 to −10)

Abbreviations: ECC, endogenous creatinine clearance; fDGF, functional delayed graft function; OR, odds ratio; RR, risk ratio; 95% CI, 95% confidence interval.

Kidney Injury Markers Compared to TFS for Prediction of fDGF

At POD 1, AUCs (95% CI) for predicting the presence of fDGF (yes vs. no) were 0.90 (0.81; 0.99), 0.73 (0.60; 0.86), 0.77 (0.62; 0.92), 0.68 (0.53; 0.82), 0.55 (0.40; 0.71), 0.89 (0.76; 1.00), and 0.81 (0.68; 0.93), for TFS, creatinine, PCR, B2M, FE-B2M, NGAL and FE-NGAL, respectively. At POD 4, NGAL and FE-NGAL outperformed TFS with AUCs of 0.97 (0.90; 1.00) and 0.98 (0.93; 1.00), respectively, compared to 0.92 (0.86; 0.98) for TFS (**Figure 3**; **Table 4**; **Supplementary Figure S3**). At POD 10, FE-NGAL, PCR and NGAL/TIMP2 performed best for severe vs. mild to moderate fDGF prediction, as AUCs were 0.74 (0.55; 0.93), 0.76 (0.58; 0.94) and 0.72 (0.53; 0.91), respectively, compared to 0.65 (0.48; 0.82) for TFS (**Table 4**; **Supplementary Figure S3**). A complete case analysis including 32 DCD kidney transplant recipients yielded similar results (**Supplementary Tables S4, S5**).

DISCUSSION

NGAL and FE-NGAL, measured at PODs 1, 4 and 10 outperformed TFS in predicting fDGF presence and severity in 89 DCD kidney transplantation recipients. fDGF severity was strongly related to IF/TA presence in POD 10 kidney biopsies and lower kidney function at 6 weeks and 12 months after transplantation. Daily monitoring of urinary

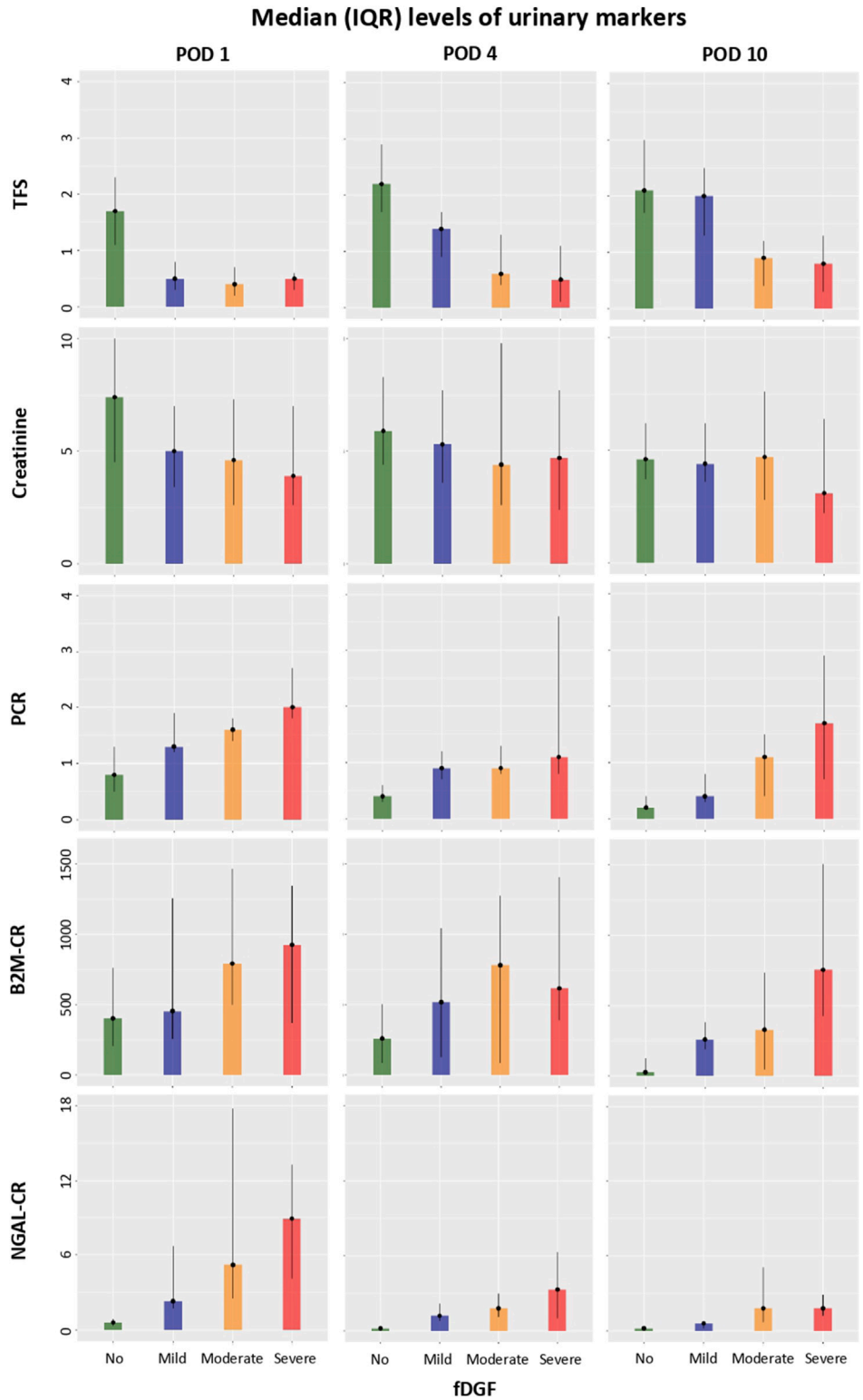


FIGURE 2 | Among 89 recipients, and stratified by fDGF duration at PODs 1, 4 and 10 after DCD kidney transplantation, the median (IQR) levels of TFS and creatinine-corrected urinary markers. *Defined based on fDGF duration as <7, ≥7 to <14, ≥14 to <21, ≥21 for no, mild, moderate and severe fDGF, respectively. Abbreviations: B2M, beta-2 microglobulin; CR, creatinine ratio; DCD, donation after circulatory death; fDGF, functional delayed graft function; NGAL, neutrophil gelatinase-associated lipocalin; PCR, protein to creatinine ratio; POD, postoperative day; TFS, tubular function slope; u, urinary.

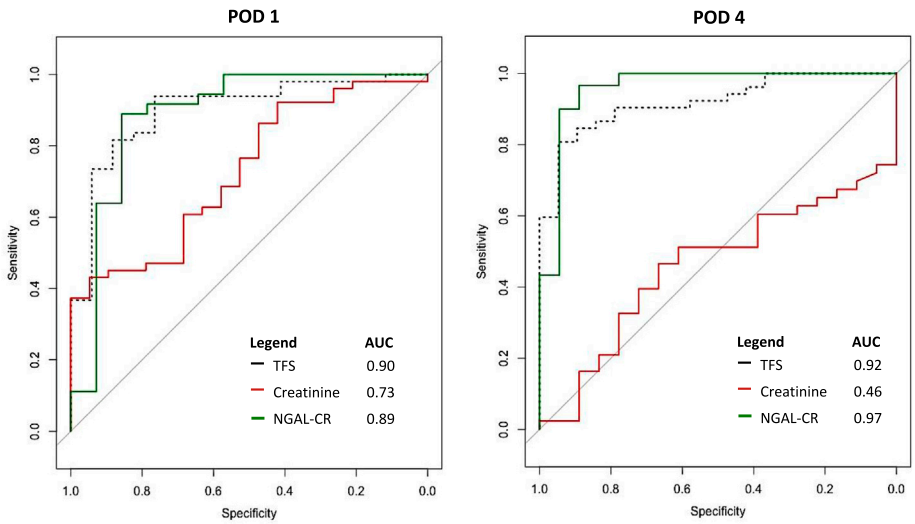


FIGURE 3 | ROC analysis for the prediction of fDGF presence (yes vs. no) by standardized TFS and standardized creatinine-corrected urinary NGAL at PODs 1 and 4 after DCD kidney transplantation. Abbreviations: AUC, area under the curve; CR, creatinine ratio; DCD, donation after circulatory death; fDGF, functional delayed graft function; NGAL, neutrophil gelatinase-associated lipocalin; POD, postoperative day; TFS, tubular function slope.

TABLE 4 | Area under the curve (95% CI) from ROC analysis for the prediction of fDGF presence at PODs 1 and 4, and fDGF severity at POD 10 by standardized TFS and standardized creatinine-corrected urinary markers at PODs 1, 4 and 10 after DCD kidney transplantation.

Urinary marker divided by SD	AUC (95% CI)		
	fDGF		Severe fDGF
	POD 1	POD 4	POD 10
TFS	0.90 (0.81–0.99)	0.92 (0.86–0.98)	0.65 (0.48–0.82)
Creatinine	0.73 (0.60–0.86)	0.46 (0.31–0.61)	0.65 (0.43–0.89)
P-CR	0.77 (0.62–0.92)	0.79 (0.65–0.94)	0.76 (0.58–0.94)
NGAL-CR	0.89 (0.76–1.00)	0.97 (0.90–1.00)	0.65 (0.30–1.00)
FE-NGAL	0.81 (0.68–0.93)	0.98 (0.93–1.00)	0.74 (0.55–0.93)
B2M-CR	0.68 (0.53–0.82)	0.67 (0.53–0.80)	0.69 (0.44–0.95)
FE-B2M	0.55 (0.40–0.71)	0.64 (0.50–0.78)	0.68 (0.43–0.94)
TIMP2-CR	0.87 (0.76–0.98)	0.88 (0.79–0.97)	0.69 (0.31–1.00)
IGFBP7-CR	0.81 (0.69–0.94)	0.77 (0.63–0.91)	0.52 (0.13–0.91)
KIM1-CR	0.51 (0.34–0.69)	0.45 (0.29–0.61)	0.64 (0.18–1.00)
CXCL9-CR	0.78 (0.62–0.94)	0.72 (0.53–0.91)	0.65 (0.32–0.97)
UMOD-CR	0.78 (0.66–0.90)	0.62 (0.47–0.78)	0.73 (0.38–1.00)

Abbreviations: AUC, area under the curve; B2M, beta-2 microglobulin; CR, creatinine ratio; DCD, donation after circulatory death; ECC, endogenous creatinine clearance; fDGF, functional delayed graft function; FE, fractional excretion; NGAL, neutrophil gelatinase-associated lipocalin; P-CR, protein to creatinine ratio; POD, postoperative day; TIMP2, tissue inhibitor of metalloproteinases-2; TFS, tubular function slope.

NGAL or FE-NGAL in the first days after kidney transplantation may provide an alternative to sequential ^{99m}TcMAG3 renography to follow PTEC function recovery and fDGF resolution.

DGF is associated with acute rejection and has an adverse impact on longer-term kidney function and patient outcomes [8, 22, 23]. Indeed, in our study the severity of fDGF was strongly associated with the presence of (donor-derived) IF/TA in the kidney biopsies, observed in 68% of those with fDGF ≥21 days compared to only 33% for the entire cohort. Distinguishing early acute rejection from a primarily insufficient renal mass remains challenging in the first weeks after kidney transplantation [9]. This is especially true in DCD kidney recipients in whom severe IRI is higher than in those receiving kidneys from living or DBD donors [1, 2]. We used the TFS as the gold standard to assess the severity of kidney injury. The TFS quantifies the tubular extraction rate of ^{99m}Tc-MAG3 through the OAT, providing insight into the overall quality and functional recovery of PTECs. Although proven accurate and effective in identifying DGF,

^{99m}Tc-MAG3 renography remains invasive, time-consuming and costly, rendering sequential TFS less appealing for routine clinical use. Our results indicate that, among both conventional and novel urinary biomarkers, FE-NGAL in particular has the potential to replace TFS allowing daily monitoring of IRI resolution.

The urinary biomarkers TIMP-2, IGFBP7, KIM-1, CXCL9, UMOD, NGAL and B2M may reflect different aspects of renal pathophysiology, although research is ongoing. For example, TIMP-2 and IGFBP7 are thought to act as markers of cellular stress and G1 cell cycle arrest, aiding in the early detection of AKI [24–26]. KIM-1 is a marker of kidney injury, primarily expressed in damaged proximal tubular cells [24, 27, 28]. CXCL9 is associated with the immune response and renal inflammation. UMOD, on the other hand, is the most abundant protein in normal urine and plays a role in kidney function and urinary tract maintenance [24].

Urinary B2M and NGAL have been well-researched as markers of proximal tubular dysfunction. B2M binds to major histocompatibility complex I (MHC-I)/human leukocyte antigen I (HLA-I) on nucleated cells [29]. NGAL is synthesized by epithelial tissues, including distal tubular epithelial cells [29]. In normal kidneys, B2M and NGAL undergo unhindered glomerular filtration and are almost entirely reabsorbed by PTECs [27, 29, 30]. After any surgical procedure that causes damage to the epithelial tissue, both plasma B2M and NGAL will increase. Following PTEC injury, reabsorption of B2M and NGAL is impaired, increasing urinary excretion. Since B2M and NGAL are normally reabsorbed via the apical PTEC membrane, and ^{99m}Tc-MAG$_3$ (used to determine the TFS) at the basolateral membrane, we hypothesized that these markers specifically would be equally accurate in the prediction of fDGF. However, since B2M (as compared to NGAL) is more abundantly present in tissues, blood levels of B2M will increase more than NGAL following surgery. The subsequent high fractional urinary excretion of B2M, independent of PTEC function, disrupts the interpretability of urinary B2M as a marker of PTEC injury. Indeed, we found that compared to TFS, NGAL-CR or FE-NGAL were stronger predictors than B2M-CR for the presence of fDGF at POD 1 and POD 4, and severe fDGF at POD 10. Thus, NGAL-CR and FE-NGAL may provide an alternative to ^{99m}TcMAG3 renography for following fDGF resolution and anticipating poorer long-term kidney function.

Sequential measurements of NGAL-CR or FE-NGAL in the first days following kidney transplantation can be used to monitor the recovery of PTEC function. Through this, transplant recipients prone to experience severe fDGF may be identified early on, as NGAL will not yet be reabsorbed due to PTEC dysfunction. A decrease in urinary NGAL will indicate restoration of PTEC functionality (**Figure 4**). In our DCD transplant recipient population, (FE-)NGAL levels (corrected for urinary creatinine and the population standard deviation) on separate days already predicted the presence and severity of fDGF when compared to the TFS as the gold standard. Sequential monitoring of NGAL will be more informative, especially considering the fluctuating nature and wide interpatient variation of NGAL. Of course, future

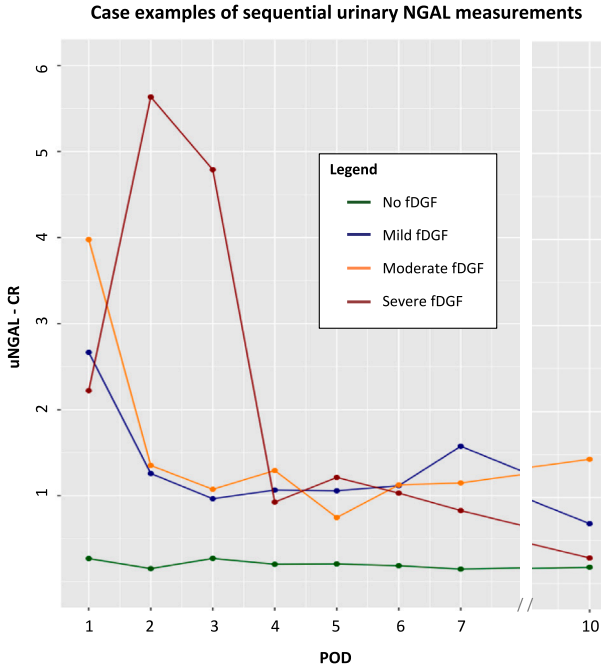


FIGURE 4 | Case examples of urinary (creatinine-corrected) NGAL measurements among 4 representative DCD kidney transplantation recipients with no, mild, moderate or severe fDGF*. *Defined based on fDGF duration as <7, ≥7 to <14, ≥14 to <21, ≥21 days for no, mild, moderate and severe fDGF, respectively. Abbreviations: CR, creatinine ratio; DCD, donation after circulatory death; fDGF, functional delayed graft function; NGAL, neutrophil gelatinase-associated lipocalin; POD, postoperative day; u, urinary.

research in larger populations is needed to determine reference levels and to interpret when a relative increase or decrease in NGAL would be clinically relevant. Furthermore, NGAL levels should always be interpreted in the context of other clinical characteristics such as diuretic volume. Decreasing urinary NGAL levels, indicating recovery of PTEC function, could guide the indication for or timing of kidney transplant biopsy to exclude the occurrence of another acute rejection episode and subsequent treatment. Therefore, in the future, NGAL testing may also improve long-term outcomes.

The current body of evidence is, however, insufficient to support large-scale implementation of routine NGAL measurement. Furthermore, sequential NGAL measurement could also be beneficial for other indications other than DGF resolution monitoring, such as early AKI monitoring, for instance during treatment with potentially nephrotoxic chemotherapeutics. If sequential NGAL testing is validated in future research as reliable for monitoring DGF resolution or timely diagnosis of early AKI, it could guide timely intervention and thereby significantly reduce costs by preventing severe complications [31, 32]. Finally, with the development of easier analytical techniques, such as our in-house developed multiplex liquid chromatography tandem mass spectrometry (LC-MS/MS) test, biomarker analysis costs will be reduced [15, 16].

There are several strengths to our study. First, we are comparing urinary markers to the TFS

(^{99m}TcMAG3 renography) shortly after DCD kidney transplantation for the first time. Since recipients of DCD kidneys are at higher risk for severe IRI, adequate monitoring of kidney injury is especially important in this group. Second, by using an in-house developed multiplex LC-MS/MS test, we were able to efficiently and reliably assess urinary levels of nine injury markers simultaneously. Third, by comparing previously proposed markers of kidney injury in recipients of DCD kidneys at high risk of severe IRI, we aimed to perform a hypothesis-generating study that may focus future research efforts. Fourth, there was no selection of participants in the PROTECT study since all consecutive patients scheduled to receive a DCD kidney transplant were approached to participate. This included expanded criteria donors (ECD) and kidneys allocated via the Eurotransplant Senior Program (ESP). Fifth, organ procurement techniques have significantly improved since the time of the PROTECT study (2005–2009). Nowadays, procurement techniques such as SR and NRP reduce the severity of IRI in DCD kidney transplantation. Consequently, with more heterogeneity in IRI severity among current DCD kidney recipients, the application of these biomarkers (after external validation) for early fDGF prediction may become especially relevant. Our results should ideally be validated in representative cohorts that include these different options in procurement and allocation strategies.

Nevertheless, our study has some limitations. First, this was a single-center study, which may limit the generalizability of our results to other centers or countries. However, our experience with DCD kidney transplantation allowed us to use this relatively large cohort to investigate the added value and patterns of these biomarkers in DCD kidney recipients. Second, we found a high percentage of IF/TA in the kidney transplant biopsies at POD 10. This is to be expected considering that these were DCD kidney transplant recipients who were transplanted between August 2005 and December 2009. The consecutive DCD kidneys offered included expanded criteria donors (ECD) and kidneys allocated via the Eurotransplant Senior Program (ESP). The presence of IF/TA was strongly related to the KDRI score, which is largely driven by donor age. Hence, IF/TA presence will mainly be donor-derived. The high percentage of IF/TA in these DCD kidney transplants will have affected the levels of urinary biomarkers. Hence, these results should not be generalized to cohorts other than DCD kidney transplant recipients. Furthermore, as transplantation techniques have improved since 2009, our results should ideally be validated in representative cohorts that include these different options in procurement and allocation strategies. Third, no data exist on whether the high-dose EPO administered as an intervention to a part of the study population may have affected the validity of our biomarker analyses. However, as there was no difference in the incidence or duration of DGF and/or primary nonfunction in patients treated with high-dose EPO versus placebo, we do not expect EPO administration to have affected our biomarker results. Fourth, due to the limited sample size and missing data, this study may have been underpowered to some extent, particularly to detect associations between urinary biomarker levels and concurrent kidney function and kidney function decline. This will, however, not have been inferred from our descriptive analysis assessing the patterns of these urinary biomarkers and their correlation with TFS. Third, part of the missing urine samples will be due to anuria. However, since anuria itself indicates DGF, it will be less relevant to measure biomarker levels in anuric patients. Finally, all urine samples included in our analysis were stored for 10–15 years and underwent one to two freeze-thaw cycles. However, it is important to note that LC-MS/MS tests are relatively insensitive to freezing and thawing of samples.

In conclusion, we found that NGAL and FE-NGAL, measured on PODs 1, 4 or 10 after DCD kidney transplantation in 89 recipients, performed better than the TFS in predicting the presence and severity of fDGF. fDGF severity was strongly related to the KDRI, the presence of donor-derived fibrosis in day-10 protocol kidney biopsies, and resulted in inferior kidney graft function 12 months after kidney transplantation. Daily urinary NGAL and FE-NGAL monitoring in the first days after kidney transplantation may provide an alternative to sequential ^{99m}TcMAG3 renography to follow PTEC function recovery and fDGF resolution, and may guide the timing of a kidney biopsy to exclude the occurrence of an additional acute rejection episode.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because as our data could be used to identify individuals, privacy concerns prevent us from allowing them to be publically available. Nonetheless, we are open to make our data available for collaboration conditional on agreement on privacy matters and appropriate usage of the data. For this, please contact the corresponding author. Requests to access the datasets should be directed to ER, e.n.m.rooij@lumc.nl.

ETHICS STATEMENT

The studies involving humans were approved by Ethical Committee of the Leiden University Medical Center (NCT00157300). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ER conceptualized the study design, performed the data analysis and participated in the writing of the paper; TD developed the in-house LC-MS/MS test, conducted the urinary biomarker analyses and reviewed the paper; EH conceptualized the study design, supervised the data analysis and participated in the writing and review of the paper; FR conducted the serum and urinary analyses and reviewed the paper; FD reviewed the paper; CK participated

in the original PROTECT study data collection and reviewed the paper; CC supervised the urinary biomarker analyses and reviewed the paper; JF participated in the original PROTECT study data collection, conceptualized the study design and reviewed the paper. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.13818/full#supplementary-material

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ORIGINAL RESEARCH
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En-Bloc Kidney Transplantation From Extremely Low-Weight (0.9–5.0 kg) Pediatric Donors: A Decade of Single-Center Experience

*Xianpeng Zeng[1], Qiuxiang Xia[1], Heng Li[1], Miao Wang[1], Hanying Li[1], Liang He[2], Hua Su[3], Chun Zhang[3] and Zhendi Wang[1]**

[1]Department of Urology, Huazhong University of Science and Technology Union Hospital, Wuhan, China, [2]Department of Hepatobiliary Surgery, Huazhong University of Science and Technology Union Hospital, Wuhan, China, [3]Department of Nephrology, Huazhong University of Science and Technology Union Hospital, Wuhan, China

En-bloc kidney transplantation from low-weight pediatric donors (≤5 kg) is a challenging procedure performed only in limited transplant centers. We retrospectively analyzed the data from 42 *en-bloc* kidney transplants from donors weighing less than 5 kg between September 2014 and September 2023. The mean donor body weight was found to be 3.1 ± 1.0 kg, and the minimum weight was 0.9 kg. At a mean follow-up period of 1,481 days, the graft survival rate was 76.2% and the recipient survival rate was 100.0%. Thrombosis and acute rejection were the major complications responsible for the short-term graft loss. Male recipients were more likely to experience graft loss than female ones ($P < 0.05$). Recipients with long-term (>1 year) graft survival were observed to have a high prevalence (31.3%) of delayed graft function. However, they still had satisfactory long-term graft function and limited proteinuria. Continuous graft volume growth took more than 1 year to reach a stable level. Lower donor/recipient body surface area may lead to higher delayed graft function and slower estimated glomerular filtration rate recovery ($P < 0.05$). Kidney transplant from low-weight pediatric donors is associated with a high incidence of short-term graft loss, while long-term outcomes are generally acceptable.

Keywords: ***en-bloc*, graft failure, kidney transplantation, thrombosis, acute rejection**

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***Correspondence**
Zhendi Wang,
zhendi_wang@163.com

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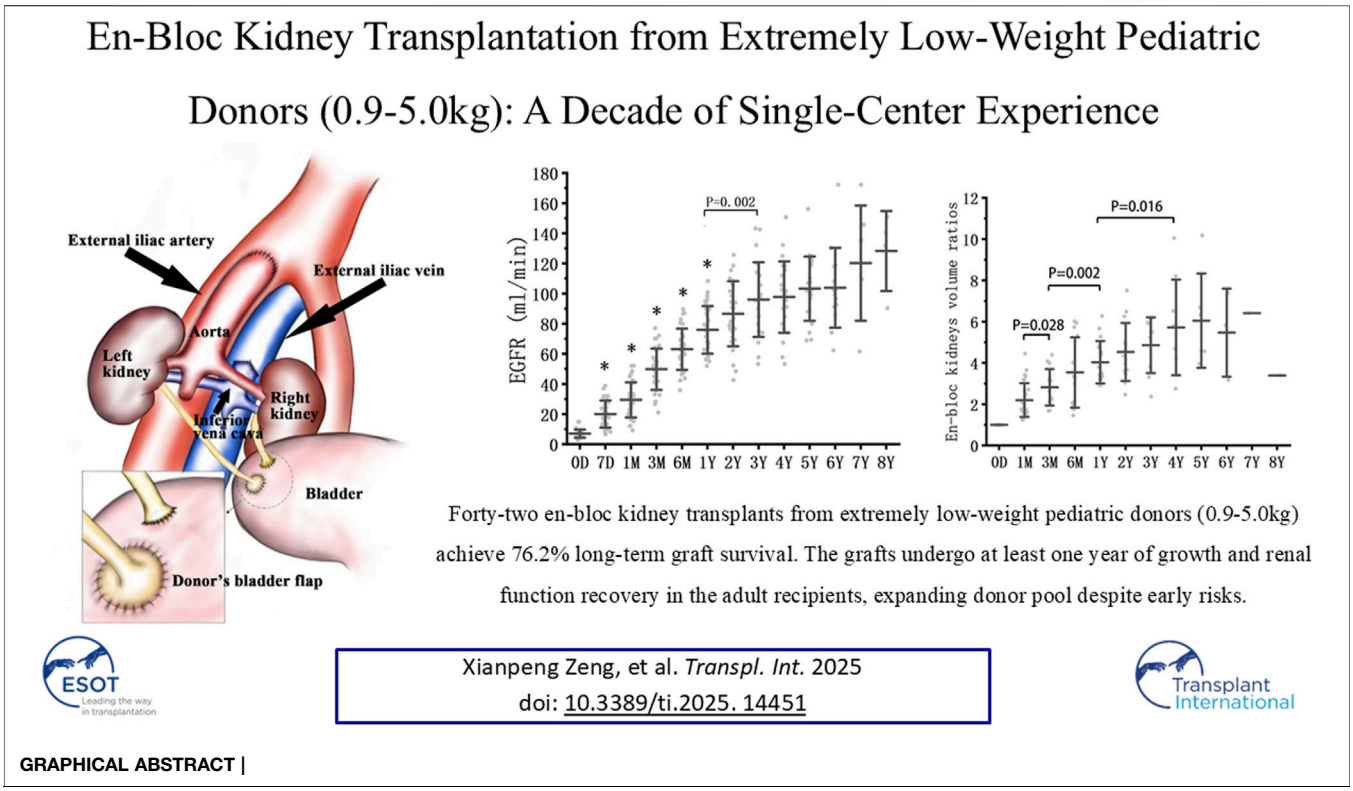
INTRODUCTION

Pediatric *en-bloc* kidney transplantation (KTx) has been a topic of interest in the medical community for over 50 years due to its potential to alleviate the shortage of donor kidneys [1, 2]. However, due to high surgical technical requirements, along with concerns about early graft loss and hyperfiltration injury, the majority of transplant centers are still reluctant to perform it. Furthermore, recipient eligibility criteria are also a subject of debate. As a result, reports of pediatric KTx with the weight of less than 5 kg (especially <2.5 kg) are very limited, although they are considered a promising source [3]. Here, we conducted a retrospective single-center study to summarize the 42 cases of *en-bloc* KTx from pediatric donors weighing between 0.9 and 5.0 kg, and analyzed the risk factors for complications. Our study aimed to provide better clinical decisions and optimal outcomes using extremely low-weight donors for *en-bloc* KTx.



GRAPHICAL ABSTRACT |

MATERIALS AND METHODS

This study included a total of 42 *en-bloc* KTx cases performed between September 2014 and September 2023. Professional human organ donation coordinators obtained written parental consent for the donation. Kidney grafts were donated by the Red Cross Society and allocated to our center by the China Organ Transplant Response System. The procedures complied with the national program of organ donation in China, the Declaration of Helsinki and the Declaration of Istanbul. This study was approved by the institutional review board of Huazhong University of Science and Technology Union Hospital (UHCT230124, **Supplementary Material S1**).

Donor and Recipient Selection

All pediatric donors weighing between 0.9 and 5.0 kg conformed to the national protocol for donation after circulatory or brain death. Organ procurement was approved by the Ethics Committee of Huazhong University of Science and Technology Union Hospital. In addition to the exclusion criteria for conventional KTx, the recipients of pediatric *en-bloc* KTx were excluded if they were: (1) patients with uncontrolled hypertension or diabetes, a history of coronary heart disease or peripheral vascular disease, a hypercoagulable state, or urinary tract abnormalities; (2) patients with panel reactive antibodies >10%, a positive lymphocytotoxicity test, secondary transplantation, or lupus erythematosus; or (3) patients with a poor compliance history [4]. Recipients with low body weight were preferred. Recipients and their relatives were informed in detail about the advantages and disadvantages of *en-bloc* KTx.

Organ Procurement

None of the livers were procured for transplantation due to the small size of the donors. A 9F or 12F sterile silicone tube without a balloon was inserted at the distal end of the abdominal aorta, with one end opening and one side opening preserved. Tube insertion depth was less than 1 cm to ensure that the cold histidine-tryptophan-ketoglutarate (HTK) solution flushing began below the level of the renal arteries. The right atrium was cut to establish the outflow to make the surgical field bloodless. The kidney surface was cooled with ice, and dissection was not started until 500 mL of HTK was perfused. Perirenal fat was kept as much as possible, and both ureters were harvested with the bladder. The inferior vena cava was dissected to the retrohepatic section and transected 1 cm above the level of the left renal vein.

Back-Table Preparation

Except for the renal vessels, all other aortic branches, the gonadal vein, and the tributaries of the vena cava were ligated. The tissues surrounding the renal arteries were completely preserved without exposing the arterial trunks. The bilateral adrenals were removed, and all vessels and tissue bundles were ligated away from the renal vessels. The anterior wall of the vena cava was cut open longitudinally from the proximal end. The distal aortic end was clamped, and the proximal aorta was perfused with cold HTK solution until the venous outflow fluid was clear of blood (**Figures 1A-C**).

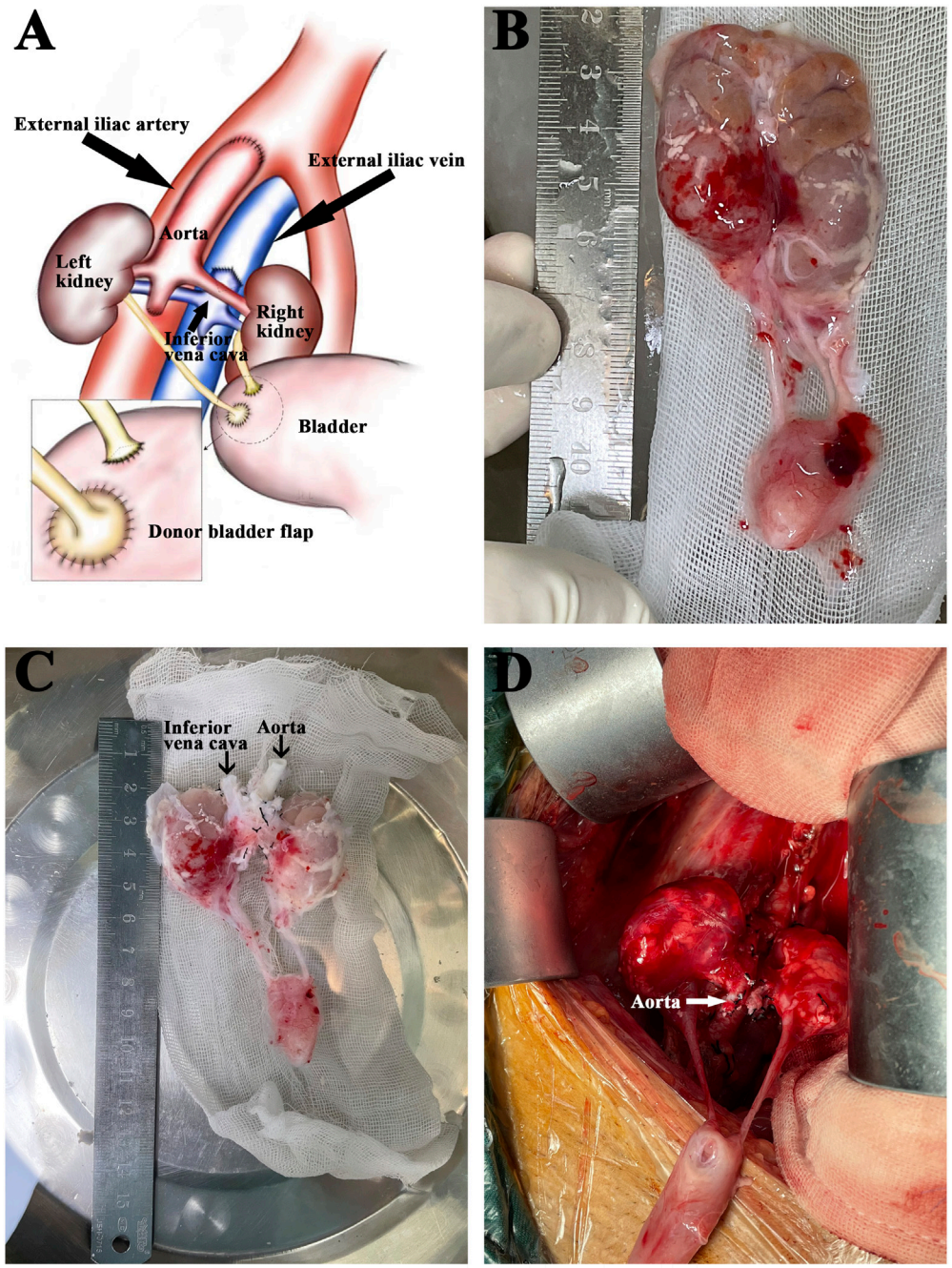


FIGURE 1 | Back-table graft preparation and kidney implantation. **(A)** Diagram of the *en-bloc* renal grafts transplanted into the recipient's right iliac. The external iliac artery was anastomosed to the donor aorta. The external iliac vein was anastomosed to the donor inferior vena cava. The recipient's bladder received separate anastomoses of the donor's short right ureter and the left ureter with a tiny bladder flap. **(B)** The explanted *en-bloc* renal grafts from a donor weighing 0.9 kg. **(C)** The same grafts after back-table preparation. **(D)** The same grafts after reperfusion.

Kidney Implantation

Laterally reversed kidneys were placed extraperitoneally in the recipient's right iliac fossa with end-to-side anastomosis to the external iliac vasculature. The proximal aorta and the V-shaped proximal vena cava of the donors were chosen for anastomosis (**Figure 1D**). After reperfusion, the Lich-Gregoir technique was used for implanting the internal ureter into the recipient bladder. The distal end of the ureter was transected appropriately. The external kidney was higher, so the external ureteroneocystostomy referred to an anastomosis between the donor small bladder patch

and the recipient bladder. A 3F ureteral stent was placed in each ureter, with the exception of two recipients, where the stent could not be accommodated due to a thin ureteral lumen. The perirenal fat of the external kidney was fixed to the posterior muscle with one stitch before closing the incision.

Immunosuppressive Regimen

All recipients received 1 mg/kg of rabbit anti-human thymocyte immunoglobulin (ATG) on day 0, followed by the same dosage on day 1 and half the dosage on day 2, for a cumulative dose of 2.5 mg/kg. Triple immunosuppressive therapy with tacrolimus (Tac), mycophenolate mofetil (MMF) and corticosteroids was maintained, with target trough levels for Tac. MMF doses were tapered from 1.5 g/d to 1.0 g/d at 1 month and beyond. Methylprednisolone was administered intravenously at 500 mg daily on days 0, 1, and 2, followed by oral prednisone tapering.

Perioperative Management

No intraoperative vasodilators were routinely administered. Low molecular weight heparin (LMWH, 2000–8000 μ/day, 5–7 days) was administered subcutaneously to the first 19 subjects but not to the remaining 23 recipients. No oral antiplatelet therapy was used. During the first 14 days following KTx, the systolic blood pressure of the recipients was maintained under 140 mmHg. Graft morphology and blood flow were examined by color doppler periodically.

Statistical Analysis

SPSS version 22 and Origin version 9 were used for statistical analyses. Mean and standard deviation were used for count data following a normal distribution, while the median was used for count data following a non-normal distribution. The Kaplan-Meier survival curve was used to evaluate graft survival. Binary logistic regression analysis was used to determine the hazard ratio of variables associated with graft loss and thrombosis. T-tests, analysis of variance and non-parametric tests were used to compare count data and $P < 0.05$ was considered statistically significant. The chi-square test and non-parametric test were used to compare the measurement data and $P < 0.05$ was considered statistically significant.

RESULTS

Donor and Recipient Profiles

In the 42 cases of KTx, the baseline characteristics of donors and recipients are summarized in **Table 1**, with the minimum donor weight being 0.9 kg. Causes of death included hypoxic ischemic encephalopathy, cerebrovascular malformation, trauma, and cerebral hemispheric cyst with gliosis. There were 41 donation after circulatory death (DCD) donors and 1 donation after brain death donor, with no anencephalic donors included. All donor-recipient lymphocytotoxicity tests yielded negative results. In conjunction with previous clinical data, among the 42 recipients' primary kidney diseases, 41 cases were glomerulonephritis without biopsy confirmation, and 1 case was IgA nephropathy. The mean follow-up time was 1,481 days (1–3,150 days). The recipient survival rate was 100%, the 1-year graft survival rate was 76.2%, and no further graft failure occurred after 1 year (**Figure 2A**).

Graft Function and Growth

The estimated glomerular filtration rate (eGFR) of long-term survival recipients increased rapidly within 1 year after KTx, reaching 63.0 ± 13.7 mL/min at 6 months, vs. 75.7 ± 16.0 mL/min at 1 year ($P = 0.001$ vs. eGFR at 6 months), and continued to increase to 93.2 ± 22.8 mL/min at 3 years ($P = 0.002$ vs. eGFR at 1 year) (**Figure 2B**).

The volume of transplanted kidneys was calculated according to the Mitrou method [5]. The *en-bloc* grafts doubled in volume within the first 3 months and could reach 3-4 times the volume at 1-2 years, after which the kidney volume reached a stable level (**Figure 2C**).

Graft Loss

Postoperative loss of the *en-bloc* grafts was due to arteriovenous thrombosis (6/10), acute rejection (3/10) and primary graft nonfunction (PNF) (1/10). When comparing the graft loss group with the graft survival group, baseline values were consistent, except for recipient gender (**Table 2**). On multivariate analysis, recipient age, donor gender, cold ischemia time (CIT) and donor/recipient body surface area (D/R BSA) ratio were not the risk factors for *en-bloc* graft loss. However, the odds ratio in female recipients compared to male recipients was 0.161 ($P = 0.036$; 95% CI, 0.029–0.884, **Supplementary Material S2**).

Of the 6 recipients with thrombosis, 2 had venous thrombosis within 24 h, and 4 had arterial thrombosis between 1 and 10 days. Donor or recipient gender, age, D/R BSA, and absence of perioperative LWMH were not risk factors for thrombosis (**Supplementary Material S3**). Two patients with venous thrombosis experienced inadequate expansion of the venous anastomotic site following reperfusion. Despite maintaining unobstructed blood flow during the procedure, the grafts were ultimately lost within 24 h, presenting with oliguria and hematuria. The onset of arterial thrombosis is characterized by anuria, pain in the graft region or lower abdomen, and elevated blood pressure. These symptoms are analogous to arterial thrombosis in KTx from adult donors. Consequently, even in the absence of pathologic confirmation, we attributed graft loss to arterial thrombosis in four recipients.

The acute rejection episodes leading to graft loss occurred at 1–3 weeks after KTx. All three recipients did not respond to steroid and post-ATG treatment. Steroid pulse therapy was initiated immediately upon suspicion of rejection, and acute rejection was subsequently confirmed via graft biopsy. Notably, a remarkably low trough concentration of Tac was consistently observed prior to the onset of rejection.

In the case of PNF, the DCD donor succumbed to hypoxic ischemic encephalopathy. Prior to organ procurement, the donor had undergone multiple cardiopulmonary resuscitations with a warm ischemia time (WIT) of 20 min. Following transplantation, the recipient developed persistent anuria and subsequently underwent bilateral nephrectomy 3 months post-KTx.

TABLE 1 | Profiles of donors and recipients for *en-bloc* kidney transplantation.

	Donors	Recipients
Age (mean)	29.4 days (4–120 days)	27.9 years (11–52 years)
Weight (mean, kg)	3.1 (0.9–5.0)	47.5 (25–64)
Gender		
Male recipients	27	16
Female recipients	15	26
Cause of death	23 hypoxic ischemic encephalopathy 16 cerebrovascular malformation 2 trauma 1 cerebral hemisphere cysticization with gliosis	
WIT (mean, min)	10.4 (6–30)	
CIT (mean, h)	11.1 (6–16)	
Primary renal disease	41 glomerulonephritis not proven by biopsy 1 IgA nephropathy	
One year graft survival		76.2%
One year recipient survival		100%

WIT, warm ischemia time; CIT, cold ischemia time.

Other Complications

Two recipients experienced arterial stenosis after KTx. One showed a decrease in eGFR in the affected kidney 18 months after KTx, and thus underwent percutaneous transluminal angioplasty and internal stent implantation, which led to a full recovery of eGFR. The other patient, who presented with hypertension in addition to stable eGFR, had satisfactory blood pressure control after taking oral antihypertensive medicines. We found urinary leakage in 11.9% of cases, which was primarily self-limiting. One case of long-segment ureteral necrosis was treated with arterial embolization of the affected external kidney, with concern for potential surgical injury risks to the blood vessels and ureter of the internal normal kidney. Two recipients on LMWH administration underwent a second surgery for hematoma removal after KTx.

Proteinuria

Among the 32 recipients with long-term graft survival, proteinuria was observed in 47.1% (8/17) at 1 month, 28.1% (9/32) at 3 months, 25.0% (8/32) at 6 months and 1 year, and 20.7% (6/29) at 2 years. In order to minimize the incidence of growth disorders and nephrotoxicity, all recipients except for two patients with severe proteinuria who received treatment were not administered any medication to reduce proteinuria within 2 years of transplantation. Despite the less rigorous follow-up in this regard, the number of recipients with urine protein levels exceeding 0.2 g/L gradually decreased (**Figure 3**).

Delayed Graft Function

Delayed graft function (DGF) refers to the cases where dialysis is required within the first week post-KTx. Of the 32 recipients with long-term graft survival, DGF occurred in 31.3%. The DGF group had significantly lower D/R BSA compared with the non-DGF group (**Supplementary Material S4**). EGFR in the DGF group was significantly lower at 7 days post-KTx ($P = 0.012$), but not at later time points. Furthermore, the volume of transplanted kidneys in the DGF group showed a faster increase compared to the non-DGF group in the first month ($P = 0.029$) (**Figure 4**).

Donors <2.5 kg vs. Donors >=2.5 kg

There were 12 recipients transplanted from donors weighing less than 2.5 kg. Compared with the other 30 cases in which the donor weight was between 2.5 and 5.0 kg, the general profiles of the two groups were similar except for the significant differences in donor weight and D/R BSA. There were no significant differences in the incidence of DGF, thrombosis, urine leakage, perirenal hematoma, acute rejection, and graft survival between the two groups (**Table 3**; **Figure 5A**). EGFR was significantly lower in donors weighing less than 2.5 kg at 3 months, but not at later time points. Moreover, the volume of transplanted kidneys showed a faster increase in the same group in the first month, while the difference gradually decreased with prolonged follow-up (**Figures 5B,C**).

DISCUSSION

To our knowledge, the 42 *en-bloc* KTx cases in this retrospective study not only include one with the lowest donor body weight (0.9 kg), but also offer an opportunity to look deep into KTx from the lowest average donor body weight ever reported [6]. With a concerning 23.8% *en-bloc* graft failure rate in donors weighing less than 5 kg, thrombosis and acute rejection are the leading causes of short-term graft loss. However, the remaining 76.2% of cases have satisfactory long-term outcomes. This study suggests that the selection of female recipients and adequate immunosuppressive exposure could potentially further reduce short-term graft loss. DGF does not affect graft survival and long-term graft function in *en-bloc* KTx from low-weight pediatric donors. Lower D/R BSA, which means greater size disparity between donor and recipient, may lead to a higher probability of receiving postoperative dialysis and slower eGFR recovery.

The small kidneys have demonstrated remarkable recovery and growth potential, which is the basis for our clinical application of the proposed procedure [7]. In terms of growth rate, small allografts showed a rapid development during the first 1–3 months, with an approximately 2-fold volume increase

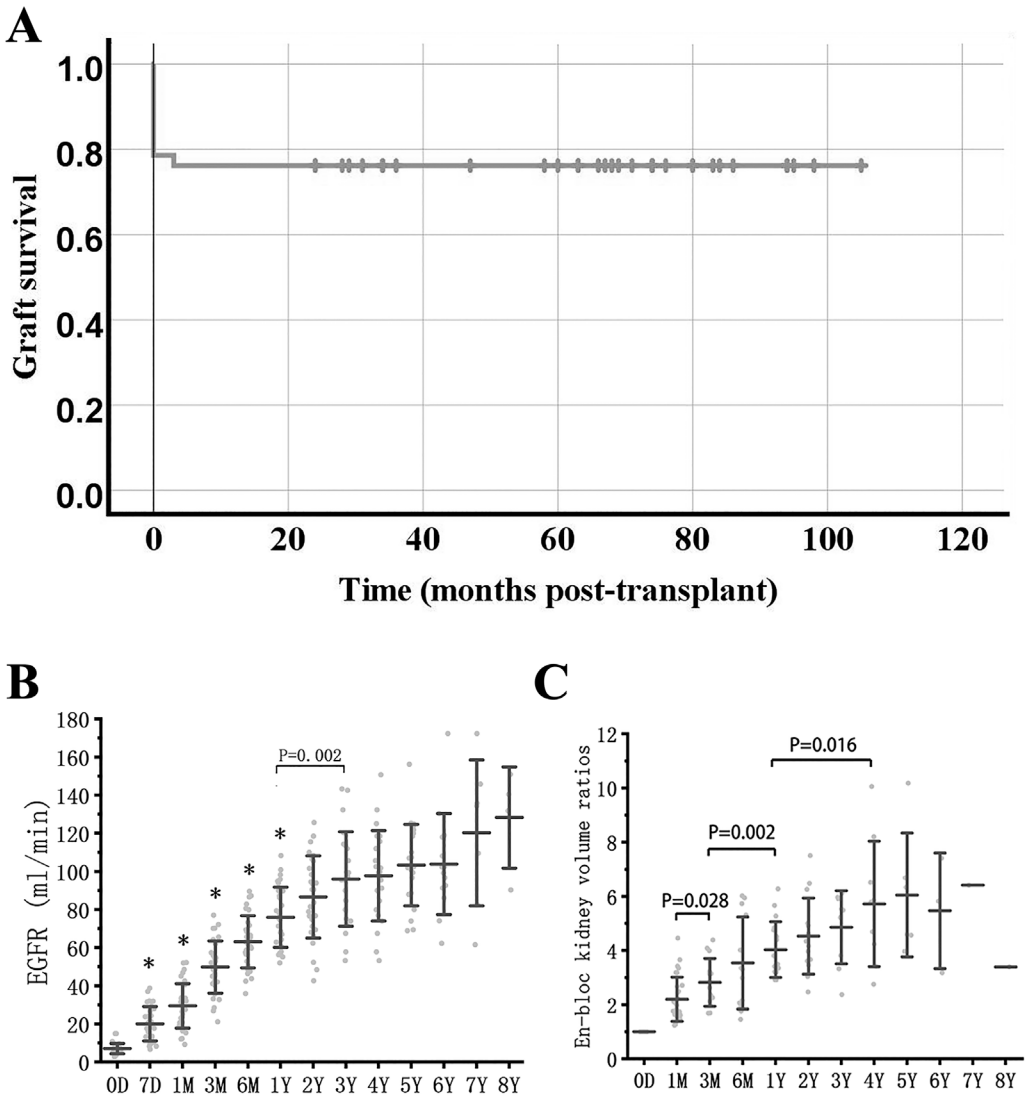


FIGURE 2 | Graft survival, function and growth. **(A)** Graft survival curve in *en-bloc* kidney transplant from donors ≤5 kg. **(B)** eGFR after *en-bloc* kidney transplant. Mean and error bars are denoted within the individual plotted values. **(C)** Volume development of the grafts after transplant. Initial volume is defined as 1. * indicates a statistically significant difference relative to the previous group.

followed by a continuous growth trend during the first 1 or even 2 years. Although *en-bloc* KTx from pediatric donors to adult recipients has been performed for over five decades, this procedure remains underutilized globally due to concerns regarding the risks of insufficient nephron mass, renal dysplasia, thrombosis, hyperfiltration injury, and urinary complications [3, 8]. Currently, the reported minimum donor weight is 1.07 kg, and the majority of scholars have reported the outcomes of kidney transplantation using donors weighing between 10 and 20 kg[6]. Although a low body weight donor *en-bloc* renal graft is considered to be one of the expanded criteria donations (ECD), previous studies reported that it has a higher anticipated eGFR than other ECD cases due to a larger reservoir and the fact that it is free of chronic injury [9]. In addition, it may take over a year following KTx to achieve *en-bloc* allograft stability in terms of eGFR, which is significantly longer than with adult grafts.

We prefer adult over pediatric patients as *en-bloc* KTx recipients for the reasons that follow. The first is an ethical consideration. It is more appropriate for the pediatric recipients to receive standard criteria donation instead of ECD. Considering the potential higher risk of short-term graft loss, higher prevalence of DGF and a longer period of eGFR recovery, *en-bloc* KTx from low-weight donors is more acceptable for adult recipients. Second, adult recipients may have more satisfactory long-term clinical outcomes [10, 11]. According to previous reports, with the exception of premature infants, the number of nephrons in full-term infants has reached adult levels,

TABLE 2 | Profiles of the graft survival group and the graft loss group in *en-bloc* kidney transplantation.

	Graft survival (n = 32)	Graft loss (n = 10)	P-value
Donor age (mean, d)	26.9	37.3	0.314
Donor weight (mean, kg)	3.1	3.4	0.455
Donor gender			1.000
Male recipients	20	7	
Female recipients	12	3	
Recipient age (mean, y)	27.0	30.8	0.259
Recipient weight (mean, kg)	46.7	50.0	0.193
Recipient gender			0.027
Male	9	7	
Female	23	3	
D-R BSA ratio	0.147	0.146	0.870
WIT (mean, min)	10.3	10.7	0.840
CIT (mean, h)	11.0	11.5	0.675
Mean time since the first *en-bloc* KTx (mean, d)	1,673	1,299	0.185

D/R BSA, donor/recipient body surface area; WIT, warm ischemia time; CIT, cold ischemia time; KTx, kidney transplantation.

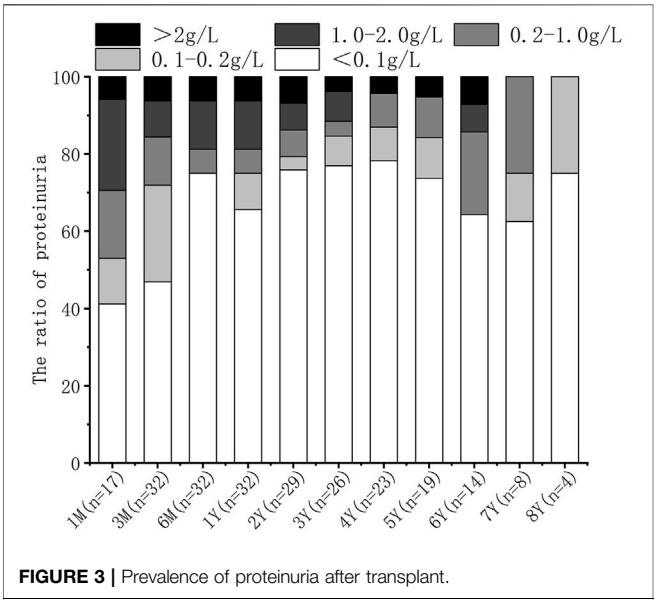


FIGURE 3 | Prevalence of proteinuria after transplant.

with subsequent growth consisting solely of nephron hypertrophy [12]. Moreover, in this study, even the adult recipients from donors weighing less than 2.5 kg were found to have non-compromised graft function and limited hyperfiltration injury. Thus, our study suggests that the small kidneys from low-weight pediatric donors may have enough adaptability to swiftly meet the needs of the recipients after transplant.

In the analysis of risk factors for short-term graft loss, we found that female recipients exhibited a significantly lower risk compared to male ones. This finding may optimize the inclusion criteria of recipients. While the underlying causes remain unconfirmed, we speculate that this may be attributed to hormonal differences. Previous research has highlighted the role of estrogen as a potent antioxidant within the renal mesangial microenvironment, promoting nitric oxide release through endothelial nitric oxide release. In the context of KTx from low-weight donors, estrogen in female recipients may mitigate donor kidney injury by inhibiting oxidative stress, preserving microvascular integrity, and reducing thrombosis occurrence [13, 14]. In addition, it has been reported that myosteatosis and sarcopenia are associated with an increased risk of mortality in both the pre-transplant waiting group and post-transplantation recipients [15]. Preoperative reduction in muscle mass is linked to a poorer prognosis following kidney transplantation [16]. In our study, the mean body weight of male recipients was 50.1 kg, while the mean body weight of female recipients was 45.9 kg. When compared to the general population, the difference in body weight between male recipients and typical men was more pronounced. This suggests that male recipients may have less muscle mass relative to normal values. Consequently, this factor could potentially contribute to the higher risk of graft loss observed in men compared to women in this study.

Pediatric *en-bloc* KTx poses challenges due to the high incidence of thrombosis in the early postoperative stage. Previous studies have reported thrombosis rates ranging from 2% to 25% within 3 days of surgery, particularly in pediatric donors weighing less than 5 kg [17]. However, the use of anticoagulation therapy during pediatric *en-bloc* KTx still remains controversial [18]. This study reveals that the perioperative administration of LMWH has the risk of perirenal hematoma, and does not contribute to the prevention of postoperative thrombosis, which is consistent with a previous study [19]. This explains our decision to administer LMWH perioperatively in the first cohort of 19 patients, whereas it was not utilized in the subsequent group of 23 patients. A delicate surgical technique is still one of the prerequisites for minimizing *en-bloc* graft loss due to thrombosis. In short, proper aortic cannula for adequate graft flushing during procurement, avoidance of excessive exposure of renal vessels during preparation, and maximization of the venous anastomosis site during implantation are major considerations during the steps [4, 20]. Unlike the adult kidney, it may be much more difficult to detect the torsion of the small grafts during the procedure. Adequate vascular surrounding tissue, keeping the

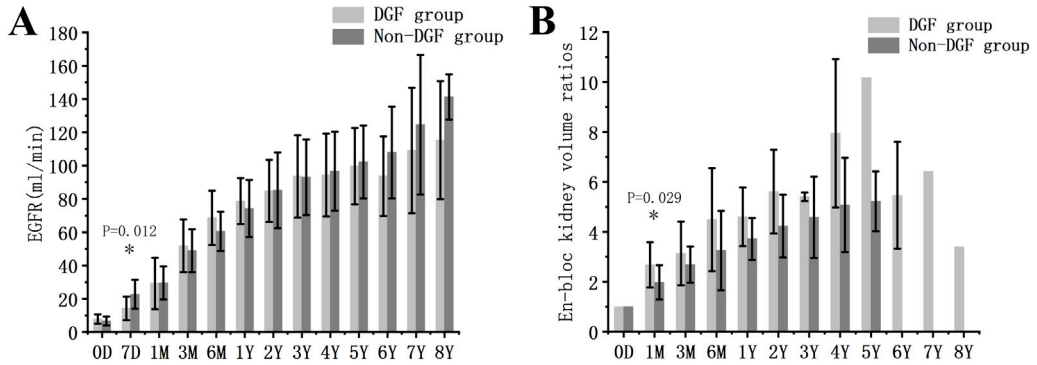


FIGURE 4 | Graft function and growth in DGF and non-DGF groups. **(A)** eGFR. **(B)** Volume development of the grafts. DGF, delayed graft function.* indicates a statistically significant difference between the DGF group and the non-DGF group.

TABLE 3 | Profiles of donors and recipients in kidney transplantation from donors weighing less than 2.5 kg and those weighing between 2.5 and 5.0 kg.

	Donor weight less than 2.5 Kg (n = 12)	Donor weight between 2.5 and 5.0 Kg (n = 30)	P-value
Donor age (mean, d)	19.6	33.3	0.154
Donor weight (mean, kg)	1.95	3.62	0.000
Donor gender			0.292
Male recipients	6	21	
Female recipients	6	9	
Recipient age (mean, y)	30.8	26.7	0.195
Recipient weight (mean, kg)	49.9	46.5	0.154
Recipient gender			0.316
Male	3	13	
Female	9	17	
D-R BSA ratio	0.114	0.160	0.000
WIT (mean, min)	10.3	10.5	0.936
CIT (mean, h)	10.9	11.2	0.801
Mean time since first *en-bloc* KTx (mean, d)	1922	1,448	0.071
DGF	5/10	5/22	0.217
Urinary leakage	0/10	5/22	0.155
Perirenal hematoma	1/10	1/22	0.534
Thrombosis	0/12	6/30	0.159
Acute rejection	2/12	2/30	0.585
Graft loss	2/12	8/30	0.696

D/R BSA, donor/recipient body surface area; WIT, warm ischemia time; CIT, cold ischemia time; KTx, kidney transplantation; DGF, delayed graft function.

bilateral ureters attached to the donor bladder before ureteral reconstruction may be helpful for prevention. Moreover, before closing the incision, it is vital to optimize the relative positions of the two grafts and permit further growth of the grafts, vessels and ureters.

In addition, pediatric donor kidneys have a much higher risk of graft loss due to acute rejection compared to adult donor kidneys [21]. As we have shown in this study, all three recipients with acute rejection experienced graft loss shortly after the procedure. The disparity may be due to the fragility of the small grafts and the fluctuation in Tac blood levels. However, it remains unknown whether the fast-growing grafts play an additional role in the fluctuation. Considering the devastating outcomes, adequate immunosuppressive exposure is vital. Thus, we suggest daily monitoring of Tac levels to assure adequate Tac exposure in the short period after KTx. Additionally, we recommend a more intensive immunosuppressive regimen as a viable strategy.

DGF may be a risk factor for long-term renal allograft failure in adult KTx outcomes. However, it may not be true in the setting of pediatric *en-bloc* KTx [22]. We found that there was no significant difference in eGFR level between the DGF group and the non-DGF group for 1 month post-KTx, and the long-term graft survival rate was also similar. However, the D/R BSA ratio may serve as an effective indicator for predicting short-term postoperative renal function recovery [23]. In this study, the recipients with higher D/R BSA were less likely to need dialysis after KTx. This suggests that recipients with lower body weight may have a smoother recovery. Significantly faster graft volume growth in the DGF group in the first month could be due to a stronger driving force in the", which are adapted to much larger recipients.

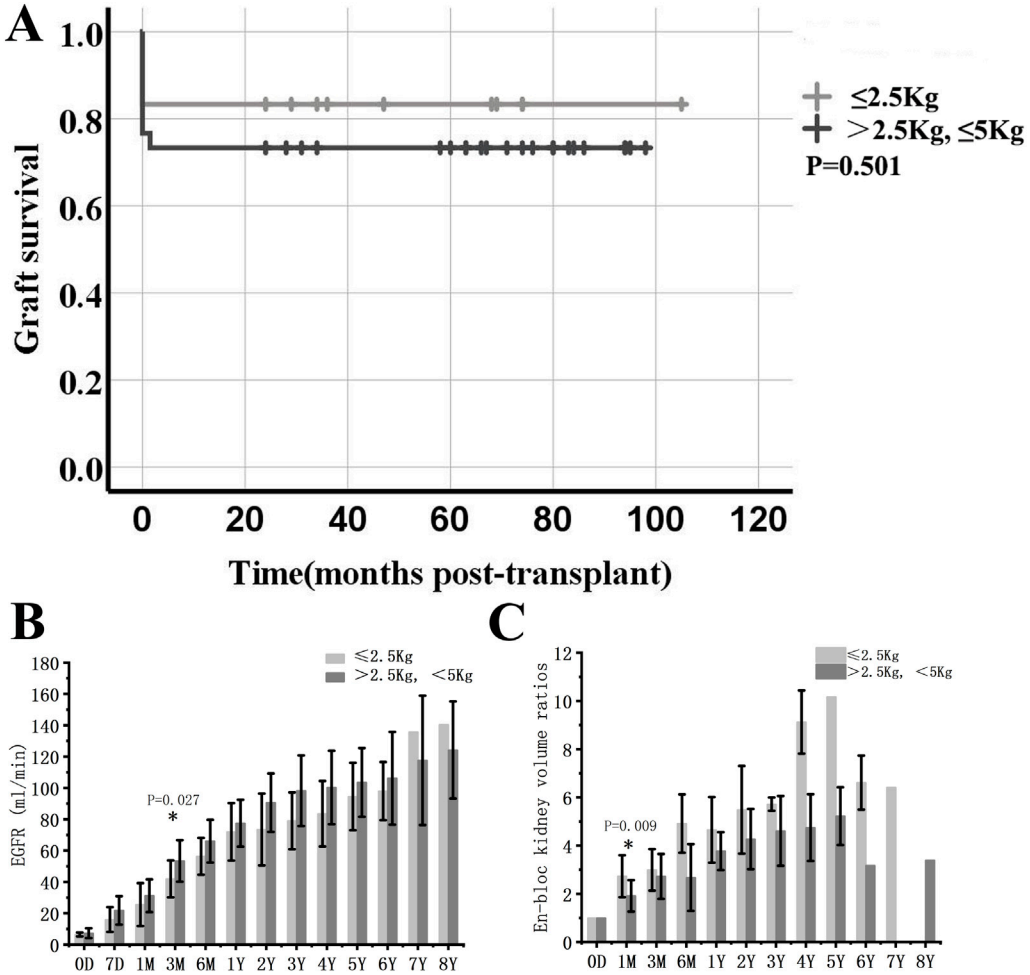


FIGURE 5 | Comparison between *en-bloc* kidney transplant from donors weighing less than 2.5 kg and those weighing between 2.5 and 5.0 kg. **(A)** Graft survival. **(B)** eGFR. **(C)** Volume development of the grafts. * indicates a statistically significant difference between donors weighing less than 2.5 kg and those weighing between 2.5 and 5.0 kg.

Pediatric *en-bloc* renal allografts are associated with a potentially higher incidence of postoperative urinary complications [24]. However, these complications can generally be successfully treated non-surgically, and there is no evidence of a negative impact on allograft survival rate [5]. Our findings are consistent with this conclusion. Except for one case of postoperative long-segment necrosis resulting from intraoperative injury, the other four cases of urinary leakage resolved by prolonging the indwelling time of the urinary catheter. The following strategies may be advantageous in minimizing ureteral complications: (1) Preserving adequate ureteric surrounding tissue during organ procurement to ensure a rich blood supply. (2) Shortening the internal ureter before ureteroneocystostomy. (3) Avoiding forceful stent placement to minimize the possibility of mechanical damage to the ureter. (4) Shortening the graft CIT [25].

Previous research found significantly higher levels of proteinuria in pediatric *en-bloc* KTx patients compared to adult KTx patients at 6 and 12 months [26]. To mitigate hyperfiltration injury, another study showed benefits of maintaining systolic blood pressure below 130 mmHg[4]. We did not detect significant glomerulosclerosis in the pathological findings of the five recipients who underwent indicative biopsy approximately 2 years after transplantation (**Supplementary Material S5**). Consequently, we conclude that hyperfiltration injury in recipients is tolerable, provided that blood pressure is rigorously maintained below 140 mmHg following kidney transplantation. Furthermore, none of the proteinuric patients developed edema or hypoalbuminemia, which is consistent with common findings in maladaptive focal segmental glomerulosclerosis [27].

In this study, donor body weight was found not to represent a risk factor for graft loss. Pediatric donors weighing less than 2.5 kg had comparable graft survival and graft function outcomes to the donors weighing between 2.5 and 5.0 kg. Although KTx from these donors poses challenges during the surgical procedure

and has a slower eGFR increase, long-term outcomes are not compromised. Given these findings, we propose that the utilization of lower body weight (<2.5 kg) kidneys should be determined based on the related experience of individual transplant teams, and the preferences of the recipients. While these grafts may present challenges, they should not be a contraindication to KTx.

Our study has several limitations, including its retrospective nature and the limited number of cases and follow-up period. In our study, the mean WIT was 10 min, and the mean CIT reached 11 h. Therefore, the use of the University of Wisconsin solution and hypothermic machine perfusion may potentially enhance postoperative outcomes. To address these limitations, a comprehensive follow-up study will be conducted over an extended period. Furthermore, it would be more interesting if the follow-up also included pathological findings.

In conclusion, although instances of graft failure and severe complications occur primarily in the initial stages, KTx from extremely low-weight donors can still expand the donor pool and have promising long-term graft function. A body weight of less than 2.5 kg should not be an absolute contraindication for kidney donation. The clinical recommendations offered in this study could further optimize the clinical outcomes of this procedure. Furthermore, considering the unique physiology, pathology and immunology in the very young and low-weight pediatric donors, this transplant environment may also offer an opportunity to study kidney development and other related issues.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Renal grafts were donated to the Red Cross Society and allocated to our center by the China Organ Transplant Response System. The procedures were in compliance with the national program of organ donation in China, the Helsinki Congress as well as the Declaration of Istanbul. This study was approved by the institutional review board (WHXHKT20230218). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

XZ: Methodology, Software, Investigation, Writing - Original Draft. QX: Formal analysis, Investigation. HeL: Supervision, Conceptualization. MW: Data Curation, Investigation. HaL: Software, Data Curation. LH: Figure Editing. HS: Pathologic analysis. CZ: Pathologic analysis. ZW: Conceptualization, Writing- Reviewing and Editing, Supervision, Project administration.

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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 authors 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.2025.14451/full#supplementary-material

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ORIGINAL RESEARCH
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Clinical and Histopathological Determinants for Kidney Allograft Survival in the Eurotransplant Senior Program (ESP) at the Time of Allocation

Tom N. Langer[1,2], Thorsten Wiech[3,4], Mercedes Noriega[3], Sergey Biniaminov[5], Tobias B. Huber[1,4], Lutz Fischer[2,6], Florian Grahammer[1,2,4†] and Malte A. Kluger[1,2†]*

[1]III. Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2]University Transplant Center (UTC), University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [3]Institute of Pathology, Nephropathology Section, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [4]Hamburg Center for Kidney Health (HCKH), University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [5]HS Analysis GmbH, Karlsruhe, Germany, [6]Department of Visceral Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

To address the shortage of organs for kidney transplantation, the Eurotransplant Senior Program (ESP) was established to enhance kidney allocation from elderly donors. This study aimed to evaluate post-transplant outcomes of deceased donor grafts and identify prognostic factors within the ESP population. We therefore analyzed patient data from 64 ESP recipients and their donors transplanted at our center between 2017 and 2022. Time-zero biopsies were analyzed using AI image analysis software for glomerular density and glomerulosclerosis. One-year patient and allograft survival rates were 96.9% and 85.9%. 5-year survival rate was 74.6%, as opposed to about 41.0% historically reported for patients on dialysis. Delayed Graft Function occurred in 29.7% of cases, with recipient coronary heart disease, BMI-disparities, and prolonged cold ischemia time as major predictors ($P < 0.05$). Histopathological analysis revealed that the degree of glomerulosclerosis and interstitial fibrosis and tubular atrophy (IFTA) were associated with graft failure in multivariable analyses ($P < 0.05$). Arteriolosclerosis (arteriolar hyalinosis) correlated with a higher risk for primary non-function ($P < 0.05$). The number of HLA mismatches was not significantly associated with graft outcome. Including prognostic baseline characteristics as well as histopathological AI analysis into individual allocation decisions during organ-acceptance process might improve allograft survival within the ESP and should prospectively be studied.

Keywords: kidney transplantation, elderly, ESP European Senior Program, AI histopathology, machine learning

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***Correspondence**
Malte A. Kluger,
m.kluger@uke.de

†These authors have contributed equally to this work

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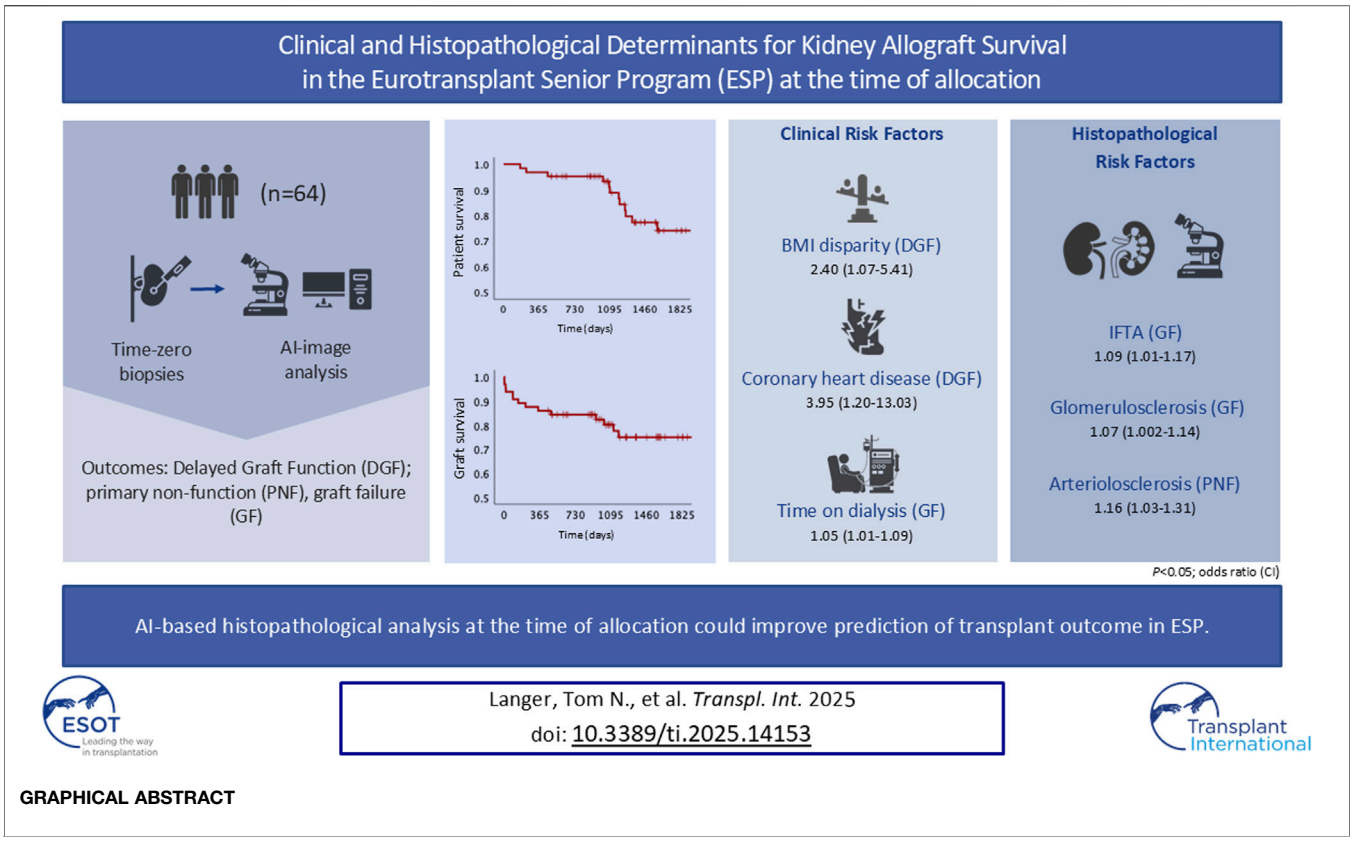
INTRODUCTION

At present, kidney transplantation represents the only treatment option for patients suffering from terminal kidney failure that offers perspectives for prolonged survival and benefits for the quality of life. In response to the demographic changes, including the rising numbers of elderly patients with end-stage kidney diseases on the waiting list but persisting shortage of donated organs, Eurotransplant established the European Senior Program (ESP) for this group in 1999. The ESP



GRAPHICAL ABSTRACT

allocates kidneys from deceased donors aged ≥65 years to elderly recipients ≥65 years of age who left the general kidney waiting list (ETKAS) for the benefit of significantly shorter waiting times. Its medical outcome is mainly based on minimizing cold ischemia time (CIT) by allocating organs locally, still based on blood group compatibility and waiting time. In contrast to the Eurotransplant Kidney Allocation System (ETKAS), the ESP does not include human leukocyte antigen (HLA) A-B-DR matching or specific immunological criteria. The latter have to be evaluated by the accepting centers, although inclusion of HLA-DR matching has recently been discussed [1]. Taken together, relevant reductions in waiting times for patients that otherwise might not even live up to their ETKAS-transplantation, as well as improved mortality rates among these elderly patients when compared to those continuing on dialysis, seem to be the major significant advantages of this program [2, 3].

Despite 25 years of experience with the ESP, selecting suitable organs from elderly donors remains a complex challenge due to the lack of extensive scientific studies identifying robust prognostic factors for satisfactory transplant outcomes. Frequently debated factors contain donor and recipient age, number of HLA mismatches, kidney re-transplantation, and body mass index (BMI) [1, 4–6]. Delayed graft function (DGF) is a significant prognostic indicator for graft survival and immunological response in ESP patients [4, 7–9]. Identifying modifiable risk factors for DGF could therefore contribute to improved outcomes in the future.

In this retrospective single-center study, we analyzed patient and graft survival in recipients of kidneys allocated via the ESP. Donor and recipient data were utilized to identify prognostic factors associated with kidney allograft survival and DGF. Furthermore, we evaluated whether the results of in-advance biopsies, that in our center are currently performed as time-zero analysis during transplantation, could potentially even further improve the prediction of the graft outcome when added to the aforementioned criteria, especially when their personnel- and time-sensitive processing could at least partially be automated. In addition, we aimed to review whether the ESP-recipients at our center in general still benefit from their transplantation.

MATERIAL AND METHODS

Study Design

From 1 September 2017 to 1 September 2022, 64 waitlisted recipients aged ≥65 years at the Hamburg University Transplant Center (UKE) received deceased donor kidneys via the ESP allocation algorithm. All renal allografts were obtained from donors after brain death, aged ≥65 years. Following the standard ESP criteria, HLA matching was not utilized during allocation. Induction immunosuppressive treatment consisted of basiliximab and steroids. Highly immunologically sensitized patients or patients with a high risk for DGF (e.g., longer CIT received thymoglobulin instead together with steroids. Maintenance

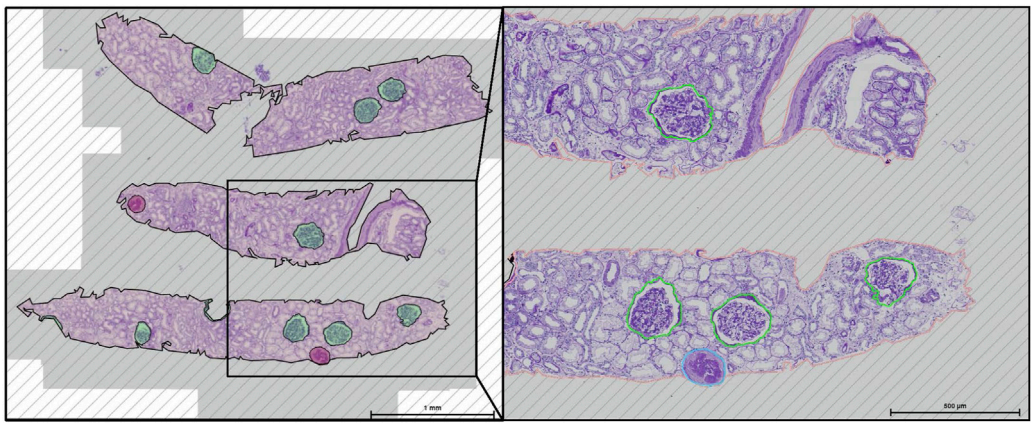


FIGURE 1 | Deep learning-based glomeruli detection in HAS KIT from periodic acid Schiff stained kidney.

immunosuppression included calcineurin inhibitors (mostly tacrolimus) and antimetabolites (mycophenolate mofetil or mTOR inhibitor) with or without steroids. From 2021 on, patients with low immunological risk were routinely placed on a steroid-free maintenance therapy from day eight after transplantation, following the HARMONY-study protocol [7].

Data Collection

Donor data was extracted from Eurotransplant's donor kidney reports. Recipient data was collected in a retrospective manner, utilizing the patient files and hospital discharge reports, with a minimum follow-up of 16 months. 18G-time-zero biopsies were performed by the implanting transplant surgeon after reperfusion. Paraffin-embedded kidney biopsies were cut into 1–2 µm sections and stained according to a standard PAS staining protocol. Slides were digitized using Zeiss AxioScan.Z1 slide scanner (ZEISS Group, Oberkochen, Germany) with a ×20 objective and retrospectively analyzed using explainable deep-learning-based software HSA KIT (HS Analysis GmbH, Karlsruhe, Germany; **Supplementary Material S1**), which calculated in a reproducible and objective manner the surface area of the renal cortex and automatically quantified glomeruli. The evaluation enabled the calculation of glomerular density and the ratio of sclerosed glomeruli to the total number of glomeruli in a biopsy section (**Figure 1**). Histological findings of these biopsies were not available prior to transplantation and did therefore not influence decisions of the transplanting team in these patients. Data on interstitial fibrosis and tubular atrophy (IFTA), arteriolosclerosis, and arterial intimal fibrosis (AIF) were obtained from post-transplant pathology reports. Follow-up data were collected from patients undergoing routine check-up appointments at the outpatient clinic.

Outcome Parameters

Recipient survival was defined as the time from transplantation until death, kidney graft failure by return to dialysis, excluding deaths with a functioning graft (DWFG). In the event of sepsis-induced multiple organ failure, documentation of dialysis therapy for at least 3 days prior to death was used for considering acute kidney injury as graft failure. DGF was defined as requiring more than one dialysis treatment within the first post-transplant week. Primary non-function (PNF) was defined for grafts never obtaining enough function to stop dialysis treatment after transplantation.

Statistical Analysis

Descriptive statistics were determined for continuous (mean ± standard deviation, median, and minimum-maximum) and categorical variables (absolute values and percentages). Two-sided t-test was used to ascertain significant differences between two groups for continuous variables. Pearson's chi-square test was applied to calculate correlations between pairs of categorical variables. The Kaplan-Meier method was employed to examine graft and patient survival and log-rank test to analyze differences in graft survival. P-values < 0.05 were considered to be statistically significant. The P-values are of descriptive nature. There was no adjustment for multiplicity. The Intraclass Correlation Coefficient (ICC) was calculated using a two-way mixed effects model with an absolute agreement model. Univariable regression analysis was conducted to determine potential prognostic factors for graft loss, PNF and DGF. Variables yielding statistical significance in the univariable analysis were evaluated through a stepwise regression process within a multivariable analysis, utilizing a binary logistic regression model. Cox proportional hazard regressions were performed univariable and multivariable in order to analyze the effect of variables on graft survival. For the multivariable model, variables with a P-value < 0.05 in univariable analysis were included, and backward stepwise selection was applied using a removal criterion of $P > 0.10$. All data were analyzed using SPSS 29.0 (IBM Corp., Armonk, NY, United States).

RESULTS

Donor and Recipients Baseline Characteristics

A total of 64 patients who underwent kidney transplantation after ESP allocation were included in this study. All organs

TABLE 1 | Demographics and clinical characteristics.

Variable	n = 64
Recipient age (years)	71.3 ± 4.3 (65–81)
Recipient sex m/f	44/20 (68.8%/31.3%)
Recipient BMI (kg/m^2)	26.8 ± 4.06 (17.7–37.5)
Recipient Comorbidities	
Hypertension	56 (87.5%)
Coronary heart disease	29 (45.3%)
Diabetes	14 (21.9%)
Past history of tumor	21 (32.8%)
Renal cell cancer	6 (9.4%)
Prostate cancer	4 (6.3%)
Colorectal cancer	4 (6.3%)
Others	7 (10.9%)
Donor age (years)	72.9 ± 6.3 (65–86)
Donor sex m/f	36/28 (56.3%/43.8%)
Donor BMI (kg/m^2)	26.7 ± 4.8 (18.4–54.9)
Donor creatinine prior to organ procurement (mg/dL)	1.02 ± 0.50 (0.43–2.81)
Donor Comorbidities	
Hypertension	34 (53.1%)
Smoking	14 (21.9%)
Diabetes	10 (15.6%)
Time on dialysis (months)	45.0 ± 24.52 (8.72–98.69)
Renal replacement therapy HD/PD	52/12 (81.3%/18.8%)
2nd kidney transplantation	7 (10.9%)
Dual kidney transplant	3 (4.7%)
Causes for kidney failure	
Nephrosclerosis or hypertensive nephropathy	17 (26.6%)
ADPKD	9 (14.1%)
IgA-nephropathy	8 (12.5%)
Diabetic nephropathy	7 (10.9%)
Nephropathy of unknown case	4 (6.3%)
Interstitial nephritis	2 (3.1%)
FSGS	2 (3.1%)
Membranous glomerulonephritis	2 (3.1%)
Membranoproliferative glomerulonephritis	1 (1.6%)
Goodpasture-syndrome	1 (1.6%)
Others	11 (17.2%)

Data are presented as absolute values (percentages) for categorical variables; mean ± standard deviation (minimum–maximum) for continuous variables. BMI, body mass index; HD, hemodialysis; PD, peritoneal dialysis; ADPKD, autosomal dominant polycystic kidney disease; FSGS, focal segmental glomerulosclerosis.

were obtained after brain death, as donations after circulatory death are currently not permitted in Germany. **Table 1** summarizes the baseline characteristics. The mean follow-up period was 49.2 ± 16.6 months. The proportion of males was higher among both recipients (68.8%) and donors (56.3%). The mean age of the recipients was 71.3 ± 4.3 years, while the donors had a mean age of 72.9 ± 6.3 years. According to the WHO definition, male recipients showed a considerable prevalence of increased bodyweight (79.5%), compared to the overall male population in Germany within the same age group (68.2%) [8]. Mean dialysis time before transplantation was 45 months. The leading cause of renal insufficiency was hypertensive nephropathy (26.6%). The mean CIT was 8.70 ± 3.0 h, and the mean warm ischemia time (WIT) was 37.5 ± 11.5 min. Due to the missing HLA matching in the ESP, 82.8% of patients had ≥4 HLA mismatches, while only 4.7% received a full-house match.

Predictors for Delayed Graft Function

DGF occurred in 19 out of 64 cases (29.7%). A minimal BMI disparity of ≤2.5 kg/m^2 between donor and recipient was associated with significantly lower prevalence of DGF (11.1%), compared to >2.5 kg/m^2 (36.9%, $P < 0.05$). Univariable analyses indicated that an unfavorable BMI match (subdivided into ≤2.5, 2.51–5.0, >5.0 kg/m^2), higher recipient BMI, presence of CHD, and prolonged CIT significantly increased the odds of DGF. Each additional hour of CIT increased DGF-risk by 24% ($P < 0.05$). **Table 2** displays the results of the uni- and multivariable analyses. In a multivariable regression model, the combination of CHD and BMI disparity reached statistical significance for the event of DGF.

Graft and Patient Outcome

Patients immunosuppressive therapy and outcome are described in **Table 3**. During the entire follow-up period, 12 patients (18.8%) died. The 1-year survival rate was 96.9%, with two patients dying within the first year and another 10 patients dying thereafter. Initially, patient survival remained nearly consistent, with a 3-year survival rate of 91.1%. After the first 3 years, the survival rate dropped, with the 5-year survival rate being only 74.0%. Seven recipients dies with a functional graft (DWFG). The primary cause of mortality was sepsis (58.3%).

Graft loss occurred in 14 patients (21.9%; DWFG excluded), with 1- and 5-year graft survival rates of 85.9% and 75.0%. Kaplan-Meier curves are shown in **Figures 2A,B**. PNF was observed in five patients. Excluding patients with PNF, the mean time to graft failure was 617.22 ± 446.83 days (89–1,177 days). Biopsy-proven rejection was observed in 14 recipients (21.9%). However, graft loss due to chronic rejection was rare, accounting for only one case. During follow-up, DSA were identified in 14 patients (21.9%), but their presence did not correlate with graft survival or rejection events. A total of 44 patients (68.8%) were hospitalized for at least 7 days due to infection-related complications. COVID-19 was diagnosed in 15 recipients (23.4%) during one of their inpatient stays. The presence of COVID-19, BK virus infection, or cytomegalovirus did not show any statistically significant correlation with mortality or graft failure.

Predictors for Graft Failure

Follow-up data at 4 weeks ($P < 0.006$), as well as at three ($P = 0.039$), six ($P = 0.006$) and twelve ($P = 0.003$) months after transplantation, demonstrated a statistically significant correlation between elevated creatinine levels and graft loss in univariable logistic regression model. The mean creatinine level at 4 weeks post-transplant in patients who later experienced graft failure was 3.44 mg/dL ± 1.71, compared to 2.09 ± 0.95 mg/dL in those who did not experience graft failure. Additionally, the length of hospitalization post-transplant emerged as a predictor for graft failure probability: the relative risk for the loss of a graft increased by 8% for each additional day spent in the hospital after transplantation ($P = 0.029$). As our study aimed to define parameters already available at the time of allocation, these

TABLE 2 | Uni- and multivariable analysis of potential risk factors for Delayed Graft Function.

Factors	Univariable analysis		Multivariable analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
BMI match (≤2,5; 2,51–5.0; >5.0 kg/m^2)	2.38 (1.10; 5.17)	0.028[a]	2.40 (1.07; 5.41)	0.035[a]
CHD	3.93 (1.25; 12.33)	0.019[a]	3.95 (1.20; 13.03)	0.024[a]
CIT (h)	1.24 (1.02; 1.50)	0.033[a]	--	--
Recipient BMI (kg/m^2)	1.20 (1.03; 1.40)	0.021[a]	--	--

BMI, body mass index; BMI match, disparity in BMI between recipient and donor; CHD, coronary heart disease; CIT, cold ischemia time; OR, odds ratio; CI, confidence interval.
[a]*Significance 0.05. -- not included.*

TABLE 3 | Immunosuppressive therapy, patient- and graft survival.

Variable	n = 64
HLA mismatch	4.4 ± 1.5
PRA positive recipient	12 (18.8%)
Induction therapy	60 (93.8%)
Basiliximab/simulect	4 (6.3%)
Antithymocyte globuline	
Use of tacrolimus as initial CNI on day eight	59 (92.2%)
Use of cyclosporine A as initial CNI on day eight	5 (7.8%)
Use of an antimetabolite (MMF/MPA) on day eight	47 (73.4%)
Use of a mTOR inhibitor on day eight	17 (26.6%)
Steroid-free immunosuppression on day eight	14 (21.9%)
Delayed graft function	19 (29,7%)
Mean hospital stay after transplantation (days)	19.0 ± 8.5 (6–47)
Death	12 (18.6%)
Cause of death	*n = 12*
Sepsis	7 (58.3%)
Cardiovascular event	1 (8.3%)
Aneurysm-related hemorrhage	1 (8.3%)
Cancer	1 (8.3%)
Unknown	2 (16.7%)
Graft failure	14 (21.9%)
Cause of graft failure	*n = 14*
Primary non-function	5 (35.7%)
As a result of infection/sepsis	2 (14.3%)
Rejection	1 (7.1%)
BK virus infection	1 (7.1%)
Cardiac decompensation	1 (7.1%)
Unknown	2 (14.3%)
Others	2 (14.3%)
Duration between transplantation and graft loss (days)	617.22 ± 446.83 (89–1,177)
NODAT	11 (17.2%)
DSA	14 (21.9%)

Data are presented as absolute values (percentages) for categorical variables; mean ± standard deviation (minimum–maximum) for continuous variables. HLA, human leukocyte antigen (Loci A, B, DR); PRA, panel reactive antibodies; CNI, calcineurin-inhibitor; NODAT, new onset diabetes after transplantation; DSA, de novo *donor-specific antibodies.*

data are presented in the **Supplementary Material S2**, along with factors that remained non-significant in univariate analysis and therefore were not included.

Focusing on kidney donors, histopathological analysis was performed for all available 51 time-zero biopsies. There was a very good agreement on glomerulosclerosis grading between the pathologist and the retrospective semi-automated deep learning quantification (ICC = 0.913; 95% Confidence Interval = 0.85–0.95). Univariable analyses identified IFTA, the percentage of arteriolosclerosis (arteriolar hyalinosis), and glomerulosclerosis as significant risk factors for graft failure (**Table 4**). Glomerular density and AIF did not reach statistical significance. When focusing on the recipients, prolonged time on dialysis was associated with increased failure rates. Patients exceeding 3 years of dialysis treatment had a 35.3% risk of graft failure, compared to a 6.6% risk for those with less than 3 years of renal replacement therapy (P = 0.006). The combination of IFTA, glomerulosclerosis, and time on dialysis reached statistical significance in a multivariable Cox proportional hazard model. The corresponding Kaplan-Meier analyses and log-rank tests are shown in **Figures 3A–C**. Additionally, arteriolosclerosis showed a significant correlation for the event of PNF (P = 0.016; odds ratio = 1.16; 95% Confidence interval = 1.03–1.31). However, the number of HLA mismatches did not significantly influence graft survival in our ESP collective.

DISCUSSION

This study aimed to identify potential prognostic factors for short- and long-term outcomes of ESP-kidney transplantations to improve organ allocation strategies within the participating transplant centers in the future. Therefore, we comparatively reevaluated those parameters proposed from previous studies [1, 4, 6] for our ESP recipients and investigated potentially predictive additional variables available at the time of the organ offer, such as the matching of baseline characteristics between donors and recipients. Finally, we used deep learning based image analysis software HSA KIT as human-machine interaction tool to retrospectively quantify histopathological data obtained from time-zero kidney biopsies and its potential as a future prospective tool prior to final organ acceptance when half-automatically integrated into the allocation process.

Our univariable analysis indicated that disparity in BMI, higher recipient BMI, CHD, and prolonged CIT significantly correlated with a higher prevalence of DGF. These factors, when modifiable, may be considered in future transplant evaluations, as existing literature has demonstrated that DGF is associated with poorer outcomes [4, 9–11]. However, due to the limited size of our patient cohort, not all variables could be included in the multivariable analysis. Previous studies have consistently shown that an increased BMI in either the recipient or the donor is associated with a higher risk of DGF and graft loss [4, 12–16].

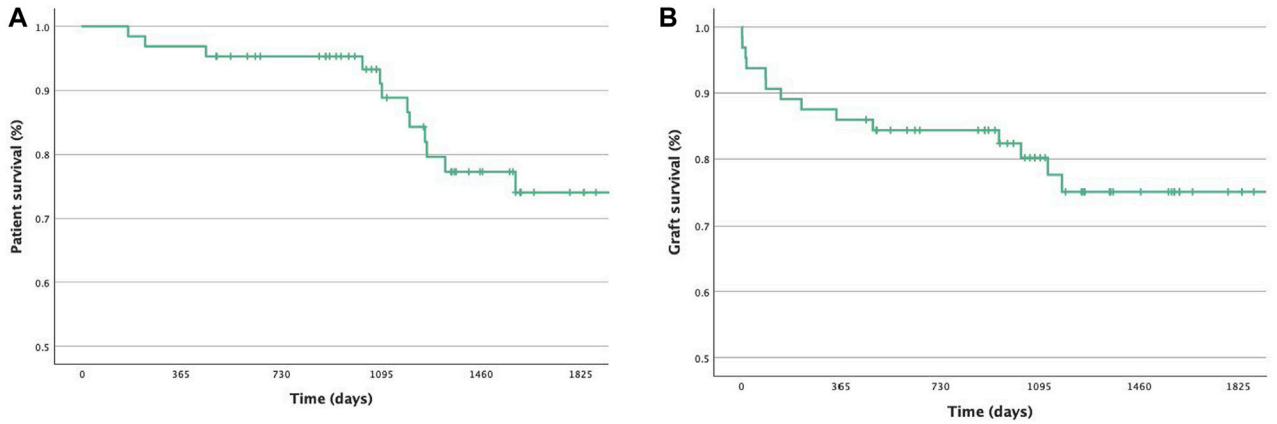


FIGURE 2 | Kaplan-Meier plots for **(A)** Patient survival during follow-up. **(B)** Graft survival during follow-up (censored due to end of observation period or death with functional graft).

But to our knowledge, this study is the first to report the impact of BMI disparities, rather than absolute values, between donor recipient pairs within the ESP as a measure that could indeed be part of an individualized allocation decision, favoring closer BMI matches to improve outcomes, as the match might indeed guide a decision for factors (absolute BMI of donor and recipient) are non-modifiable at the time of allocation.

Analyses of time-zero biopsies revealed that histopathological findings such as IFTA and the degree of glomerulosclerosis and arteriolosclerosis represented independent predictors of graft survival in ESP recipients. Our Cox proportional hazard model points to IFTA as one of the main histological factors associated with graft survival. Ouellet et al. used IFTA scoring to demonstrate that each unit increase in IFTA at 6 months is associated with a higher risk of graft loss [17]. In this respect, it is important to emphasize that validation of AI automated IFTA scoring is still in progress at our center. Our results regarding the influence of glomerulosclerosis on graft survival as the other major histopathological determinant align with findings from other studies [18–20]. In contrast to Keijbeck et al., our observations revealed a significant association between histological arteriolosclerosis and graft outcome [21]. Much to our surprise, glomerular density and AIF were not significantly associated with graft survival, while the importance of AIF in predicting kidney function after transplantation was recently demonstrated [20].

The retrospective findings of Jacobi et al. revealed that higher biopsy scores in pre-implantation biopsies from ESP kidneys were associated with an increased prevalence of PNF and higher creatinine levels 1-year post-transplant [5]. The value of preimplantation biopsies is still a matter of debate. Given that the logistics and economics (24/7 on-call nephropathologists and technical staff), as well as the resulting time delay, would only legitimate the effort if major improvements in outcome could still be expected, considering prolonged CIT already as one of the relevant determinants of DGF and prognosis. This is where semi-automated deep learning systems could help to reduce this delay. They could be operated by the cryosectioning team (technician and pathologist), typically available at transplant centers, which are usually situated at highly specialized university hospitals. In the future, this tool may not necessarily require a designated nephropathologist during routine analysis, as only the location of the analyzed area (glomerulus, blood vessel, tubulointerstitium) needs to be validated. The agreement between retrospective semi-automated quantification and pathologist grading of glomerulosclerosis was very good [22]. However, we have not yet been able to automate the analysis of time-zero biopsies for IFTA and arteriolosclerosis. This remains a promising area for future research. Nevertheless, combining automated glomerulosclerosis-scoring with IFTA assessment by a cryosectioning on duty team might be a feasible concept today already.

In addition, a biopsy only represents a limited section of the kidney, and there may be some variation in the distribution of healthy and sclerosed glomeruli. Still, final interpretation of biopsy results needs the context of clinical and laboratory findings, although we find the opportunity of utilizing quite reliable specific parameters via deep learning systems in the environment of sparse resources very intriguing as well as applicable during our routines. Taken together, such efforts must still be justified by a significant improvement of the transplant outcomes for individual patients, considering the potential benefits of knowing histopathological details compared to the effects of procedural extension of ischemia times.

Our retrospective study was not able to confirm the positive impact of HLA-DR matching on ESP-graft survival. Fijiter et al. lately reported that HLA-DR matching for ESP-recipients resulted in reduced waiting time on dialysis (2.6 vs. 4.1 years) and improved graft survival, despite an increase in CIT (12.0 vs. 10.6 h) [1]. Furthermore, Koch et al. assert that HLA matching is even beneficial for organs from donors aged 75 and older [6]. In

TABLE 4 | Uni- and multivariable analysis of potential risk factors for graft failure using Cox Regression.

Factors	Univariable analysis		Multivariable analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
IFTA (%)	1.04 (1.006; 1.07)	0.021[a]	1.08 (1.03; 1.41)	0.002[a]
Glomerulosclerosis (%)	1.05 (1.01; 1.09)	0.025[a]	1.07 (1.02; 1.12)	0.011[a]
Time on dialysis (months)	1.02 (1.002; 1.04)	0.031[a]	1.05 (1.02; 1.09)	0.004[a]
Arteriolosclerosis (%)	1.05 (1.01; 1.09)	0.011[a]	--	--
Arterial intima fibrosis (%)	1.01 (0.98; 1.05)	0.483	--	--
HLA-MM	0.99 (0.66; 1.50)	0.969	--	--

IFTA, Interstitial fibrosis and tubular atrophy; Glomerulosclerosis - ratio of sclerosed glomeruli to total number of glomeruli; HLA-MM, number of human leucocyte antigen mismatches; HR, Hazard ratio; CI, confidence interval.

[a]*Significance 0.05. – not included.*

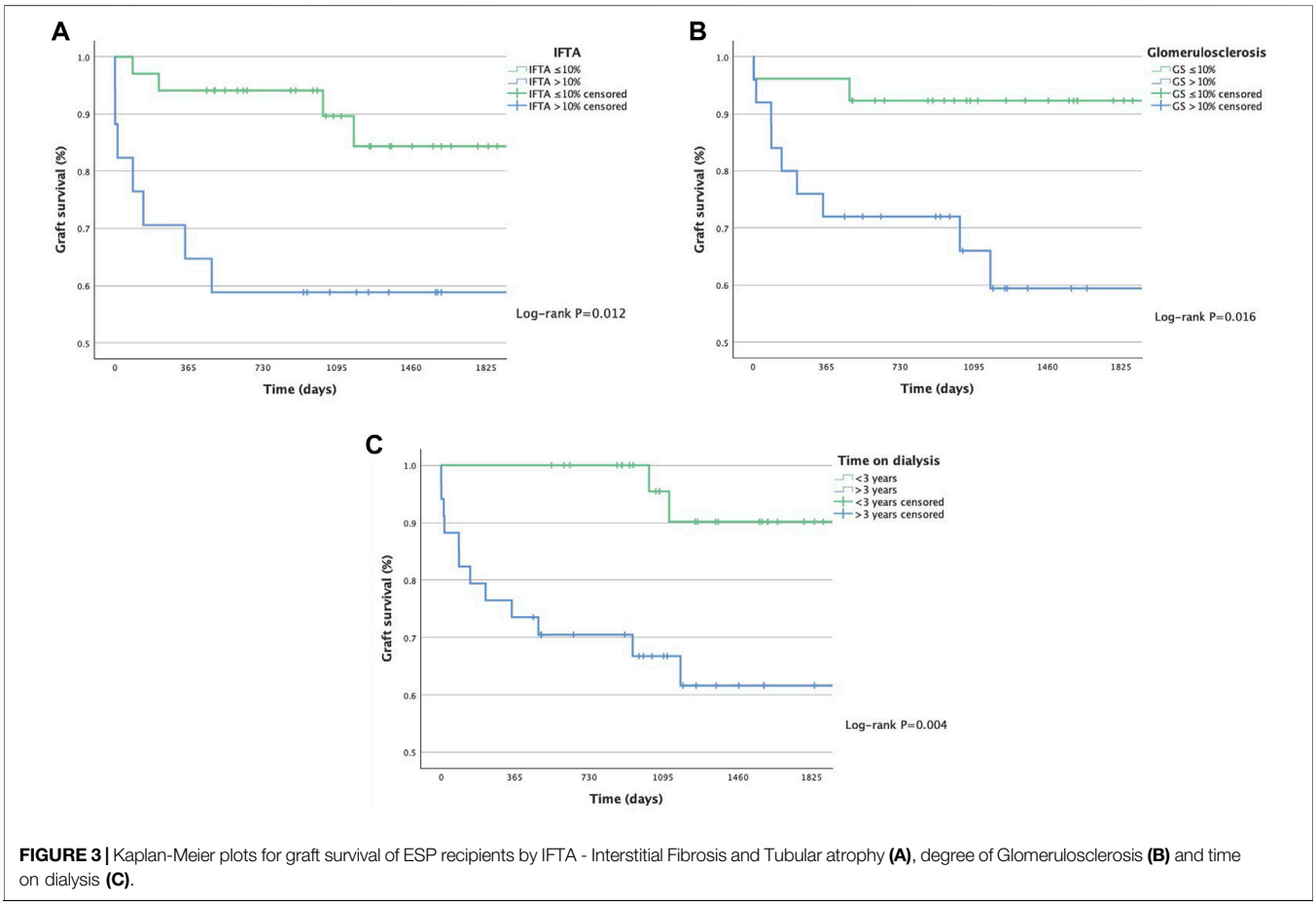


FIGURE 3 | Kaplan-Meier plots for graft survival of ESP recipients by IFTA - Interstitial Fibrosis and Tubular atrophy **(A)**, degree of Glomerulosclerosis **(B)** and time on dialysis **(C)**.

contrast, our findings indicate that prolonged CIT is associated with an increased risk of DGF, whereas better HLA match in our recipients did not correlate with improved outcomes. Several other studies also confirmed that extended CIT correlates with a higher incidence of DGF and graft loss [4, 11, 22, 23]. The increased susceptibility of older organs to damage from cold ischemia underscores the importance of minimizing CIT. The reduction in waiting time resulting from prospective HLA-DR matching may be the reason for better outcome, as our retrospective study again pronounces the negative impact of prolonged dialysis duration on later graft survival, as reported in the literature before [24].

DGF-rates, graft and patient survival in our study were comparable to those reported in similar studies evaluating the ESP. One- and 5-year graft survival rates ranged between 84%–87% and 63%–77%. Patient survival rates were 92%–94% and 65%–73% [4, 5, 25]. The incidence of DGF ranged between 19%–41.1% [4–6, 23, 25]. Excluding cases of PNF in our cohort, patient

and graft survival rates remained stable throughout the initial 3 years, with a notable increase in mortality thereafter. Death with a functional graft occurred in 58.3% of deceased patients, which is also in line with recent ESP observations [4, 5, 16, 23]. Compared to one- and 5-year survival rates of elderly dialysis patients with end-stage kidney disease, recipients still benefited from a transplantation within the ESP. In our cohort, the 5-year survival rate for recipients aged between 65–74 years was 74.6%, as opposed to 41.0% reported for patients on dialysis [3].

In our elderly cohort of transplant recipients, sepsis was identified as the primary cause of death. This once again highlights the unmet need for individually assessed and optimized levels of immunosuppression, considering initial renal disease and immunological burden by prior immunization, immunosenescence, and the patient's history of infections. Our results suggest that implementing less-potent immunosuppressive regimens might be advantageous, although no specific correlations of immunosuppressive therapy with patient or graft survival could be detected. In contrast to findings in previous ESP studies, in our cohort graft survival and DGF were not associated with rejection events [16]. However, the incidence of graft loss due to chronic rejection was low, and the limited number of chronic rejection cases precluded our statistical analysis from detecting potentially significant results. Taken together, follow-up care should especially evaluate the individual risk for infections and the adjustment of the immunosuppressive regimen as long as measures for individualized immunosuppressive guidance [26] cannot routinely be used.

The primary limitation of our study, next to its retrospective setup, is the relatively small sample size in terms of events for statistical testing. This constraint may have prevented identifying relationships between post-transplant outcomes and baseline characteristics such as age, diabetes mellitus, re-transplantation, and number of HLA mismatches. These factors were significant determinants of graft survival in prior ESP studies [4, 6, 16]. Our analysis of glomerular density did not yield statistically stable information regarding graft survival. An alternative approach might involve correlating glomerular density from biopsies and graft volume, which could facilitate the calculation of the total number of glomeruli in terms of "transplanted functional tissue" as a potential predictor of later transplant outcomes. These limitations could be addressed by multi-center studies with larger cohorts to prospectively validate the prognostic factors identified in this study for use during allocation. Moreover, we are quite aware that deep-learning-driven quantification would need to be validated and adapted for the use of fast-track HE-stained frozen sections, which, according to the manufacturer, would generally be technically realizable, but not yet included in our analysis.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on reasonable request by a qualified investigator for three years after the date of publication from the corresponding author.

ETHICS STATEMENT

The requirement of ethical approval was waived by Ethikkommission der Aerztekammer Hamburg, Weidestrasse 122b, Hamburg, Germany for the studies on humans because retrospectively analyzed anonymous data obtained during standard medical care without any additional sampling usually receive a waiver from our board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements. The human samples used in this study were acquired from no additional sample analyses performed, anonymous evaluation of digital routine-care data that already existed.

AUTHOR CONTRIBUTIONS

TL, FG, and MK established the study design. TL, FG, and MK performed literature research. TL collected the data and performed the statistical analyses. TW and MN performed nephropathological analysis, TW developed, validated and supervised the machine-learning processes, SB provided the software and technical support for automated histopathological analyses. TL, FG, and MK wrote the initial draft of the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

SB is the founder of HS Analysis.

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

GENERATIVE AI STATEMENT

The authors 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.2025.14153/full#supplementary-material

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Role of Lymphopenia in Early prediction of Infection Following Orthotopic Liver Transplantation in Cirrhotic Patients

Mikhael Giabicani[1,2], Clara Timsit[1], Léa Copelovici[1], Pauline Devauchelle[1], Marion Guillouët[1], Marina Hachouf[1], Sylvie Janny[1], Juliette Kavafyan[1], Stéphanie Sigaut[1], Tristan Thibault-Sogorb[1], Safi Dokmak[3], Federica Dondero[3], Mickael Lesurtel[3,4], Olivier Roux[5], François Durand[4,5] and Emmanuel Weiss[1,4]*

[1]Department of Anaesthesiology and Critical Care, Beaujon Hospital, DMU Parabol, AP-HP Nord, and Université Paris Cité, Paris, France, [2]Centre de Recherche des Cordeliers, Sorbonne Université, Université, Paris Cité, Inserm, Laboratoire ETREs, Paris, France, [3]Departement of HPB Surgery & Liver Transplantation, AP-HP, Beaujon Hospital, DMU DIGEST, Université Paris-Cité, Clichy, France, [4]Université Paris-Cité, Inserm, Centre de Recherche sur l'Inflammation, UMR 1149, Paris, France, [5]Service d'Hépatologie, AP-HP, Hôpital Beaujon, DMU DIGEST, Centre de Référence des Maladies Vasculaires du Foie, FILFOIE, ERN RARE-LIVER, Clichy, France

Infections remain a main cause of morbidity and mortality following orthotopic liver transplantation (OLT). Patients with end-stage liver cirrhosis exhibit a deregulation of their immune response, making them more susceptible to infections. From a prospective database, we retrospectively assessed the ability of preoperative lymphopenia, as a marker of this immune dysregulation, to predict the occurrence of early postoperative bacterial infections during post-OLT ICU hospitalization in patients with cirrhosis. Between January 2011 and December 2021, we included 445 patients. Post-OLT infections occurred in 92 patients (21%) and were mainly represented by bacteriemia (39%), pneumonia (37%) and surgical site infection (30%). Preoperative lymphocyte count $\leq 1.150 \times 10^9$/L was identified as an independent risk factor, as well as preoperative encephalopathy, intraoperative RBC transfusion >2 and intraoperative maximum norepinephrine dose >0.5 $\mu g.kg^{-1}.min^{-1}$ (all $p < 0.05$). Bootstrap analysis validated these results ($p < 0.05$). The risk factors were integrated into the PRELINFO score which was associated with the risk of infection ($p < 0.05$). The depth of preoperative lymphopenia was also associated with the risk of infection and postoperative correction of lymphopenia was slower in patients who developed an infection than in those who did not. Preoperative blood lymphocyte count should be incorporated into the assessment of the risk of early post-OLT bacterial infections.

Keywords: infection, lymphopenia, cirrhosis associated immune dysfunction, orthotopic liver transplantation (OLT), cirrhosis

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***Correspondence**
Mikhael Giabicani,
mikhael.giabicani@aphp.fr

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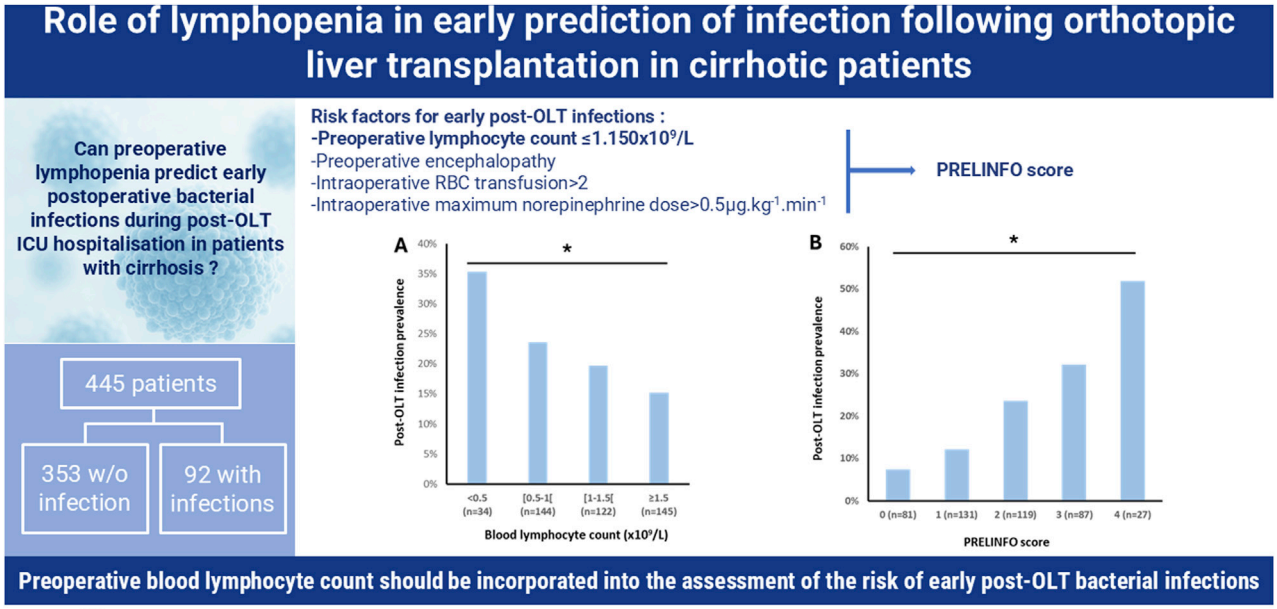
Abbreviations: OLT, orthotopic liver transplantation; ICU, intensive care unit; MELD, Model for End-Stage Liver Disease; ACLF, Acute-on-Chronic Liver Failure; CAID, cirrhosis associated immune dysfunction; RBC, red blood cell; BPSS, blind protected bronchial sampling; BAL, bronchoalveolar lavage; CFU, colony-forming units; SSI, surgical-site infections; UTI, urinary tract infections; ESBL-E, Extended-Spectrum β-Lactamase-producing *Enterobacteriaceae*; PRELINFO, PRediction of EarLy INfection Following Orthotopic liver transplantation; HCC, hepatocellular carcinoma.



GRAPHICAL ABSTRACT |

INTRODUCTION

The occurrence of infection following orthotopic liver transplantation (OLT) remains one of the main postoperative complications affecting patient morbidity and mortality [1, 2]. The incidence of infectious complication after OLT reported in literature ranges from 20% to 70% [3–5] and in more than 2/3 of cases, these infections are of bacterial origin [6, 7]. Especially when they occur during intensive care hospitalization, infections following OLT increase the risk of early death, the duration under mechanical ventilation and the intensive care unit (ICU) length of stay [3, 4]. Then, in an era where the trend is towards personalized medicine and "fast-tracking" strategy bypassing a systematic ICU hospitalization after OLT [8–10], early identification of patients at risk to develop infection after OLT would be useful to tailor their perioperative management and their immunosuppressive regimen.

Different risk factors for bacterial infections after OLT have been proposed in the literature including poor clinical conditions of the recipient (high Model for End-Stage Liver Disease (MELD) score, Acute-on-Chronic Liver Failure (ACLF), sarcopenia...), the complexity of the surgical procedure (blood transfusion, cold ischemia time, duration of surgery, type of biliary anastomosis...), and postoperative risk factors (type of immunosuppression therapy, ICU length of stay, biliary complication...) [11]. The recipient's immune system could also play a particularly important role [12]. Patients with end-stage liver cirrhosis, which is the main indication for OLT in Europe [13], are known to exhibit a deregulation of their immune response described under the term "cirrhosis associated immune dysfunction" (CAID) [14, 15]. One of the consequences of CAID is lymphopenia, which has led to the absolute blood lymphocyte count being considered one of the simplest surrogate markers for assessing CAID [16–18]. However, the impact of absolute lymphopenia on the early onset of bacterial infections after OLT has been poorly studied.

The aim of this study was to analyze the ability of preoperative blood lymphocyte count to predict the occurrence of early postoperative bacterial infections after OLT in patients with cirrhosis.

PATIENTS AND METHODS

Study Design and Patients

We performed a retrospective monocenter (Beaujon Hospital, Clichy, France) observational study from a prospective database from January 2011 to December 2021. This study was conducted in accordance with both the Declarations of Helsinki and Istanbul and was approved by the local ethics committee, which waived the need for written informed consent (Institutional Review Board—IRB 00006477—of HUPNVS, Paris 7 University, AP-HP— 13-020).

All patients older than 18 years who received an OLT for underlying cirrhosis were included. The non-inclusion criteria were: history of previous liver transplantation, multiple organ

transplantation (combined liver-kidney, liver-lung or liver-heart transplantation), primary graft non-function [19], immediate preoperative infection (including ACLF patients with an infectious trigger) or suspicion of intraoperative infection, and unknown preoperative blood lymphocyte count.

Data Collection

For each patient, clinical and biological data were recorded preoperatively, intraoperatively and postoperatively during ICU stay. Preoperative data included demographic parameters, etiology and severity of underlying liver disease as assessed by MELD score and ACLF before OLT. Intraoperatively, data such as duration of surgery, blood loss and number of packed red blood cell (RBC) units transfused during surgery, cold and warm ischemia times, type of biliary reconstruction (duct-to-duct or Roux-en-Y anastomosis) and reperfusion syndrome were recorded.

Biological data included biochemical, hematological and bacteriological data. Blood cell count data were collected retrospectively from the immediate preoperative period until postoperative day 7 using medical charts.

Patients were followed up during their postoperative ICU stay to record: usual ICU severity score (SAPS II) at admission, postoperative morbidity: infection occurring during the ICU hospitalization and time between OLT and infection, acute renal failure and duration of renal-replacement therapy, mechanical ventilation duration, vasopressor infusion duration and ICU length of stay. Only the first episode of post-OLT infection was considered. Mortality was assessed at day 30 and day 90 after OLT.

Definitions

Pretransplant lymphopenia was defined as a preoperative blood lymphocyte count $<1.50 \times 10^9$/L.

The criteria used to define ACLF were those published by Moreau et al. [20].

All bacterial infections occurring during ICU hospitalization were investigated. Importantly, all infections were diagnosed on the basis of a clinical suspicion that was confirmed by the isolation of a bacteria from microbiological culture. We have used the same definitions in our work as those published in a previous study [21].

The diagnosis of pneumonia was based on Infectious Disease Society of America guidelines [22]. It was consistently suspected on clinical criteria (2 or more of the following characteristics: temperature >38.3°C or <36°C, leukocyte count >10 G/L or <4 G/L, and purulent respiratory secretions) and radiological findings (new lung infiltrate on chest radiography). It was confirmed by a lower respiratory tract microbiological sample (blind protected bronchial sampling (BPSS) or bronchoalveolar lavage (BAL)). The diagnostic thresholds for BPSS and BAL quantitative cultures were 10^3 Colony-Forming Units (CFU)/mL and 10^4 CFU/mL respectively.

Surgical-site infections (SSI) were defined according to the CDC National Nosocomial Infections Surveillance criteria [23] as superficial, deep or organ/space. In this study, only deep and organ/space SSI were considered. Their diagnosis was made on the basis of clinical (at least 1 of the following signs or symptoms: fever >38°C, localized pain or tenderness) and biological (leukocytosis, liver exams abnormalities) signs. It was confirmed by the isolation of bacteria from biliary fluid, from peritoneal fluid containing >250 polymorphonuclear cells/mm^3, or from an intra-abdominal abscess or collection. All these microbiological samples were obtained aseptically when surgical or radiological drainages were performed, or by percutaneous aspiration. No culture of fluid obtained through a previous drain was considered.

Urinary tract infections (UTI) were diagnosed based on the guidelines of the Infectious Disease Society of America [24]. Of note, asymptomatic bacteriuria and uncomplicated UTI were not considered in this work. Complicated UTI were defined by the presence of signs and symptoms compatible (new onset or worsening of fever, rigors, altered mental status, malaise or lethargy, flank pain, costo-vertebral angle tenderness, hemodynamic instability, leukocytosis) with no other source of infection along with a significant growth of a uropathogen ($\geq 10^3$ CFU/mL). Catheter-related UTI diagnosis was requiring signs and symptoms in presence of indwelling urinary catheters and presence of $\geq 10^3$ CFU/mL in a single catheter urine specimen or in a midstream urine in case of urinary catheter removal in the previous 48 h.

Finally, bacteremia was defined as a positive peripheral blood culture bottle result together with clinical and biological signs of infection.

Antimicrobial Prophylaxis Protocol

All patients received an intraoperative antimicrobial prophylaxis. According to our local protocol and following the results of our previous studies [21, 25], the patients received either cefoxitin or a targeted antimicrobial prophylaxis tailored to cover ESBL-E (Extended-Spectrum β-Lactamase-producing *Enterobacteriaceae*) colonizing bacteria in case of known preoperative rectal carriage (carbapenems, piperacillin-tazobactam or cefoxitin according to the antibiotic susceptibility testing). The duration of prophylaxis was limited exclusively to the intraoperative period for all patients.

Immunosuppressive Regimen

For all patients, immunosuppressive regimen consisted in a triple therapy combining glucocorticoids, mycophenolate mofetil and either tacrolimus (in case of normal renal function) or basiliximab (in case of acute or chronic renal failure). All patients received an intravenous bolus of 5 $mg.kg^{-1}$ glucocorticoids intraoperatively and on admission to the ICU, followed by a daily dose reduction. At D7, all patients received only 20 mg glucocorticoids. Mycophenolate mofetil was administered enterally at a dose of 1,500 mg twice daily, or 1,000 mg twice daily intravenously when enteral administration was not possible. For patients receiving basiliximab, a 20 mg dose was administered intravenously on D0 and D4. For these patients, tacrolimus was introduced no later than D7. For patients receiving tacrolimus initially, treatment was initiated at a daily dose of 0.025 $mg.kg^{-1}$ administered by enteral route. Dosage was adjusted daily according to residual tacrolimus

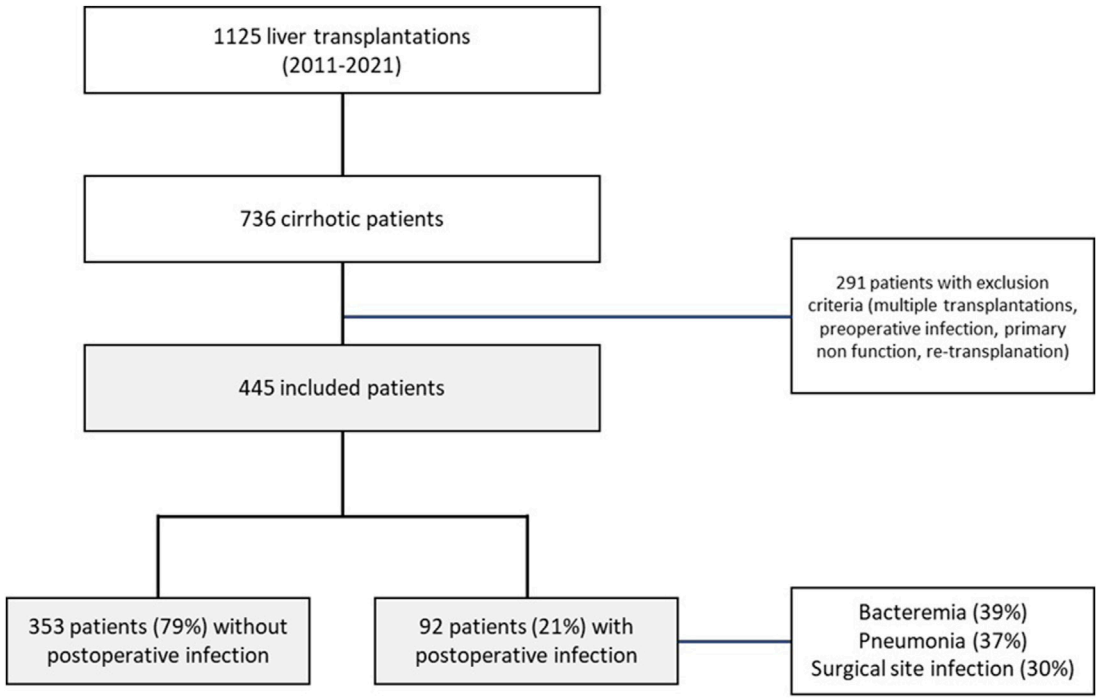


FIGURE 1 | Flow chart.

blood levels. The target residual tacrolimus level was individually adjusted according to the patient's hematocrit and protidemia (ranging, in extreme cases, from 3 to 11 μg/L).

Outcome Variables

The primary outcome was the occurrence of a bacterial infection during the ICU hospitalization following OLT. Secondary outcomes were mechanical ventilation duration, vasopressor infusion duration, ICU length of stay, need for and duration of renal-replacement therapy and ICU mortality within 30 days and 90 days.

Statistical Analyzes

Data were compared using Mann-Whitney U test or Kruskal-Wallis test for continuous variables and using Fisher's exact test for qualitative variables. Variables achieving a p value <0.05 in univariate analysis were introduced into a multivariable logistic regression model with backward elimination (exit $p = 0.05$) in complete cases. Potential collinearity between variables was checked, and the more clinically relevant variable was retained in the case of collinearity. Significant continuous variables identified in the univariable analysis were dichotomized to optimize their sensitivity and specificity using the Youden index with the creation of ROC curves. Then, variables with p values <0.05 by multivariable logistic regression were included in an infection risk score, using the beta coefficient to build the score. A bootstrap analysis with 2000 resampling was used to confirm the result of the multivariable logistic regression model.

Results are expressed as number and percentage or median and interquartile range. All tests were 2-sided and used a significance level of 0.05. Data handling and analysis were performed with SPSS 22.0 (SPSS Inc., Chicago, IL).

RESULTS

Patients' Characteristics

Of the 1,125 patients transplanted at Beaujon Hospital during the study period, 736 had underlying cirrhosis. After exclusion of the 291 patients who did not meet the inclusion criteria, 445 patients were finally included in the study (**Figure 1**). Population characteristics are displayed in **Table 1**. The main cause of liver disease was alcohol-related cirrhosis (55%) and median MELD score on the day of transplantation was 14 [10–20]. Thirty-five (8%) patients underwent liver transplantation for ACLF. Donor information is presented in **Supplementary Table S1**.

Post-OLT infection occurred in 92 patients (21%) during ICU hospitalization, including 9 patients who developed septic shock. These infections were mainly represented by bacteriemia (39%), pneumonia (37%), surgical site infection (30%) and UTI (26%). Species involved in post-OLT infections were mainly *Enterobacterales* and *Enterococci*. The median time between OLT and infection was 5 [4–7] days. Two patients developed a fungal infection in addition to the bacterial infection, and no patient developed a viral infection. There was no difference in the occurrence of post-OLT infection between patients receiving

TABLE 1 | Patients' main characteristics and univariate analysis.

Characteristics	All (n = 445)	Post-LT infection (n = 92)	Absence of post-LT infection (n = 353)	*p*
Baseline characteristics				
Age (years)	57 [51–63]	58 [50–63]	57 [52–63]	0.897
Male sex, n (%)	343 (77)	70 (76)	273 (77)	0.799
BMI ($kg.m^{-2}$)	26 [24–30]	27 [24–31]	26 [24–30]	0.425
Malnutrition, n (%)	164 (37)	42 (47)	122 (35)	**0.050**
Diabetes, n (%)	105 (24)	23 (25)	82 (23)	0.736
HIV coinfection, n (%)	8 (2)	2 (2)	6 (2)	0.763
COPD, n (%)	8 (2)	2 (2)	6 (2)	0.767
Cause of cirrhosis, n (%)				
• Excessive alcohol consumption	243 (55)	55 (60)	188 (53)	0.263
• Metabolic syndrome	115 (26)	27 (29)	88 (25)	0.389
• HCV infection	119 (27)	20 (21)	99 (28)	0.224
• HBV infection	61 (14)	7 (8)	54 (15)	0.056
• Auto-immune hepatitis	19 (4)	5 (5)	14 (4)	0.535
• Cholestatic liver disease	15 (3)	3 (3)	12 (3)	0.948
HCC, n (%)	222 (50)	36 (39)	186 (53)	**0.021**
• HCC compensated cirrhosis	133 (30)	19 (21)	114 (32)	**0.030**
Preoperative ascites, n (%)	140 (31)	33 (36)	107 (30)	0.307
Preoperative encephalopathy, n (%)	169 (38)	49 (53)	120 (34)	**<0.001**
Decompensated cirrhosis, n (%)	312 (70)	73 (79)	239 (68)	**0.030**
History of SBP, n (%)	76 (17)	22 (24)	54 (15)	**0.050**
Severity of cirrhosis				
• MELD	14 [10–20]	17 [11–23]	13 [10–19]	**0.001**
• MELD≥ 25, n (%)	57 (13)	21 (23)	36 (10)	**<0.001**
• ACLF, n (%)	35 (8)	15 (16)	20 (6)	**<0.001**
Pre-LT blood count				
Hemoglobin (g/L)	119 [101–138]	112 [96–132]	121 [103–138]	**0.011**
Hemoglobin<11 g/L, n (%)	149 (33)	44 (48)	105 (30)	**<0.001**
Platelets ($x10^9$/L)	91 [67–130]	81 [61–127]	95 [69–132]	0.077
Leucocytes ($x10^9$/L)	5.20 [4.00–6.80]	5.15 [3.80–7.08]	5.20 [4.05–6.80]	0.966
Neutrophils ($x10^9$/L)	3.10 [2.29–4.20]	3.15 [2.42–4.45]	3.10 [2.20–4.10]	0.333
Lymphocytes ($x10^9$/L)	1.13 [0.74–1.60]	0.98 [0.60–1.47]	1.20 [0.78–1.66]	**0.006**
Lymphocytes≤1.15 × 10^9/L, n (%)	231 (52)	61 (66)	170 (48)	**0.002**
Monocytes ($x10^9$/L)	0.57 [0.40–0.80]	0.59 [0.37–0.80]	0.57 [0.40–0.79]	0.635
Intraoperative characteristics				
Surgery duration (min)	315 [270–360]	320 [270–387]	310 [274–360]	0.375
Cold ischemia time (min)	425 [357–536]	432 [374–540]	420 [351–535]	0.570
Warm ischemia time (min)	45 [38–53]	45 [36–55]	45 [39–52]	0.904
Blood loss (mL)	1,000 [500–1,500]	1,000 [788–2000]	900 [500–1,400]	**<0.001**
Blood loss≥750	254 (57)	63 (68)	191 (54)	**<0.001**
RBC transfusion, n (%)	223 (50)	60 (65)	163 (46)	**<0.001**
Number of RBCs units transfused (U)	1 [0–2]	2 [0–4]	0 [0–2]	**<0.001**
RBCs transfusion>2U (%)	99 (22)	36 (39)	63 (18)	**<0.001**
Reperfusion syndrome, n (%)	224 (50)	50 (54)	174 (49)	0.348
Maximum norepinephrine dose ($\mu g.kg^{-1}.min^{-1}$)	0.55 [0.29–0.93]	0.71 [0.48–1.19]	0.50 [0.25–0.85]	**<0.001**
Maximum norepinephrine dose>0.5 $\mu g.kg^{-1}.min^{-1}$, n (%)	239 (54)	66 (72)	173 (49)	**<0.001**
Biliary reconstruction, n (%)				
• Duct-to-duct	408 (92)	86 (93)	322 (91)	0.792
• Roux-en-Y anastomosis	6 (1)	1 (1)	5 (1)	0.792

Mann-Whitney U test used for continuous variables. Chi-square test used for categorical variables. Results are expressed as number (percentage) or median [interquartile range]. p-value <0.05 was considered significant. p-values in bold are significant. BMI, body mass index; HIV, Human Immunodeficiency Virus; COPD, chronic obstructive pulmonary disease; HCV, hepatitis C virus; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; SBP, spontaneous bacterial peritonitis; MELD, Model for End-stage Liver Disease; ACLF, Acute on Chronic Liver Failure; RBC, red blood cells.

basiliximab (31%) and those receiving tacrolimus (69%) as an immunosuppression induction regimen: 24% and 19% respectively ($p = 0.163$).

Complications and mortality are presented in **Supplementary Table S2**. Patients who developed post-OLT infection had higher 30-day and 90-day mortality rates than those who did not. Moreover, durations of mechanical ventilation, vasopressor infusion and ICU stay were longer, and renal-replacement therapy requirement was more frequent among patients who developed an infection. Fifteen patients (3%) had died by postoperative day 90. Among them, 13 patients had died during ICU-hospitalization.

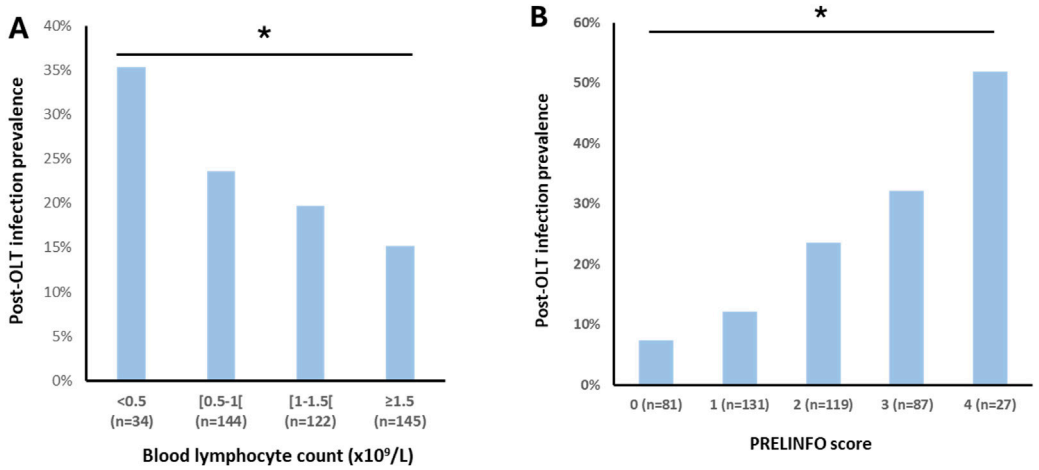


FIGURE 2 | Post-OLT infection prevalence according to preoperative blood lymphocytes count (panel **(A)**) and PRELINFO score (panel **(B)**) Chi-square test used. Bold stars indicate statistically significant difference ($p < 0.05$).

TABLE 2 | Features associated with the primary endpoint by a multivariable logistic regression model.

Risk factor	OR [IC 95%]	*p*	Point
Preoperative encephalopathy	1.764 [1.047–2.974]	**0.033**	**1**
Lymphocytes≤1.15 × 10^9/L*	1.836 [1.064–3.168]	**0.029**	**1**
RBCs transfusion>2U**	2.160 [1.242–3.755]	**0.006**	**1**
Maximum norepinephrine dose>0.5 μg.kg^{-1}.min^{-1}**	2.457 [1.406–4.296]	**0.002**	**1**

*Multivariable logistic regression with backward elimination (exit p = 0.05). Results presented as OR [CI 95%] (p). p-value <0.05 was considered significant. p-values in bold are significant. RBC, red blood cells. * Immediate preoperative data. **Intraoperative data.*

Association Between Preoperative Blood Lymphocyte Count and Prevalence of Post-OLT Infections

Univariate analysis investigating factors associated with post-OLT infections is displayed in **Table 1**. Patients who developed a post-OLT infection had a lower preoperative blood lymphocyte count than those who did not. Patients with a blood lymphocyte count of less than 1.150 × 10^9/L had an almost 2-fold increased risk of post-operative infection.

We further analyzed the prevalence of post-OLT infections in different sub-groups according to the preoperative blood lymphocyte count: <0.5 × 10^9/L, between 0.5 and 1.0 × 10^9/L, between 1.0 and 1.5 × 10^9/L and ≥1.5 × 10^9/L. Results are displayed in **Figure 2A**. The lower the preoperative blood lymphocyte count, the higher the prevalence of post-OLT infections ($p = 0.047$).

Risk Factors for Post-OLT Infections

By multivariate regression analysis with backward elimination, preoperative encephalopathy, lymphocytes ≤1.150 × 10^9/L, intraoperative RBC transfusion >2 U and intraoperative maximum norepinephrine dose >0.5 μg.kg^{-1}.min^{-1} were independent predictors of post-OLT infections (**Table 2**). Bootstrapping methods (2000 resampling) confirmed that they were all independent predictors of post-OLT infections. Results are displayed in **Supplementary Table S3**.

The sensitivity and specificity associated with the risk of post-OLT infection of each criterion were as follows: preoperative encephalopathy: 53% and 66%; blood lymphocyte count <1.150 × 10^9/L: 66% and 52%; intraoperative RBC transfusion >2 U: 39% and 82%; intraoperative maximum norepinephrine dose >0.5 μg.kg^{-1}.min^{-1}: 72% and 51%, respectively.

PRELINFO Score

Since the beta coefficients were roughly similar for these 4 risk factors of post-OLT infections, a score of one point was then attributed to each of them to build the PRELINFO (PRediction of EarLy INfection Following Orthotopic liver transplantation) score. The PRELINFO score ranged from 0 to 4 points. The prevalence of post-OLT infections for PRELINFO score of 0, 1, 2, 3 and 4 points was respectively 7.4%, 12.2%, 23.5%, 32.2% and 51.9% ($p < 0.05$) (**Figure 2B**). Thus, patients with a score of 0 (18%) or 1 (12%) had a low risk, while patients with a score of 3 (20%) or 4 (6%) had a medium or high risk of post-OLT infections.

We assessed the specific contribution of adding lymphopenia to the score in predicting infectious risk. Among patients who developed a postoperative infection (n = 92), 66% (n = 61) were effectively reclassified into a higher-risk category when lymphopenia was included in the score: including 24% (n = 22) in low-risk groups (0–2), and 42% (n = 39) in higher-risk groups (3–4). Conversely, among patients who did not develop a

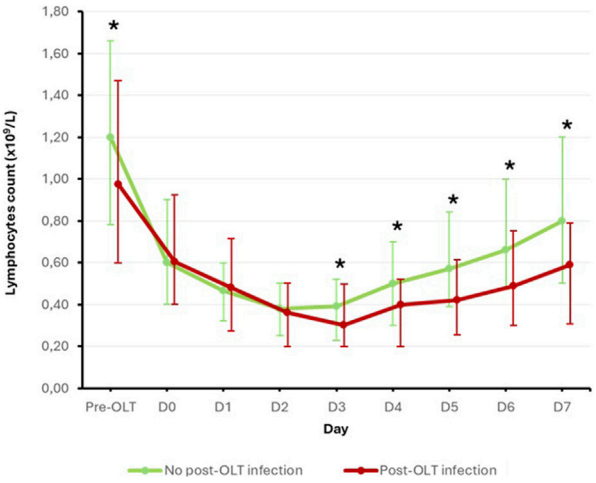


FIGURE 3 | Evolution of blood lymphocyte count according to the primary endpoint Mann-Withney U test used. Results are presented as median [interquartile range]. Bold star indicates statistically significant difference ($p < 0.05$).

postoperative infection (n = 353), 30% were erroneously reclassified to a higher-risk category in the low-risk groups, and 18% in higher-risk groups.

Evolution of Blood Lymphocyte Count According to the Primary Endpoint Before and After OLT

Finally, to better understand the role of preoperative lymphopenia in the occurrence of post-OLT infections, we analyzed the evolution of blood lymphocyte count before and within the first 7 days after OLT in the two subpopulations according to the occurrence of the primary endpoint. Results are displayed in **Figure 3**. All patients experienced a decrease in their blood lymphocyte count in the first few days post-OLT. Interestingly, while blood lymphocyte counts decreased to similar levels from D0 to D2 in both groups, patients in the post-OLT infection group showed a statistically slower resurgence in blood lymphocyte counts from D3 onwards than patients in the no post-OLT infection group.

Subgroup Analysis

In our cohort, 312 patients (70%) were transplanted for decompensated liver disease and 133 (30%) for HCC with compensated cirrhosis. HCC was a protective factor for the occurrence of post-OLT infection (OR = 0.57 [0.361–0.922], $p = 0.021$). In the HCC-compensated subgroup, 19 (14%) patients developed a post-OLT infection, and preoperative lymphocyte count was not significantly associated with infection risk ($p = 0.870$). We conducted a secondary analysis in the decompensated cirrhosis subgroup (n = 312) which included 73 (23%) patients who developed a post-OLT infection. The results confirmed the data obtained on the main population and are presented in **Supplementary Tables S4, S5**. As in the main cohort, the PRELINFO score was associated with the occurrence of post-LT infection ($p < 0.001$).

DISCUSSION

In our study, 21% of patients presented at least one bacterial infectious complication during the hospitalization in intensive care following OLT for cirrhosis. These infections, mainly represented by bacteremia, pneumonia and surgical site infection, occurred in median 5 days after the transplantation. In our cohort, we have highlighted that the lower the preoperative blood lymphocyte count, the higher the prevalence of infections. In multivariate analysis, preoperative blood lymphocyte count $\leq 1.150 \times 10^9$/L was found to be an independent risk factor for early post-OLT infections as was preoperative MELD ≥25, and intraoperative RBC transfusion >2 U during the liver transplantation. These parameters were integrated into a predictive score for early bacterial infections following liver transplantation: the PRELINFO score. The higher the score, the greater the risk of early post-OLT infections. Finally, the kinetics of lymphocyte count during the first seven days after OLT differed between patients who would develop a postoperative infection and those who would not. The preoperative lymphocyte count was lower, and while all patients were similarly lymphopenic in the early postoperative days, the recovery from lymphopenia was slower in the post-OLT infection group.

Infections are a major cause of morbidity and mortality after OLT [3, 12, 26, 27]. The incidence of infections reported in the literature can reach 80% in the year following the transplantation [26]. In the vast majority of cases (around 70% of cases), these are bacterial infections [12, 26], and the early postoperative period seems to be particularly at risk [1, 28]. Indeed, 20%–40% of patients would develop a bacterial infection within the first month following the OLT [3, 12]. In our study, we found similar results to the literature in terms of infected sites (bacteremia, pneumonia and surgical site infections) [3, 12, 28] but the infection rate was in the low range of what is described in the literature (21%). This can be explained on the one hand by the fact that only infections occurring during ICU hospitalization (median length of stay 8 days [6–12]) have been collected, and on the other hand by the study population which was relatively selected (inclusion of cirrhotic patients exclusively, exclusion of re-transplantations, multiple transplantations, patients with immediate preoperative infection or suspicion of intraoperative infection especially). Possibly for the same reasons, we observed fewer septic shocks than described by Laici et al. in 2018 [3] who found that post-OLT infections were complicated by a septic shock in almost a quarter of cases and were responsible for almost half of the deaths occurring at day 90.

There were several reasons for analyzing the relationship between lymphopenia and post-OLT infections. First, the risk of bacterial infection after solid organ transplantation seems to increase with the degree of immunosuppression [29]. The latter depends not only on the immunosuppressive treatments introduced after the transplantation but also on the pre-

existing level of immunosuppression specific to each patient [1, 12]. Since a few years, alterations in the immune response in cirrhotic patients have been described under the term "Cirrhosis-Associated Immune Dysfunction," for which lymphopenia is thought to have an important role [14, 15, 30]. Moreover, preoperative lymphocyte count was associated with the occurrence of infection in the subgroup of patients with decompensated cirrhosis and was not in the subgroup of patients transplanted for HCC with compensated cirrhosis. This is consistent with the fact that CAID, and hence lymphopenia, is more pronounced in patients with advanced cirrhosis. Thus, the absolute lymphocyte count could be a simple and accessible marker to easily assess the basal immunosuppression state of cirrhotic patients before OLT. Recently, lymphopenia at the time of the liver transplantation has been associated with short-term mortality [31]. In this study, the authors found that patients with very low preoperative lymphocyte count (<500/μL) had a higher risk of mortality, particularly sepsis-related mortality, and of bacteremia within 180 days post-OLT. However, this study was not designed to assess the relationship between preoperative blood lymphocyte count and early postoperative infections. Our results provide new data by considering all bacterial sepsis and focusing on the early postoperative period, known to be particularly at risk [1, 28]. Moreover, we demonstrate an effect of lymphopenia on the occurrence of infections from a higher lymphocyte threshold ($<1.15 \times 10^9$/L). Another study showed that patients who developed infection after OLT had a higher neutrophil-to-lymphocyte ratio the day before the sepsis than those who did not, suggesting that a low postoperative lymphocyte count is associated with the risk of infection after OLT [32]. However, this study failed to show an effect between preoperative neutrophil-to-lymphocyte ratio or preoperative lymphocyte count and postoperative infections. In our study, we analyzed the preoperative neutrophil-to-lymphocyte ratio which was less accurate than lymphocyte count in predicting early postoperative infections (data not shown). Finally, Riff et al. found similar results with regard to the kinetics of post-OLT lymphocyte count in patients with cirrhosis [33]. Thus, to the best of our knowledge, our study is the first to show a clear association between preoperative lymphocyte count and the occurrence of early post-OLT bacterial infections in patients with cirrhosis.

Secondly, treatments limiting immune rejection have a major role in the postoperative immunosuppression state [29, 34]. The main immunosuppressive treatments used after OLT (glucocorticoids, tacrolimus and mycophenolate mofetil) all cause qualitative or quantitative lymphocyte alterations, thus worsening the potential pre-existing lymphopenia. Although the risk of post-OLT rejection is becoming low, in particular by the improvement in immunosuppressive treatments, the balance between risk of infection and risk of transplant rejection remains challenging [35]. A recent national survey, assessing perioperative management practices, found that 30% of OLT centers modified the immunosuppressive regimen (mainly by reducing tacrolimus or corticosteroid doses) in case of postoperative suspected sepsis [36]. In the future, studies will be needed to determine the value of preoperative blood lymphocyte count to individualize the immunosuppressive regimen in the immediate postoperative period based on the assessment of post-OLT infection risk.

Among the other risk factors for post-OLT infections described in the literature, the MELD score is inconsistently found. Some studies did not find an association between MELD and the occurrence of surgical site infection [37], pneumonia or bacteremia after OLT [38]. Conversely, a study published in 2013 by Avkan-Oguz et al. found an association between a MELD score >20 and the onset of bacterial infections within 30 days after the OLT, whatever the infected sites [5]. In our study, in multivariate analysis, the history of encephalopathy was a more accurate factor than MELD in reflecting the impact of cirrhosis severity on the risk of post-OLT infection. Intraoperative RBC transfusion has also been studied by other teams and is frequently described as a predictor of post-OLT infections [5, 6, 39, 40]. However, while the immunosuppressive role of transfusion is recognized [41], the significant thresholds in terms of transfusion volume vary between studies [5, 6]. While Avkan-Oguz et al. [5] considered a transfusion threshold greater than 6 U as a risk factor for post-OLT infections, the threshold we used in our study (>2U) was based on the evaluation of the Youden index and can be explained by a very low median of intraoperative transfusion in our cohort (1 U [0–2]). Rarely described in the literature in this way, we also show in our study a link between intraoperative severity represented by the maximum norepinephrine dose and the risk of early post-OLT infection. Other risk factors have been described in the literature such as undernutrition, renal-replacement therapy, the need for retransplantation, history of COPD or even a Roux-en-Y anastomosis biliary reconstruction [3, 5, 37]. Interestingly, the type of biliary reconstruction was not identified as a risk factor in our work, but this is most likely linked to the very high predominance of duct-to-duct reconstruction in our cohort (92%).

We propose a simple, pragmatic score for bedside use in the immediate post-OLT period to assess the risk of early infection. The preoperative blood lymphocyte count and the PRELINFO score could be used to adapt the immunosuppression regimen and indicate closer monitoring of the development of bacterial infection or even pursue more prolonged antibiotic prophylaxis in patients most at risk.

Our study has several limitations. First, it is a monocentric study. Although many characteristics of our population are consistent with what is reported in the literature, the results we present need to be validated in an external cohort. To be used in everyday practice, the PRELINFO score would need to be validated in another prospective, multicenter study. In order to limit this bias, we used a bootstrap analysis providing an internal validation of the multivariable logistic regression model. Second, although the database was completed prospectively, some specific data of our work have been collected retrospectively potentially biasing the results. Furthermore, we cannot rule out the possibility that certain data that we were unable to collect may have affected lymphocyte levels (e.g., certain patient-specific treatments) or

the risk of infection (e.g., cumulative dose of tacrolimus or mycophenolate mofetil, hypogammaglobulinemia). Finally, the study period is relatively long. It is possible that medical and surgical practices have evolved over time, thus influencing the results we have observed.

In conclusion, early bacterial infections after OLT for cirrhosis are a relatively frequent phenomenon and represent a real challenge in terms of morbidity and mortality in the early post-operative period. We highlight several known risk factors and the role of preoperative lymphopenia in the occurrence of these infections. These results suggest that preoperative blood lymphocyte count should be incorporated into the assessment of the risk of post-OLT bacterial infections, and that further studies should be carried out to clarify its use in defining the immunosuppression regimen in the early postoperative period.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. Requests to access these datasets should be directed to MG mikhael.giabicani@aphp.fr.

ETHICS STATEMENT

The studies involving humans were approved by Institutional Review Board of HUPNVS, Paris 7 University, AP-HP— 13-020. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

MG, CT, and LC participated in research design, in the writing of the paper, in the performance of the research and in data analysis. PD, MGu, MH, SJ, JK, SS, SD, FD, ML, and OR participated in the performance of the research. TT-S participated in the performance of the research and in data analysis. FDu and EW participated in research design, in the writing of the paper, in the performance of the research and in data analysis. 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.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.14372/full#supplementary-material

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A Retrospective Test-Negative Case-Control Study to Evaluate Influenza Vaccine Effectiveness in Preventing Influenza Among Immunocompromised Adults With a Solid Organ Transplant

Manon L. M. Prins[1,2], Ernst D. van Dokkum[3,4], Aiko P. J. de Vries[5], Maarten E. Tushuizen[6], Danny van der Helm[5], Edwin M. Spithoven[7], Irene M. van der Meer[8], J.H. Marc Groeneveld[9], Leo G. Visser[1], Saskia le Cessie[10], Albert M. Vollaard[11] and Geert H. Groeneveld[1,2]*

[1]LUCID, Subdepartment of Infectious Diseases, Leiden University Medical Center, Leiden, Netherlands, [2]Division Acute Internal Medicine, Department of Internal Medicine, Leiden University Medical Center, Leiden, Netherlands, [3]Department of Public Health and Primary care, Leiden University Medical Center, Leiden, Netherlands, [4]Health Campus The Hague, Leiden University Medical Center, The Hague, Netherlands, [5]Leiden Transplant Center, Leiden University Medical Center, Leiden, Netherlands, [6]Department of Gastroenterology and Hepatology, Leiden University Medical Center, Leiden, Netherlands, [7]Department of Internal Medicine, Amphia Hospital, Breda, Netherlands, [8]Department of Nephrology, Haga Teaching Hospital, The Hague, Netherlands, [9]Department of Nephrology, Haaglanden Medical Center, The Hague, Netherlands, [10]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands, [11]Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, Netherlands

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***Correspondence**
Manon L. M. Prins,
m.l.m.prins@lumc.nl

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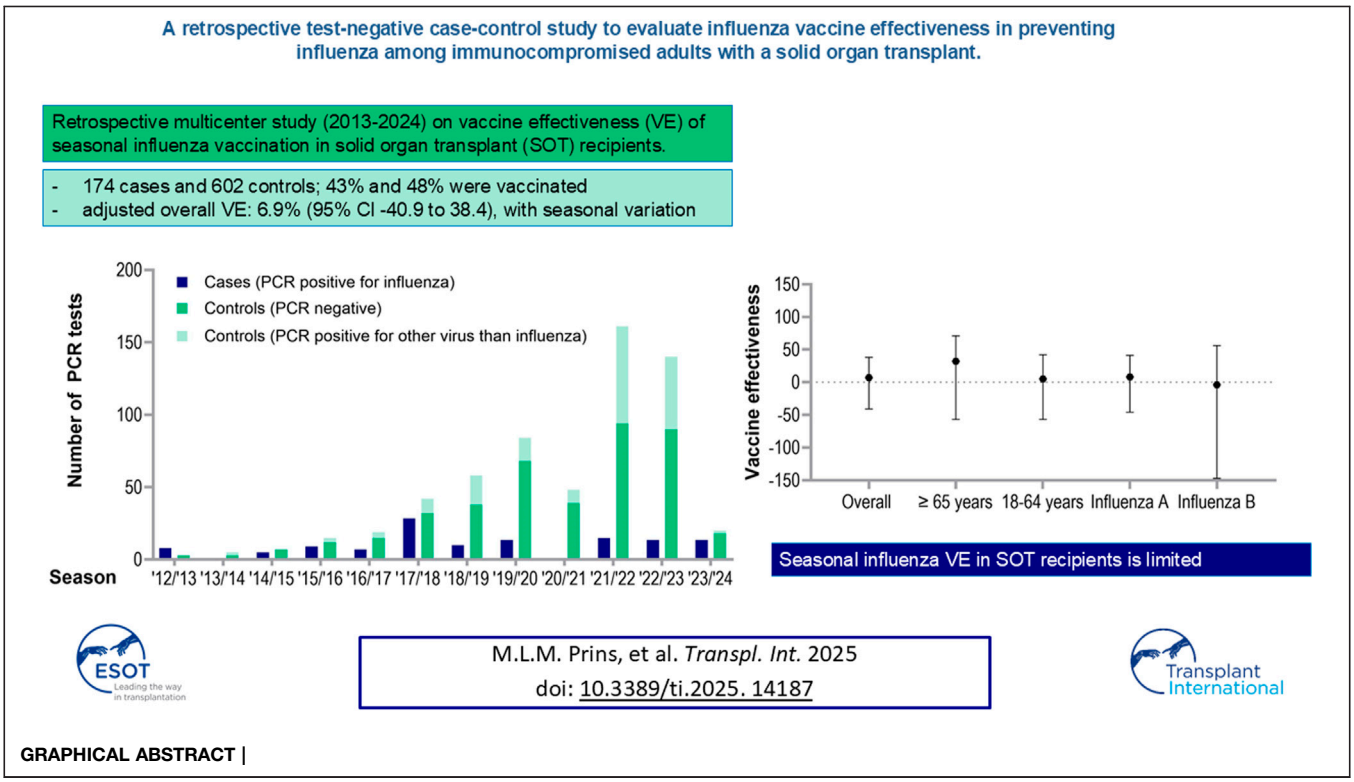
Vaccination may prevent influenza in solid organ transplant (SOT) recipients. This study evaluates the influenza vaccine effectiveness (VE) in this high-risk population in the Netherlands. We also compared disease progression and 30-day mortality between vaccinated and unvaccinated influenza patients. In this multicenter, test-negative case-control study, SOT recipients with respiratory symptoms were included when tested for viral respiratory infections during the respiratory seasons between 1 January 2013 and 1 July 2024. Cases had a positive influenza PCR, while controls tested negative. Influenza vaccination in cases (74/174) and controls (291/602) were compared after adjusting for potential confounders. VE was calculated as (1-adjusted odds ratio) x 100. The overall VE was 6.9% (95% CI –40.9 to 38.4), with considerable variation across seasons. For those aged ≥65 years, VE was higher (32.4%, 95% CI –56.5–70.8) compared to those aged 18–64 years (4.8%, 95% CI –56.5 to 42.1). The adjusted VE against influenza A [7.5% (–46.0 to 41.3)] was higher than against influenza B (–3.8% (–146.7 to 56.3)). No differences in influenza-related complications were observed between the vaccinated and unvaccinated cases. The observed seasonal influenza vaccine effectiveness in adult SOT recipients is limited; further investigation for improvement is warranted.

Keywords: influenza, influenza vaccine effectiveness, influenza vaccination, Netherlands, solid organ transplant patients



GRAPHICAL ABSTRACT |

INTRODUCTION

Influenza viruses are globally among the most common causes of respiratory infections in both immunocompetent and immunocompromised individuals, like recipients of a solid organ transplant (SOT) [1]. The prevalence of seasonal influenza among viral pathogens in SOT recipients may vary annually, depending on the types and intensity of circulating viruses, vaccine coverage (i.e., the percentage of a specific population that has received the vaccine), vaccine efficacy related to vaccine-match and dosage of influenza vaccines, type of transplant, and adherence to non-pharmacological interventions [2]. National data from Finland suggests a substantial increased likelihood of detecting laboratory-confirmed influenza and hospitalization due to influenza in kidney transplant recipients compared to the general population [3].

While infection in healthy, immunocompetent individuals may present as a mild and self-limiting condition [4], SOT recipients have an increased risk of influenza-related complications, including secondary bacterial pneumonia, acute graft rejection and mortality [2, 5–8]. Moreover, SOT patients with influenza have a significantly elevated risk of hospitalization, up to 70% [3, 7, 9].

Annual seasonal vaccination is the primary measure for preventing influenza [2] and is universally recommended for SOT recipients [10]. Nevertheless, vaccination rates among SOT recipients are reported to be low in both US and European settings and nearly half of SOT recipients were unvaccinated in registries from the US and Denmark [11, 12].

Lifelong use of immunosuppressive medication affects the lymphocyte function of SOT recipients, thereby leading to an immunocompromised status. Several mechanisms are known, depending on the specific immunosuppressive drug used: reduced T-cell activity, direct suppression of B-cells or antibody production, suppression of cytokine production or inhibition of immune cell proliferation and differentiation. The amount of impairment depends on several factors, such as type of transplant, type of immunosuppression such as mycophenolate or co-stimulation blockers, use of T-cell depleting agents in the year before vaccination and time since transplantation [2, 13]. Consequently, the immunogenicity of the influenza vaccine in SOT recipients is reduced compared to immunocompetent persons, reported as reduced serologic immune responses to influenza vaccines and lower seroprotection rates, based on hemagglutination-inhibition (HI) titers [6, 13–21]. In addition to the immunological (surrogate) marker, two other clinical outcome measures are commonly used for the protective effects of vaccines: vaccine efficacy and vaccine effectiveness (VE). Vaccine efficacy refers to how well a vaccine performs in controlled settings (e.g., clinical trials), while VE describe its performance in real-world conditions. Ultimately the VE is the most relevant outcome. The immune response does not always correlate with the clinical effectiveness of a vaccine. In addition, the VE of the influenza vaccine varies yearly, with mismatches negatively

affecting its effectiveness [22]. In the general population, influenza VE ranged from 19% to 59%, with lower percentages among people above 65 years [23–31]. However, studies on the VE of the influenza vaccine in SOT recipients are lacking and therefore its effectiveness remains controversial. In several epidemiological studies, the benefit of influenza vaccination in SOT recipients is only reported in relation to disease progression and the occurrence of complications, such as pneumonia, graft outcomes, intensive care unit (ICU) admission and mortality [9, 12, 19, 32, 33].

The aim of this study is to determine the influenza VE among immunocompromised adult SOT recipients in the Leiden transplantation region in the Netherlands.

MATERIALS AND METHODS

Study Design

We performed a multicenter, retrospective test-negative case-control study [34] to estimate VE of seasonal influenza vaccination in SOT recipients. Patients in the Leiden University Medical Center (LUMC), one of seven transplantation centers in the Netherlands, and its seven affiliated shared-care hospitals (Alrijne Hospital, Amphia Hospital, Groene Hart Hospital, Haga Hospital, Haaglanden Medical Center, Reinier de Graaf Hospital, Spaarne Hospital), were eligible. The study period was between 1st January 2013, and 1st July 2024.

Study Participants

All adult patients (≥18 years) who received a SOT (kidney, liver, pancreas, islet cells of Langerhans, or a combination of these), and underwent diagnostic testing for influenza in an outpatient setting or within 24 h after hospital admission, were included. Other types of SOT, such as heart or lung transplants, were not included, as these are not performed at the LUMC. The standard protocol in our center mandates SOT recipients to contact the hospital (academic hospital or the nearest affiliated hospital, depending on the duration post-transplantation and the hospital were the patient is monitored) if they experience fever or respiratory symptoms. Influenza diagnostics via polymerase chain reaction (PCR) are readily available during the respiratory virus season in the emergency departments or outpatient clinics. We included only symptomatic patients. The indication for PCR test was determined by the treating physician and hospital.

The respiratory virus season in the Netherlands spans from week 40 in 1 year to week 20 in the following year (early October to mid-May) [35]. Subjects enrolled outside this season were excluded from analysis to avoid bias by calendar time [22]. Patients could be included only once a season, but could be included multiple times if they were tested for influenza during multiple seasons. They were classified as cases if there was at least one positive test during the respiratory virus season; otherwise they were controls. For cases, outcomes up to 30 days following the first positive test were studied, for controls outcomes after the first negative test.

Patients were defined as vaccinated if they had received the seasonal influenza vaccine (standard dose) in the ongoing respiratory virus season, prior to PCR testing. Patients were defined as unvaccinated if no influenza vaccine was received in the current season prior to PCR testing.

Data Collection

In the Netherlands, the seasonal influenza vaccine, standard-dose trivalent (season 2013/14–2018/19) or quadrivalent (since 2019/20) vaccine, is administered to risk groups by general practitioners (GP), primarily in the months October and November. Influenza vaccination is free of charge. After receiving a standard-dose influenza vaccination, the GP documents the type and date/month of this vaccination in their GP electronic information system. Therefore, data regarding influenza vaccination history was obtained by contacting the patient's GP, either through a letter/email or by phone. In cases where the vaccination history was not accurately recorded at the GP, the patient was contacted directly. Patients were excluded from analysis if no information was available regarding their vaccination status.

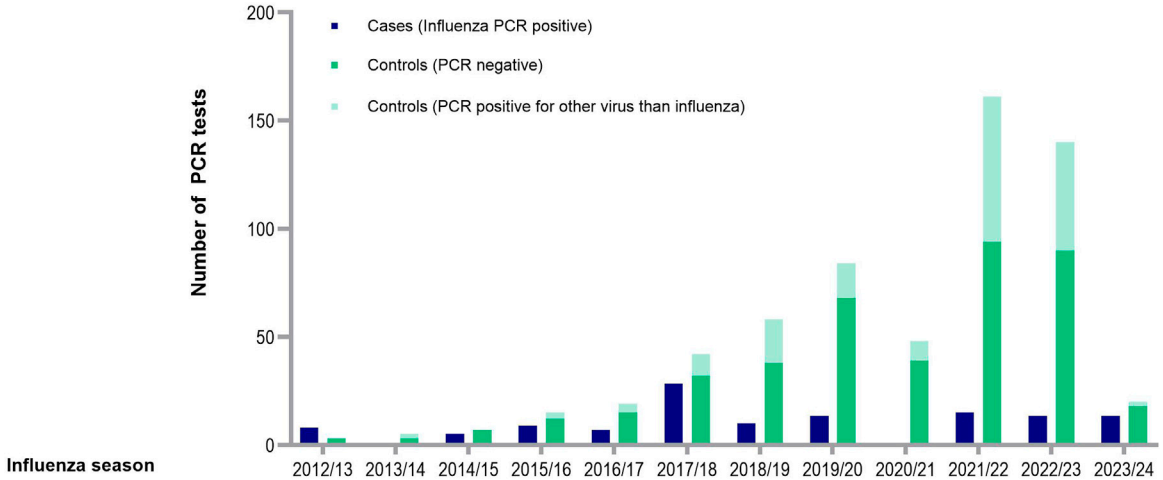
In addition, we retrieved detailed clinical information from the electronic healthcare records, including baseline demographics, test results for (other) respiratory pathogens, comorbidities, and use of immunosuppressive agents. Comorbidity was categorized into cardiovascular disease (CVD), chronic pulmonary disease and diabetes mellitus (DM). The degree of immunosuppression was determined by the type of induction, maintenance and/or rejection therapy. Patients were considered to be highly immunosuppressed if they were treated with triple therapy and/or had received lymphocyte depleting agents (anti-thymocyte globulin and/or alemtuzumab) in the preceding 6 months.

Outcome Measures

The primary outcome is the adjusted influenza VE over the whole period in preventing the occurrence of laboratory-confirmed influenza in patients with a SOT. Adjusted VE by season, age group and by influenza subtype were also determined. Secondary to this, we compared course of disease (hospital length of stay, ICU-admission, need for mechanical ventilation) and 30-day mortality between vaccinated and unvaccinated lab-confirmed influenza patients.

Sample Size

The influenza vaccination rate for the entire target population has varied from 50% to 57% in the Netherlands in recent years [36]. The VE in the overall vaccinated population in the Netherlands ranged from 31% to 57% [23, 24]. Based on that data, our hypothesis is that the VE in SOT recipients is around 40%, and the vaccination rate in this group is 50%. This VE corresponds to an odds ratio (OR) of 0.6 and a vaccination rate of 0.375 in the influenza-positive group. Based on an expected case/control ratio of 1/3, the required sample size is 165 cases and 495 controls to detect a VE of 40% with a power of 80% and an alpha of 0.5.



Influenza season	2012/13	2013/14	2014/15	2015/16	2016/17	2017/18	2018/19	2019/20	2020/21	2021/22	2022/23	2023/24
VE in SOT recipients	NA*	NA*	99	95	-517	-16	-35	-30	NA*	-32	38	30
VE in general population in the Netherlands	62&	65&	-11&	44&	48&	42&	27&	48^	NA*	34^	52#	50#
Incidence influenza in SOT recipients (%)	0.80	0.07	0.53	0.79	0.45	2.08	0.62	0.85	0	0.823	0.74	0.73

FIGURE 1 | Number of cases and controls, incidence of influenza in SOT recipients and influenza vaccine effectiveness each respiratory season. Presented in the figure are the amount of cases and controls each respiratory season. Below the figure, the adjusted VE in SOT recipients is presented each respiratory year, compared to the yearly influenza VE in the general population in the Netherlands, reported by the National Institute for Public Health and the Environment. In addition, incidence of influenza cases is calculated among all SOT recipients still alive during a respiratory season at January 1 of that season. *NA because no cases were detected (2020/2021) or the sample size was too small (2012/2013, 2013/2014). &Reported by the National Institute for Public Health and the Environment. ^Adjusted for the confounders age, history of chronic pulmonary disease, history of rejection therapy, hospital of inclusion, season, use of cell division inhibitors, highly immunosuppressed status. Abbreviations: NA, not applicable; VE, vaccine effectiveness; PCR, polymerase chain reaction; SOT, solid organ transplant.

Statistical Analysis

Continuous variables were reported as means and standard deviations (SD) or as median and interquartile range (IQR), depending on distribution. Categorical variables were reported as numbers and percentages. Baseline differences between groups were evaluated using the independent T-test, Mann-Whitney U test and Chi-squared test, with significance set at $p < 0.05$. VE was calculated as (1-adjusted OR) x 100% and reported as percentages. The OR is the ratio of the odds of being vaccinated versus not vaccinated with a standard vaccine dosage against influenza among cases and controls. Adjusted ORs and 95% confidence intervals (95% CI) were calculated using multiple logistic regression, with influenza PCR results as the outcome and vaccination status as the primary variable at interest. A univariate logistic regression analysis identified factors independently associated with influenza status, with variables showing $p < 0.10$ included in the multivariable model (age, history of chronic pulmonary disease, history of rejection therapy, hospital of inclusion, season), alongside clinically relevant factors (use of mycophenolic acid [cell division inhibitors] or highly immunosuppressed status). Incidences were calculated by dividing the number of new influenza cases during a respiratory season by the total number of individuals who underwent organ transplantation at the LUMC and were still alive on January 1 during that season, multiplied by 100. All calculation were made using SPSS statistics 25.0 for Windows.

Reporting and Ethics

The study was done in accordance with Good Clinical Practice Guidelines. The study was approved by the Institutional Review Board of the LUMC (nWMODIV2_2022034) and the need for informed consent was waived. The study was described according to the STROBE checklist for observational studies.

RESULTS

After excluding 30 patients due to missing vaccination data, 776 participants were included in the analysis: 174 cases and 602 controls. Of all the participants, 207 were included more than once, including 29 cases and 178 controls. Among the controls, 183 had a positive PCR result for another viral pathogen, while 419 patients had a negative result (**Figure 1**). Of the patients with positive PCR, SARS-CoV-2 (59%), respiratory syncytial virus (16%) and rhinovirus (13%) infections were most common. Most controls underwent PCR testing in 2022 (28.7%), followed by 2023 (15.1%),

TABLE 1 | Characteristics of patients included in the analysis.

	Overall (n = 776)	Influenza negative/ controls (n = 602)	Influenza positive/ cases (n = 174)	p[a]
Male sex	459 (59.1)	360 (59.8)	99 (56.9)	0.49
Age, mean (SD)	59.7 (13.4)	60.8 (13.3)	56.2 (13.3)	<0.001
BMI, mean (SD)	25.9 (5.1)	25.8 (5.0)	26.2 (5.6)	0.42
Type of influenza				
A	129 (16.6)	-	129 (74.1)	-
B	45 (5.8)		45 (25.9)	
Month of testing				<0.001
January	149 (19.2)	99 (16.4)	50 (28.7)	
February	133 (17.1)	92 (15.3)	41 (23.6)	
March	141 (18.2)	103 (17.1)	38 (21.8)	
April	89 (11.5)	77 (12.8)	12 (6.9)	
May	34 (4.4)	32 (5.3)	2 (1.1)	
October	61 (7.9)	60 (10.0)	1 (0.6)	
November	66 (8.5)	65 (10.8)	1 (0.6)	
December	103 (13.3)	74 (12.3)	29 (16.7)	
Pre-existing cardiovascular disease	649 (83.6)	506 (84.1)	143 (82.2)	0.56
Pre-existing lung disease	227 (29.3)	186 (30.9)	41 (23.6)	0.06
	119 (15.3)	99 (16.4)	20 (11.5)	0.11
Asthma/COPD	142 (18.3)	119 (19.8)	23 (13.2)	0.05
Other[b]				
Pre-existing diabetes	309 (39.8)	241 (40.0)	68 (39.1)	0.82
Empiric antibiotics	189 (24.4)	155 (25.7)	34 (19.5)	0.09
Time between transplantation and PCR in years, median (IQR)	7 (3–13)	7 (3–13)	6 (2–12)	0.01
Type transplantation				0.41
Kidney	642 (82.7)	503 (83.6)	139 (79.9)	
Pancreas	2 (0.3)	2 (0.3)	-	
Islets of Langerhans	2 (0.3)	1 (0.2)	1 (0.6)	
Liver	105 (13.5)	77 (12.8)	28 (16.1)	
Kidney & pancreas	13 (1.7)	8 (1.3)	5 (2.9)	
Kidney & liver	11 (1.4)	10 (1.7)	1 (0.6)	
Kidney & islets of Langerhans	1 (0.1)	1 (0.2)	-	
Type induction[c]				0.88
IL-2 inhibitor	440 (87.8)	336 (88.0)	103 (86.6)	
Alemtuzumab	47 (6.1)	36 (9.4)	12 (10.1)	
No. of				0.32
Immunosuppressive	68 (8.8)	50 (8.3)	18 (10.3)	
agents	402 (51.8)	322 (53.5)	80 (46.0)	
1	305 (39.2)	229 (38.0)	76 (43.7)	
2				
3				
Type of				
immunosuppressive	675 (87.0)	522 (86.7)	153 (87.9)	0.67
agents	612 (78.9)	479 (79.6)	133 (76.4)	0.37
Corticosteroids	449 (57.9)	343 (57.0)	106 (60.9)	0.35
Calcineurin inhibitors	52 (6.7)	41 (5.9)	11 (6.8)	0.82
Cell division inhibitors	48 (6.2)	37 (6.1)	12 (7.9)	0.93
MTOR inhibitors				
Lymphocyte depleting agents				
Rejection therapy	151 (19.5)	108 (17.9)	43 (24.7)	0.047
<6 months ago	12 (1.5)	10 (1.7)	2 (1.1)	
Once	139 (17.9)	98 (16.3)	41 (23.6)	
Never	625 (80.5)	494 (82.1)	131 (75.3)	
Type of rejection therapy				
Solumedrol	124 (16.0)	88 (14.6)	36 (20.7)	0.10
Alemtuzumab	36 (4.6)	25 (4.2)	11 (6.3)	0.26
ATG	31 (4.0)	21 (3.5)	10 (5.7)	0.22
Other[d]	39 (5.0)	28 (4.7)	11 (6.3)	0.37
Time between rejection therapy and PCR in years, median (IQR)	6 (2–16)	2 (6–15)	6 (3–18)	0.07
Hospital of inclusion				<0.001
Hospital 1	26 (3.4)	21 (3.5)	5 (2.9)	
Hospital 2	88 (11.3)	78 (13.0)	10 (5.7)	
Hospital 3	43 (5.5)	41 (6.8)	2 (1.1)	
Hospital 4	171 (22.0)	147 (24.4)	24 (13.8)	
Hospital 5	54 (7.0)	47 (7.8)	7 (4.0)	
Hospital 6	249 (32.1)	143 (23.8)	106 (60.9)	
Hospital 7	45 (5.8)	41 (6.8)	4 (2.3)	
Hospital 8	100 (12.9)	84 (14.0)	16 (9.2)	
Vaccinated	365 (47.0)	291 (48.3)	74 (42.5)	0.18
Time between vaccination and PCR in months, mean (SD)	2.8 (1.8)	2.8 (1.8)	2.6 (1.5)	0.53

(Continued in next column)

(*Continued*)

Data are presented per episode. In total, 207/776 (26.7%) patients were included more than one time. Data are presented as no. (%) unless otherwise indicated.
Abbreviations: IL-2, interleukine-2; SD, standard deviations; IQR, interquartile range; BMI, body mass index; MTOR, mammalian target of rapamycin; ATG, anti-thymocyte globulin.
[a]Independent T-test, Chi-squared test or Mann-Whitney U test.
[b]Other types of lung diseases are active lung cancer, bronchiectasis, cystic fibrosis, pulmonal hypertension, sarcoidosis, tuberculosis, obstructive sleep apnea syndrome (OSAS).
[c]Valid percentages are presented (numbers do not always add up to 776 as there are some missing data).
[d]Other types of rejection therapy are OKT3 (muromonab), plasmapheresis, IVIG, rituximab, switch to tacrolimus, addition of third agent).

2021 (14%) and 2020 (12.6%). Among the cases, 74% tested positive for influenza A and 26% tested positive for influenza B. The influenza A subtype was not determined. Estimated yearly incidence of influenza among transplant recipients is presented in **Figure 1** and ranged between 0% (2020/21) and 2.08% (2017/2018).

The demographic characteristics of the participants are presented in **Table 1**. Cases were slightly younger than controls and the percentage of cases varies by month. Overall, 47% of the participants were vaccinated: 43% of cases (74/174) and 48% of controls (291/602). Among patients aged 65 years and older, 168 out of 365 (46%) were vaccinated, compared to 147/411 (36%) individuals under the age of 65.

Overall Vaccine Effectiveness and for Each Individual Season

After adjusting for the previously mentioned confounders, the adjusted VE over the whole period was 6.9% (95% CI -40.9 to 38.4). VE for individual seasons varied widely (**Figure 1**). Nonetheless, this study was not powered to analyze these yearly VE's, leading to wide confidence intervals. In the 2020/2021 season, no VE could be determined as no individuals tested positive for influenza. Similarly, VE could not be calculated for

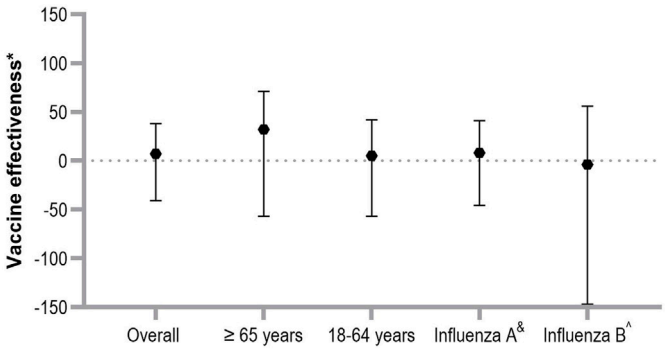


FIGURE 2 | Estimation of vaccine effectiveness against laboratory confirmed influenza. Overall VE in SOT recipients, VE by age group and by influenza virus subtype. Errors bars represent 95% CI. *Corrected for age, history of chronic pulmonary disease, history of rejection therapy, hospital of inclusion, season, use of cell division inhibitors, highly immunosuppressed status. [&]Only cases with influenza A subtypes were included; cases with influenza B virus subtypes were excluded. ^Only cases with influenza B virus subtypes were included; patients with influenza A virus subtype were excluded. Abbreviations: OR, odds ratio; VE, vaccine effectiveness; CI, confidence interval.

TABLE 2 | Course of disease in patients who tested positive for influenza.

	Overall (n = 174)	Vaccinated (n = 74)	Unvaccinated (n = 100)	p[a]
Admission in the hospital	112 (64.4)	51 (68.9)	61 (61.0)	0.28
Hospital length of stay, median (IQR)	3.0 (2.0–5.0)	3.0 (2.0–4.0)	3.0 (2.0–7.0)	0.25
ICU-admission	6 (3.4)	2 (2.7)	4 (4.0)	0.61
Need for mechanical ventilation	5 (2.9)	2 (2.7)	3 (3.0)	0.92
30-day mortality	3 (1.7)	1 (1.4)	2 (2.0)	0.75
Rejection	2 (1.1)	0	2 (2.0)	0.22

Data are presented as no. (%) unless otherwise indicated.
[a]*Chi-squared test, Fisher's exact test or Mann-Whitney U test.*

the 2012/2013, 2013/2014 and 2014/2015 seasons due to small sample sizes. After excluding this three seasons, the adjusted VE was 4.3% (95% CI −46.6 to 37.5).

Vaccine Effectiveness by Age Group and by Influenza Virus Type

Among individuals aged 18–64 years, the adjusted VE from 2013 to 2024 was 4.8% (95% CI −56.5 to 42.1), compared to a VE of 32.4% (95% CI −56.5–70.8) among those aged 65 years and older (**Figure 2**). The total adjusted VE against influenza A was 7.5% (95% CI −46.0 to 41.3), while the total adjusted VE against influenza B was −3.8% (95% CI −146.7 to 56.3).

Course of Disease in Patients Who Tested Positive for Influenza

Overall, 112 influenza-positive patients (64.4%) were hospitalized, with a median stay of 3 days (IQR 2–5 days) (**Table 2**). Six patients (3.4%) required ICU admission, five of whom needed mechanical ventilation. Overall, the all-cause 30-day mortality among lab-confirmed influenza cases was 1.7%. The course of disease for vaccinated SOT recipients was similar to that of unvaccinated patients. ICU admission, mechanical ventilation, 30-day mortality and treatment for rejection after influenza illness (1.7%) did not differ between vaccinated and unvaccinated patients (**Table 2**).

DISCUSSION

In this retrospective test-negative case-control study, the observed adjusted VE against influenza infection of the standard-dose seasonal influenza vaccine in SOT recipients was low over the years 2013–2024 in the Netherlands, with a most optimal adjusted VE of 6.9%. Compared with VE in people below 65 years, the adjusted VE in patients above 65 years was higher (4.8% versus 32.4%, respectively). The VE against influenza B was lower than against influenza A (−3.8% versus 7.5%, respectively). We also showed that influenza-related complications did not differ between the vaccinated and unvaccinated influenza cases.

Data on vaccine effectiveness for preventing influenza infection in adults with immunocompromised status are scarce. Most research has concentrated on assessing the humoral antibody responses by measuring influenza-specific antibody levels, associated with protection in healthy adults, using standard HI assays [37–40]. However, these antibody concentrations are surrogate markers of vaccine efficacy and if these are also protective in SOT recipients is unknown. Therefore, it remains important to determine VE as the primary outcome measure, rather than relying on the immunological response.

Previous immunogenicity studies have reported a lower humoral response to influenza vaccination in SOT recipients compared with healthy controls [15, 18, 21]. Our study is among the first to demonstrate and quantify the clinical impact of this

known reduced immunological vaccine response in SOT recipients.

In the Netherlands, the effectiveness of the (inactivated) influenza vaccine ranged from −11% to 65% in the past decade in the general population [23–27]. Our findings suggest that VE against influenza in SOT recipients is low compared to the general healthy population. Similarly, a study by Hughes et al reported an adjusted VE of 5% against influenza-associated hospitalizations among eight categories of immunocompromised adults during the 2017–2018 season, compared to 41% among non-immunocompromised adults [41].

Numerous studies have shown that the estimates of VE in the general population are higher in subjects under the age of 65 years than in those aged 65 years or older [30, 31]. In contrast, we found a higher VE in those aged 65 years or older compared to those aged 18–64 years. This finding aligns with data from the Dutch National Institute for Public Health and the Environment, which also reported higher VE in the older population compared to the younger population [23–27, 42, 43]. A possible explanation could be differences in exposure, healthcare-seeking behavior or disease severity between these age groups. Younger patients with (mild) symptoms may be less likely to seek hospital care than older individuals. This could lead to undocumented mild infections, which might attenuate VE estimates. The low annual incidences of influenza observed in our population, compared to the general Dutch population, supports the idea that there may be more mild cases among vaccinated individuals or high levels of vaccination in household contacts of SOT recipients that may prevent secondary transmission. However, the incidence rates in the general population reflects influenza-like illness (ILI) reported by GP's, rather than laboratory-confirmed influenza reported by hospitals. Since not everyone with ILI seek hospital care, this may account for the lower incidences of influenza observed in our population.

In earlier influenza seasons, PCR was less widely used than in the (post-) COVID-19 seasons, where PCR on RSV/SARS-CoV-2/influenza was likely done more routinely to all patients with equal severity of disease (who where not tested before COVID pandemic). However, this would not have had an impact on the VE. Lower threshold for PCR testing may result in testing less severely ill patients, resulting in more influenza negative patients (controls). However, the ratio of vaccinated to unvaccinated individuals in a population with fewer cases does not change (as doctors are unaware of the vaccination status of the patient), and the OR and consequently the VE remains unaffected (OR= ((a/b)/(c/d)), where "a" represents the number of vaccinated cases, "b" the number of unvaccinated cases, "c" the number of vaccinated controls, and "d" the number of unvaccinated controls).

Our results showed that influenza-related outcomes -such as hospital length of stay, need for ICU admission and/or mechanical ventilation, 30-day mortality and rejection- did not differ between the vaccinated and unvaccinated influenza cases. However, this only applies to those who presented at the hospital. Due to the retrospective design of the study, we cannot accurately quantify the extend of illness prevented by the influenza vaccine. However, we do instruct SOT recipients to contact the hospital in case of respiratory infection symptoms. Studies evaluating the impact of antecedent influenza vaccination in SOT recipients with influenza disease are scarce. One study that assessed the impact of the 2010–2011 seasonal influenza vaccination on illness severity among SOT recipients with influenza disease reported similar results [19]. The study indicated that receiving the influenza vaccine was not associated with a decreased risk of hospitalization, ICU admission, mortality or severe disease. In contrast to our study, it did find an association with shorter hospital stay. In addition, Kumar et al reported that receiving the influenza vaccine in the current season was associated with a lower incidence of ICU admission in a multivariate model among 616 patients with a SOT or hematopoietic stem cell transplantation [9].

The observed reduced influenza VE in SOT patients in comparison to the healthy population warrants further investigation aimed at improving the VE or investigation to explore alternative strategies to protect this vulnerable group. Various methods had been previously evaluated to improve vaccine immunogenicity in immunocompromised patients, including adjuvanted vaccines [44], the use of high-dose (HD) influenza vaccines [45–48], administration of a booster-dose (BD) [21, 49], intradermal vaccination [50–52] and adjusting immunosuppression to target [53]. Most of these measures have not resulted in clinically significant increases in immunogenicity compared with single standard-dose intramuscular strategies [54]. Of these strategies, HD (especially those four times the standard dose) and BD vaccines seem to be the most promising for enhancing immunogenicity and are generally well tolerated [54].

Several limitations should be considered when interpreting these results. First, the wide confidence intervals surrounding the VE estimate limit the strength of our conclusion. However, the upper bound of the confidence interval still remains below the VE observed in the healthy population. Second, VE fluctuate annually, depending on the degree of antigenic match between vaccine strains and circulating strains [22]. Our study focused on the adjusted VE over 11 respiratory seasons, as yearly sample sizes were insufficient for reliable calculating, introducing some heterogeneity. Third, the observational design of the study also introduces potential confounding. Although we adjusted for all known confounding variables, residual confounding still exist. The test-negative design required that cases seek medical attention, which might not occur for mild symptoms. However, SOT recipients are more likely to contact the hospital for mild symptoms compared to the general population, as they are advised to do so in the presence of fever or symptoms of a viral respiratory infection. Moreover, during the COVID-19 pandemic and the subsequent years, patients were more inclined to seed medical care and get tested for respiratory viruses more readily, which likely mitigates the risk of underestimating VE. Next, the timing of vaccination was not accounted for due to the often unknown exact dates of vaccine administration at many GP offices. Lastly, our criteria for being considered vaccinated were fairly stringent, requiring individuals to have received the seasonal influenza vaccine in current respiratory season before PCR testing.

Those vaccinated in the previous season were considered unvaccinated. Less stringent criteria would likely lower the VE estimate, as studies indicate a progressive decline in antibody titers within a year after vaccination [37, 49, 55, 56]. Additionally, VE tends to drop during the season, beginning around 100 days post-vaccination [30]. Thus, vaccinated patients receiving their influenza vaccination longer ago (e.g., those who present to the hospital between May and October) were less protected against influenza disease, which consequently should influence the VE estimate. However, since individuals between week 20 and week 40 were excluded, we believe that the impact of waning immunity on our estimates limited.

The test-negative design represents a strength of our study. By ensuring that all laboratory-confirmed cases and test-negative controls sought care in the same healthcare settings for similar sets of symptoms, we reduce bias related to community-level variations in vaccine coverage. In addition, cases and non-cases will typically originate from the same communities. Another advantage of this design is the reduction in disease misclassification, as cases are confirmed through laboratory testing. Furthermore, we assessed vaccination history by contacting GP's, who were unaware of their patients' respiratory infections when verifying vaccination status, thereby reducing misclassification of vaccine history as a potential source of bias. Selection bias, which could arise from physicians' clinical decision-making regarding testing for influenza, is also mitigated. Since patients' vaccine history is generally unknown to treating physicians in hospitals- who typically rely on GPs for such records- we further limit potential biases in vaccine status that could affect outcomes.

In conclusion, the results of our study demonstrate that seasonal effectiveness of the standard-dose influenza vaccine against laboratory confirmed influenza in adult SOT recipients is limited. Despite the low precision and limitations of a retrospective analysis, our findings prompt further investigations aimed at improving VE in SOT recipients. New vaccine formulations or a different vaccination strategy may increase VE. In addition, more prospective data with larger sample size on such regional VE estimates are needed, as it could help convince both doctors and patients of the benefits of vaccination. This data collection should not only focus on influenza VE, but also on burden of disease and VE of other vaccine-preventable infections in SOT recipients, such as COVID-19 and RSV. If the low VE and low burden of disease due to influenza were to be confirmed, annual vaccination campaigns focusing on single pathogens may be questioned and use of combination-vaccines including influenza, COVID-19 and RSV would be preferred to limit the number of vaccinations and healthcare consultations.

DATA AVAILABILITY STATEMENT

The data will be made available on reasonable request.

ETHICS STATEMENT

The requirement of ethical approval was waived by Institutional Review Board of the LUMC for the studies involving humans because It concerns a study not subject to the WMO as the individuals are not subjected to procedures or were imposed with behavioral rules. It is an observational study. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board also waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/ next of kin. In addition, it concerns such large groups that the research is reasonably not feasible through obtaining individual consent. Additionally, it was expected that some patients will have already passed away, making it impossible to obtain consent from them.

AUTHOR CONTRIBUTIONS

MP, GG, AdV, SC, LV, AV, and MT participated in research design. MP, ED, DH, ES, IM, and JG participated in the performance of research. MP, SC, and ED participated in data analysis. MP and ED participated in writing the article. GG, AdV, LV, SC, AV, MT, ES, IM, and JG participated in revision of the article. All authors contributed to the article and approved the submitted version.

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BRIEF RESEARCH REPORT
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CTLA4 Single-Nucleotide Polymorphisms Influence the Risk of HSV and VZV Infection in Kidney Transplant Recipients: A Prospective Cohort Study

Natalia Redondo[1,2*†], *Isabel Rodríguez-Goncer*[1,2†], *Tamara Ruiz-Merlo*[1,2†], *Francisco López-Medrano*[1,2,3†], *Esther González*[4], *Natalia Polanco*[4], *Ana Hernández-Vicente*[4], *Rafael San Juan*[1,2,3†], *Amado Andrés*[3,4†], *José María Aguado*[1,2,3†] *and Mario Fernández-Ruiz*[1,2,3†]

[1]*Unit of Infectious Diseases, Hospital Universitario "12 de Octubre", Instituto de Investigación Sanitaria Hospital "12 de Octubre" (imas12), Madrid, Spain,* [2]*Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Madrid, Spain,* [3]*Department of Medicine, School of Medicine, Universidad Complutense, Madrid, Spain,* [4]*Department of Nephrology, Hospital Universitario "12 de Octubre", Instituto de Investigación Sanitaria Hospital "12 de Octubre" (imas12), Madrid, Spain*

Herpesviruses are able to modulate adaptive T-cell-mediated responses to establish latency within the host. Reactivation of herpes simplex virus (HSV)-1/2 and varicella zoster virus (VZV) is a frequent and potentially serious complication among kidney transplant recipients (KTRs). The ability of clinical criteria to identify KTRs at increased risk of α-herpesvirus (HSV/VZV) infection is limited. We investigated the effect of two single nucleotide polymorphisms (SNPs) in the cytotoxic T-lymphocyte antigen 4 *(CTLA4)* gene in a single-center cohort of 204 KTRs. After a median follow-up of 3.1 years, 34 of them (16.7%) experienced 22 episodes of zoster and 15 episodes of HSV-1/2 infection. Homozygous carriers of the minor allele of rs231775 had a higher cumulative incidence of α-herpesvirus infection (23.5% for GG versus 7.6% for AA/AG carriers; *P*-value = 0.011) and a lower infection-free survival (log-rank *P*-value = 0.037). After multivariable adjustment by clinical factors (including use of valganciclovir prophylaxis and acute rejection as time-dependent variables), the GG genotype of *CTLA4* (rs231775) SNP was associated to the study outcome (adjusted hazard ratio: 3.21; 95% confidence interval: 1.44–7.16). In conclusion, genetic polymorphisms in the co-inhibitory T-cell receptor CTLA-4 may be detrimental for the immune control of latent HSV/VZV infection in KTRs.

Keywords: herpesvirus, kidney transplantation, single-nucleotide polymorphism, cytotoxic T-lymphocyte antigen 4, HSV

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***Correspondence**
Natalia Redondo,
natalia.redondo.imas12@h12o.es

†ORCID:
Natalia Redondo
orcid.org/0000-0001-9356-8102
Isabel Rodríguez-Goncer
orcid.org/0000-0003-2150-5748
Tamara Ruiz-Merlo
orcid.org/0000-0002-8261-6057
Francisco López-Medrano
orcid.org/0000-0001-5333-7529
Rafael San-Juan
orcid.org/0000-0003-3446-1991
Amado Andrés
orcid.org/0000-0003-0238-1364
José María Aguado
orcid.org/0000-0002-9520-8255
Mario Fernández-Ruiz
orcid.org/0000-0002-0315-8001

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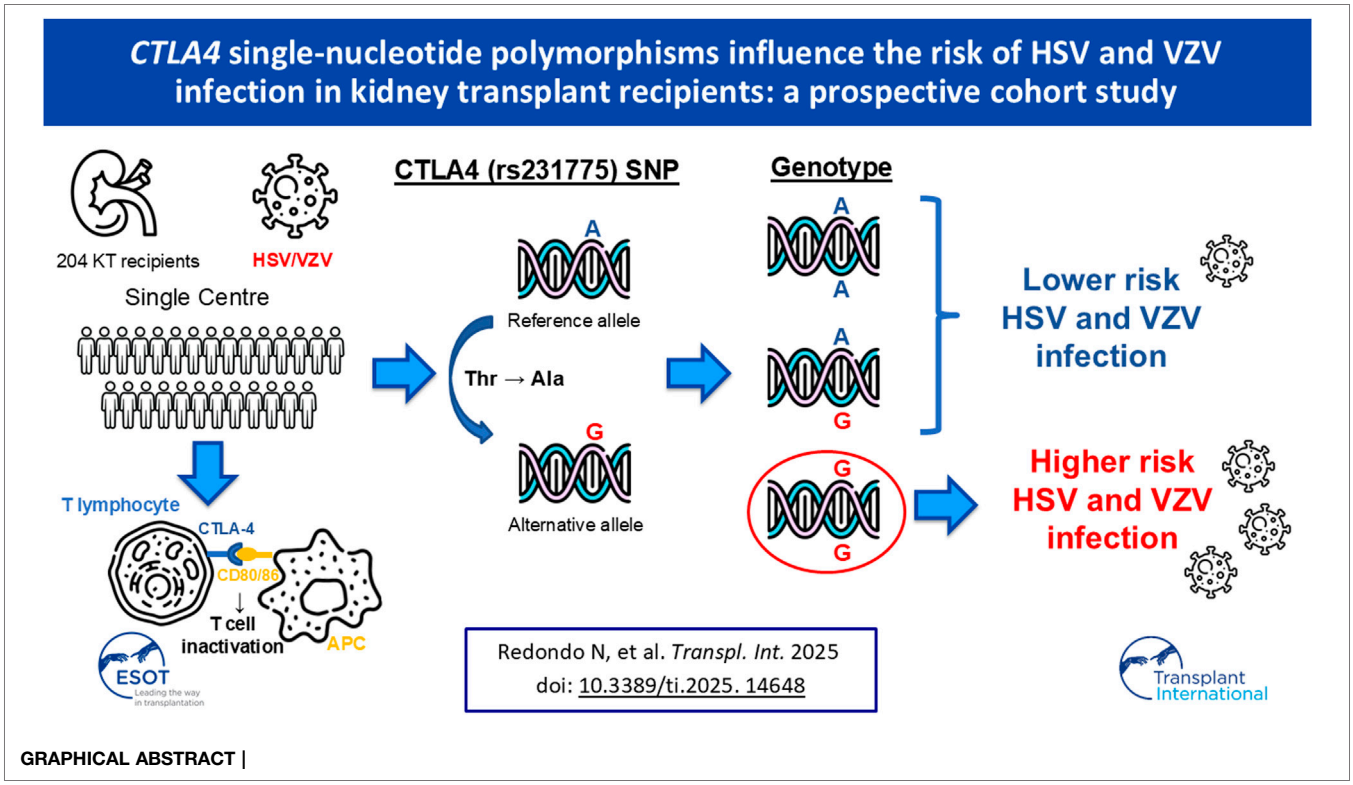
Abbreviations: CMV, cytomegalovirus; CTLA-4, cytotoxic T-lymphocyte antigen 4; D, donor; GP, general practitioner; HSV, herpes simplex virus; HZ, herpes zoster; HZ/su, HZ subunit vaccine; IQR, interquartile range; IHC, immunohistochemistry; IRR, incidence rate ratio; KTR, kidney transplant recipient; PCR, polymerase chain reaction; R, recipient; PBMC, peripheral mononuclear cell; SD, standard deviation; SNP, single-nucleotide polymorphism; SOT, solid organ transplantation; VZV, varicella-zoster virus.



GRAPHICAL ABSTRACT |

INTRODUCTION

Herpes simplex viruses type 1 and 2 (HSV-1/2) and varicella zoster virus (VZV) are ubiquitous α-herpesviruses able to establish life-long infection and to reactivate under certain circumstances, such as immunosuppression. Solid organ transplant (SOT) recipients are more prone to experiencing reactivation of α-herpesviruses as compared to the general population. The clinical spectrum may range from minor mucocutaneous forms—orolabial or genital vesicular lesions or localized herpes zoster (HZ)— to disseminated disease with central nervous system (CNS) and visceral involvement [1–3]. In addition to older age, use of valganciclovir as prophylaxis against cytomegalovirus (CMV) and increase of immunosuppressive therapy due to previous rejection episodes [1, 4–6], the factors governing the development of post-transplant HSV/VZV reactivation remain poorly characterized.

Single nucleotide polymorphisms (SNP) in genes coding for immune molecules confer a differential susceptibility to viral pathogens. The contribution of host genetics is highlighted after SOT due to the additive effect of iatrogenic immunosuppression. Therefore, SNP genotyping has emerged as a complementary tool for risk stratification in this population [7].

Herpesviruses are able to modulate adaptive T-cell-mediated responses to maintain latency, with CMV as the most notorious example [8]. The expression of co-inhibitory T-cell receptors plays a relevant role in the virus-host interaction [9–11]. Cytotoxic T-lymphocyte antigen 4 (CTLA-4) and its homologous CD28 are two immunoglobulin superfamily members with a shared ability to bind CD80/B7.1 and CD86/B7.2 but opposed biological functions. CTLA-4 suppresses T-cell receptor signaling, contracts the expanded T-cell populations by inhibiting T-cell proliferation and interleukin-2 secretion, and promotes the suppressive functions of Tregs [12–14]. Different SNPs in the *CTLA4* gene have been accordingly investigated in the context of cancer or autoimmune diseases [15] or infection, such as hepatitis C [16] or dengue [17].

The effect of genetic polymorphisms in *CTLA4* on the risk of infectious complications in the specific setting of SOT has been assessed in some previous studies [18–21]. Jiang et al. reported a protective role for the GG genotype of rs231775 on the recurrence of hepatitis B virus infection after liver transplantation [19]. Other study revealed that the presence of the mutant genotypes of rs231775 and rs3087243 were associated with a lower CMV disease-free survival in kidney transplant recipients (KTRs) as compared with heterozygous and wild genotypes [20]. In addition, a meta-analysis established a correlation between two *CTLA-4* SNPs and the risk of post-transplant infection [21]. Of note, this previous research yielded some discrepant results in the sense of the association found (protective or deleterious).

Due to the alleged impact of *CTLA4* SNPs on the host susceptibility and the lack of specific data, we aimed to explore the effect of two *CTLA-4* SNPs (rs5742909 and rs231775) on the incidence of α-herpesvirus infection (HSV/VZV) in a cohort of KTRs.

MATERIALS AND METHODS

Study Population and Design

The present study was based on a prospectively maintained cohort of consecutive KTRs at our institution between November 2014 and December 2016 [22]. The research was performed in accordance with the ethical standards outlined in the Declarations of Helsinki and Istanbul. All the patients provided informed consent and the local Clinical Research Ethics Committee approved the study protocol (number 14/030). The project was developed according to the STREGA statement recommendations.

The study outcome was the occurrence of α-herpesvirus (HSV-1/2 and VZV) infection during the follow-up period. Participants were enrolled at the time of transplantation and followed-up until graft loss, death or December 2018, whichever occurred earlier. None of the included KTRs received the HZ subunit vaccine (HZ/su) during the study period, since this product was approved in Spain in 2020. Descriptions of immunosuppression and prophylaxis regimens are provided as **Supplementary Methods**. Attending physicians were not made aware of the genotyping results.

Study Definitions

Mucocutaneous HSV-1/2 infection was diagnosed by the presence of painful vesicular or ulcerative lesions on orolabial, genital or perianal areas, with or without confirmation by polymerase chain reaction (PCR), cell culture or immunohistochemistry (IHC). The diagnosis of visceral disease required compatible clinical manifestations involving the gastrointestinal tract (esophagitis, gastritis or hepatitis), ocular structures (conjunctivitis, keratitis or uveitis) or CNS (meningitis, encephalitis or stroke) associated to a positive result of PCR assay, culture or IHC in an appropriate sample [1]. The diagnosis of HZ was also clinical (characteristic pruritic papulovesicular rash with a dermatomal distribution), and virological or IHC confirmation was not required. Disseminated HZ was defined by lesions involving ≥2 non-contiguous dermatomes or varicella-like syndrome. Complicated HZ comprised ocular or CNS disease or any other visceral involvement with virological and/or IHC documentation [3]. Clinical diagnoses were made by transplant nephrologists, ID physicians or general practitioners (GPs) (with subsequent reevaluation at the transplant outpatient clinic). Additional definitions are available in **Supplementary Methods**.

CTLA4 SNP Genotyping

Genotyping was retrospectively performed from whole blood specimens collected at inclusion and stored at −80°C until analysis. DNA was extracted with the KingFisher™ Duo Prime system (Thermo Fisher Scientific, Waltham, MA) using the MagMax™ DNA Multi-Sample Ultra 2.0 kit, following the manufacturer´s instructions. *CTLA4* (rs5742909, rs231775) genotyping was performed by TaqMan technology (Thermo Fisher Scientific) in a QuantStudio 3 system (Applied

TABLE 1 | Demographics and clinical characteristics of the study cohort (n = 204).

Variable	
Age, years [mean ± SD]	54.6 ± 15.7
Gender (male) [n (%)]	146 (71.6)
Body mass index, kg/m^2 [mean ± SD]	25.9 ± 9.5
Ethnicity [n (%)]	
Caucasian	177 (86.8)
Hispanic	17 (8.3)
African	6 (2.9)
Asian	4 (2.0)
Current or prior smoking history [n (%)]	81 (39.9)
Pre-transplant chronic co-morbidities [n (%)]	
Hypertension	175 (85.8)
Diabetes mellitus	58 (28.4)
Non-coronary heart disease	35 (17.2)
Chronic lung disease	27 (13.2)
Coronary heart disease	21 (10.3)
Peripheral arterial disease	21 (10.3)
Solid or hematological malignancy or melanoma	20 (9.8)
Previous solid organ transplantation [n (%)]	28 (13.7)
Underlying end-stage renal disease [n (%)]	
Diabetic nephropathy	35 (17.2)
Glomerulonephritis	55 (27.0)
Polycystic kidney disease	24 (11.8)
Hypertensive nephropathy	18 (8.8)
Congenital nephropathy	8 (3.9)
Reflux nephropathy	7 (3.4)
Unknown	25 (12.3)
Other	32 (15.7)
CMV serostatus [n (%)]	
D+/R+	148 (72.5)
D+/R-	23 (11.3)
D-/R+	22 (10.8)
D-/R-	7 (3.4)
D unknown/R+	4 (2.0)
Positive HCV serostatus [n (%)][a]	15 (7.4)
Positive HIV serostatus [n (%)][b]	2 (1.0)
Positive VZV serostatus [n (%)][c]	186 (95.4)
Pre-transplant renal replacement therapy [n (%)]	180 (88.2)
Hemodialysis	148/180 (82.2)
Continuous ambulatory peritoneal dialysis	32/180 (17.8)
Time on dialysis, months [median (IQR)]	17.2 (8.9–35.4)
Age of donor, years [mean ± SD]	53.8 ± 15.5
Gender of donor (male) [n (%)]	109 (53.4)
Type of donor [n (%)]	
DBD donor	128 (62.7)
DCD donor	46 (22.6)
Living donor	29 (14.2)
Cold ischemia time, hours [median (IQR)]	18.0 (10.1–23.0)
Number of HLA mismatches [median (IQR)]	4 (3–5)
Induction therapy [n (%)]	
ATG	94 (46.1)
Basiliximab	83 (46.7)
None	27 (13.2)
Primary immunosuppression regimen [n (%)]	
Prednisone, tacrolimus and MMF/MPS	196 (96.1)
Prednisone, tacrolimus and azathioprine	8 (3.9)
Conversion to mTOR inhibitor during follow-up [n (%)]	19 (9.3)
Time to conversion, days [median (IQR)]	232 (118–321)
Anti-CMV prophylaxis with valganciclovir [n (%)]	113 (55.4)
Duration of prophylaxis, days [median (IQR)]	103 (91–147)
Post-transplant complications [n (%)]	
Delayed graft function	99 (48.5)
New-onset diabetes	24 (11.8)
CMV infection [n (%)]	114 (55.9)
CMV disease [n (%)]	22 (10.8)

(Continued on following page)

TABLE 1 | (*Continued*) Demographics and clinical characteristics of the study cohort (n = 204).

Variable	
Renal artery stenosis	40 (19.6)
Acute graft rejection	25 (12.3)
Time to the first episode, days [median (IQR]	134 (28.5–291.5)
T-cell-mediated acute rejection	16 (7.8)
Borderline T-cell-mediated rejection	8 (3.9)
Antibody-mediated acute rejection	5 (2.5)
Graft loss	8 (3.9)
All-cause death	11 (5.4)

ATG, antithymocyte globulin; CMV, cytomegalovirus; D, donor; DBD, donation after brain death; DCD, donation after circulatory death; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; IQR, interquartile range; MMF/MPS, mycophenolate mofetil/mycophenolate sodium; mTOR, mammalian target of rapamycin; R, recipient; SD, standard deviation; VZV, varicella zoster virus.
[a]*At the pre-transplant evaluation. Data not available for 5 patients.*
[b]*At the pre-transplant evaluation. Data not available for 2 patients.*
[c]*At the pre-transplant evaluation. Data not available for 7 patients.*

Biosystems, Foster City, CA). SNP and allele (genotype) calling was made by a standard end-point analysis with the aid of a commercial genotype-calling software (TaqMan™ Genotyper Software v1.0.1) and the QuantStudio Design and Analysis Software v1.5.1 (both from Applied Biosystems).

Statistical Analysis

Quantitative data were shown as the mean ± standard deviation (SD) or the median with interquartile range (IQR). Deviation from the Hardy-Weinberg equilibrium for each SNP was evaluated by the χ^2 test with one degree of freedom. Comparisons of the cumulative incidence of α-herpesvirus infection according to the different SNP alleles or genotypes, either individually or in combination, were performed by the χ^2 test or the Fisher's exact test. Incidence rates per 1,000 patient-days and the corresponding incidence rate ratio (IRR) were calculated with 95% confidence interval (95 CIs). Survival probabilities were estimated by the Kaplan-Meier method and differences between groups were compared by the log-rank test. Univariable Cox regression was used to identify variables with *P*-value < 0.09, which were entered into a multivariable model that included the selected *CTLA4* SNP as the variable of interest. The exposure to valganciclovir prophylaxis and the occurrence of acute rejection were entered as time-dependent covariates. Since the completeness of the institutional database was very high, no imputation for missing data was performed. Statistical analysis was performed using SPSS v21 (Statistical Package for Social Sciences, Chicago, IL).

RESULTS

Study Cohort and Outcomes

We included 204 KTRs (**Table 1**). After a median follow-up period of 3.1 years (IQR: 2.6–3.6), 34 patients (16.7%) developed 37 episodes of α-herpesvirus infection, yielding an incidence rate of 0.17 cases per 1,000 patient-days (95% CI: 0.12–0.23). The median interval between transplantation and the first episode was 454.5 days (IQR: 47.5–1639.8). In detail, 16.2% (6/37), 21.6% (8/37) and 62.2% (23/37) of episodes occurred in the early (first month), intermediate (1–6 moths) and late post-transplant periods (≥6 months), respectively.

There were 22 episodes of VZV infection in form of HZ confined to a single dermatome, the diagnosis of which was based solely on clinical manifestations. All of them occurred in KTRs that were VZV-seropositive before transplantation. The 15 episodes of HSV-1/2 infection included mucocutaneous disease in form of orolabial (9 cases) or genital herpes (4 cases), HSV esophagitis and HSV pharyngitis with facial palsy (one case each). There were no cases of visceral or disseminated disease. The diagnosis of HSV-1/2 infection was confirmed by cell culture (5/15 [33.3%]), IHC (2/15 [13.3%]) or clinical findings alone (8/15 [53.3%]).

Association Between *CTLA4* SNPs and α-Herpesvirus Infection

All the SNP genotype frequencies were in Hardy-Weinberg equilibrium (data not shown). First, we investigated whether the presence of specific alleles within the *CTLA4* gene was correlated with the cumulative incidence of α-herpesvirus infection. There were no significant differences in the allele distribution of the *CTLA4* (rs5742909) SNP between KTRs that experienced or did not experience the study outcome (*P*-value = 0.967). In contrast, the presence of the minor allele G of the *CTLA4* (rs231775) SNP was significantly more common among KTRs with α-herpesvirus infection (*P*-value = 0.005) (**Supplementary Table S1** of **Supplementary Material**). Subsequently, we tested both dominant and recessive models. Only carriers of the G allele in a homozygous state experienced a higher incidence of infection (23.5% [8/34] for GG versus 7.6% [13/170] for AA/AG; *P*-value = 0.011), suggesting a recessive effect (**Table 2**). The incidence rates were 0.375 (95% CI: 0.180–0.690) and 0.137 (95% CI: 0.180–0.690) episodes per 1,000 patient-days for the GG and AA/AG genotypes, respectively (*P*-value = 0.004), with an IRR of 2.73 (95% CI: 1.18–5.82; *P*-value = 0.013). Time-to-event Kaplan-Meier curves for time to first episode of α-herpesvirus infection according to the genotype of rs231775 are shown in **Supplementary Figure S1**. There were no differences in the length of follow-up according to the genotype (median of 3.5 [IQR: 1.7–3.8] years for the GG genotype versus 3.0 [IQR: 2.6–3.6] years for AA/AG genotypes; *P*-value = 0.848).

α-Herpesvirus Infection-Free Survival

We plotted α-herpesvirus infection-survival curves according to the genotype of rs231775 (**Figure 1**). KTRs that were homozygous or heterozygous for the reference allele (AA/AG) were significantly more likely to remain free from infection as compared to GG carriers (log-rank *P*-value = 0.037). After multivariable adjustment by gender, pre-transplant diabetes mellitus, use of valganciclovir as CMV prophylaxis, cold ischemia time and occurrence of acute rejection, the GG genotype of *CTLA4* (rs231775) SNP remained associated to α-

TABLE 2 | Cumulative incidence of α-herpesvirus infection according to dominant and recessive models for candidate *CTLA4* SNPs.

Gene (SNP database ID number)	Model	Genotype	α-herpesvirus infection, n (%)		*P*-value
			No (n = 170)	Yes (n = 34)	
CTLA4 (rs5742909)	Dominant	CC	139 (81.8)	28 (82.4)	0.935
		CT/TT	31 (18.2)	6 (17.6)	
	Recessive	CC/CT	166 (97.6)	33 (97.1)	1.000
		TT	4 (2.6)	1 (2.9)	
CTLA4 (rs231775)	Dominant	AA	85 (50.0)	19 (55.9)	0.531
		AG/GG	85 (50.0)	15 (44.1)	
	Recessive	AA/AG	157 (92.4)	26 (76.5)	0.011
		GG	13 (7.6)	8 (23.5)	

ID, identification; SNP, single-nucleotide polymorphism; CTLA, cytotoxic T-lymphocyte antigen.

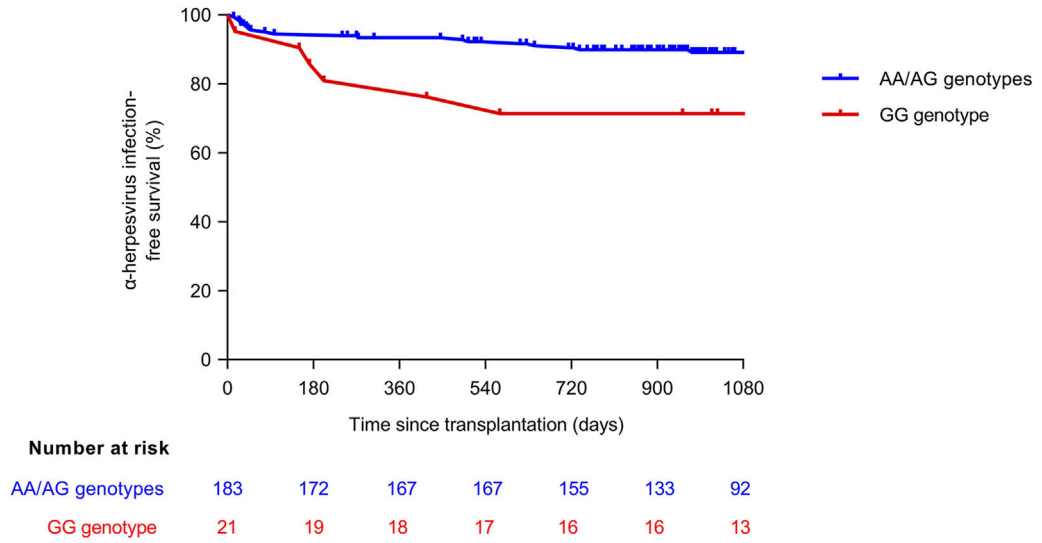


FIGURE 1 | Kaplan-Meier α-herpesvirus infection-free survival curves according to the genotype of *CTLA4* (rs231775) SNP (log-rank *P*-value = 0.037). CTLA, cytotoxic T-lymphocyte antigen; SNP, single-nucleotide polymorphism.

herpesvirus infection (adjusted hazard ratio: 3.21; 95% CI: 1.44–7.16; *P*-value = 0.004) (**Table 3**).

DISCUSSION

We have shown an association between the presence of the minor allele of rs231775 in the *CTLA4* gene and the susceptibility to α-herpesviruses in KTRs. Homozygous carriers of the G allele faced a more than three-fold increase in the incidence of HSV-1/2 and VZV infection, typically in form of mucocutaneous disease and unidermatomal HZ secondary to viral reactivation. This impact was still significant after controlling for well-established risk factors, such as valganciclovir prophylaxis or over-immunosuppression due to recent treatment for acute rejection [1, 4–6].

The *CTLA4* (rs231775) SNP consists of a nonsynonymous A/G substitution that implies the change from threonine to alanine, which lead to lower expression levels of membrane-bound CTLA-4 [23, 24]. In keeping with this effect, the GG genotype has been associated with a lower mortality in sepsis patients, a finding presumably attributable to a less pronounced sepsis-associated immunoparalysis [25]. Limited evidence is available regarding the risk of post-transplant infection. In pediatric heart transplant recipients, Ohman et al. reported a significant (albeit modest) association between AA/AG genotypes and the late occurrence of viral infection at the univariable level, but not in the adjusted Cox model. Although the authors did not provided data on specific viral pathogens, it is likely that most episodes were due to primary infection rather than reactivation, in view of the age of the cohort [26]. Iravani Saadi et al. performed a meta-analysis on the basis of 9 studies that found a protective effect for the A allele of the rs231775 SNP (odds ratio: 0.77; 95% CI: 0.59–0.95). Unfortunately, no details on the type of SOT or infection were provided, or whether the genotyping was performed in the donor or the recipient, which limited the possibility of drawing clear conclusions [21].

TABLE 3 | Univariable and multivariable Cox regression models to predict the occurrence of α-herpesvirus infection.

	No α-herpesvirus infection (n = 170)	α-herpesvirus infection (n = 34)	*P*-value	Univariable analysis			Multivariable analysis		
				HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age of recipient, years [mean ± SD]	54.7 ± 15.9	54.1 ± 14.9	0.411	1.05[a]	0.83–1.33	0.675			
Gender (male) [n (%)]	117 (68.8)	29 (85.3)	0.052	4.86	1.15–20.59	0.032	2.24	0.84–5.98	0.107
Body mass index, kg/m^2 [mean ± SD]	25.2 ± 3.7	26.0 ± 10.4	0.623	0.99[b]	0.95–1.04	0.883			
Non-Caucasian ethnicity [n (%)]	150 (88.2)	27 (79.4)	0.172	0.55	0.21–1.46	0.231			
Current or prior smoking history [n (%)]	68 (40.0)	13 (38.2)	0.848	1.04	0.47–2.31	0.929			
Pre-transplant hypertension [n (%)]	146 (86.4)	29 (85.3)	0.791	1.15	0.33–3.84	0.821			
Pre-transplant diabetes mellitus [n (%)]	47 (27.6)	11 (32.4)	0.579	2.18	0.99–4.79	0.054	1.05	0.49–2.26	0.905
Pre-transplant coronary heart disease [n (%)]	16 (9.4)	5 (14.7)	0.358	1.95	0.74–5.13	0.178			
Pre-transplant chronic lung disease [n (%)]	20 (11.8)	7 (20.6)	0.172	2.20	0.88–5.51	0.092			
Pre-transplant peripheral arterial disease [n (%)]	18 (10.6)	3 (8.8)	1.000	1.33	0.39–4.45	0.641			
Pre-transplant malignancy [n (%)]	16 (9.4)	4 (11.8)	0.751	1.79	0.62–5.23	0.284			
Pre-transplant renal replacement therapy [n (%)]	149 (87.6)	31 (91.2)	0.772	0.98	0.29–3.27	0.972			
Time on dialysis, months [median (IQR)]	17.3 (8.8–35.3)	13.1 (9.3–35.9)	0.950	0.99[b]	0.99–1.01	0.384			
Diabetic nephropathy as ESRD [n (%)]	31 (18.2)	4 (11.8)	0.361	0.97	0.33–2.82	0.954			
Glomerulonephritis as ESRD [n (%)]	44 (25.9)	11 (32.4)	0.438	1.06	0.44–2.55	0.890			
Polycystic kidney disease as ESRD [n (%)]	22 (12.9)	2 (5.9)	0.382	0.30	0.04–2.22	0.238			
Previous solid organ transplantation [n (%)]	22 (12.9)	6 (17.6)	0.426	1.07	0.43–2.63	0.887			
Mismatched CMV serostatus (D+/R-) [n (%)]	19 (11.3)	4 (12.5)	0.769	1.11	0.39–3.21	0.846			
Positive CMV serostatus (R+) [n (%)]	145 (85.3)	30 (88.2)	0.792	1.97	0.47–8.37	0.356			
Positive HCV serostatus (R+) [n (%)]	11 (6.6)	4 (12.1)	0.281	1.83	0.55–6.14	0.327			
Positive VZV serostatus (R+) [n (%)]	154 (94.5)	32 (100.0)	0.360	21.73	0.01–723, 315	0.457			
Age of donor, years [mean ± SD]	53.7 ± 15.4	54.4 ± 16.4	0.400	1.98[a]	0.87–1.38	0.432			
DCD donor [n (%)]	40 (23.5)	6 (17.6)	0.454	0.88	0.36–2.16	0.779			
Living donor [n (%)]	26 (15.3)	3 (8.8)	0.426	0.24	0.03–1.74	0.156			
Cold ischemia time, hours [median (IQR)]	17.3 (9.1–22.3)	19 (13.7–23.1)	0.148	1.07[b]	1.02–1.14	0.013	1.04	0.99–1.09	0.083
Number of HLA mismatches [median (IQR)]	4 (3–5)	5 (3–5.3)	0.340	1.17[b]	0.86–1.58	0.326			
Induction therapy with ATG [n (%)]	77 (45.3)	17 (50.0)	0.615	0.81	0.40–1.65	0.569			
Induction therapy with basiliximab [n (%)]	69 (40.6)	14 (41.2)	0.949	1.17	0.53–2.58	0.694			
No induction therapy [n (%)]	24 (14.1)	3 (8.8)	0.581	0.61	0.19–1.99	0.410			
CMV antiviral prophylaxis [n (%)][c]	94 (55.3)	19 (55.9)	0.950	0.32	0.09–1.18	0.088	0.29	0.08–1.11	0.071
PBLSs at month 1, x 10^3 cells/μL [median (IQR)]									
CD3$^+$ T-cell count	0.857 (0.306–1.443)	0.673 (0.194–1.317)	0.363	1.00[b]	0.99–1.00	0.517			
CD4$^+$ T-cell count	0.495 (0.155–0.991)	0.378 (0.127–0.754)	0.273	1.00[b]	0.99–1.00	0.302			
CD8$^+$ T-cell count	0.278 (0.129–0.278)	0.273 (0.101–0.538)	0.763	1.00[b]	0.99–1.00	0.745			
Acute rejection during the first 12 months [n (%)][c]	18 (10.6)	4 (11.8)	0.768	7.12	1.58–32.58	0.011	7.79	1.67–36.31	0.009
GG genotype of *CTLA4* (rs231775) SNP [n (%)]	13 (7.6)	8 (23.5)	0.011	2.95	1.18–7.39	0.021	3.21	1.44–7.16	0.004

ATG, antithymocyte globulin; CMV, cytomegalovirus; D, donor; DCD, donation after circulatory death; ESRD, end-stage renal disease; HCV, hepatitis C virus; HR, hazard ratio; IQR, interquartile range; PBLSs, peripheral blood lymphocyte subpopulations; R, recipient; SD, standard deviation; VZV, varicella zoster virus.

[a]*HR per 10-year increment.*

[b]*HR per unitary increment.*

[c]*Time-dependent covariate.*

We are not aware of previous studies that have investigated the effect of genetic polymorphisms in *CTLA4* on the risk of post-transplant HSV-1/2 or VZV infection. Thus, the present results should be considered hypothesis-generating only. The mechanistic explanation is not straightforward, since the rs231775 G allele has been shown to reduce the inhibitory function of CTLA-4 through decreased cell surface expression and ligand affinity [23, 24]. This should result in the improved immune control of latent α-herpesvirus infection. Nevertheless, the frequency of the GG genotype of *CTLA4* (rs4553808) SNP—which is mapped within the promoter region and also alters its transcription rate—was significantly higher in Chinese KTRs that developed viral infection as compared to those without [18]. It may hypothesized that a lower baseline CTLA-4 expression on polyclonal activated T-cells would render more effective the induction of phenotypically exhausted virus-specific $CD8^+$ T-cells, which is one of the immune evasion tactics displayed by HSV and VZV [27, 28]. This susceptibility would be specific for α-herpesviruses, since we have found no association between *CTLA4* SNPs and the incidence of CMV infection (data not shown).

Our study is limited by the relatively low number of KTRs that developed infection and the lack of severe cases. Since no data on the baseline HSV-1/2 serostatus was available, we cannot rule out that some episodes were secondary to primary infection rather than reactivation, which would imply a differential role for virus-induced immune evasion. In addition, we lack granular data on the receipt of immunosuppressive therapy before transplantation. Nevertheless, neither the presence of glomerulonephritis as ESRD nor previous SOT (as two surrogate markers for pre-transplant immunosuppression) had an apparent impact on the event of interest. Most episodes of shingles and orolabial HSV infection were diagnosed solely based on clinical findings, and some of them by GPs (although with prompt referral to the transplant outpatient clinic). However, previous studies have reported that GPs have good clinical judgment for the diagnosis of herpes zoster [29]. Finally, the assessment of the confounding effect associated to the use of valganciclovir prophylaxis may have limited by the relatively low number of KTRs in this subgroup.

Future investigations should provide a functional insight into the immune and cellular mechanisms eventually involved in the association observed between *CTLA4* polymorphisms and susceptibility to α-herpesvirus infection among KTRs. In the current setting of increasing availability of the HZ/su vaccine for the immunocompromised population, it might be worth exploring whether carriers of the risk-genotype would additionally benefit from extended antiviral prophylaxis during the early post-transplant 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 Clinical Research Ethics Committee of the University Hospital "12 de Octubre" (reference 14/030). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

NR and MF-R designed the study, performed statistical analyses and wrote the manuscript. TR-M collected patient samples. NR and TR-M performed laboratory experiments. IR-G, FL-M, EG, NP, RS, and AA performed patient recruitment and data collection. IR-G, FL-M, RS, AA, and JA critically reviewed the manuscript and provided significant input and feedback. 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.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2025.14648/full#supplementary-material

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Editorial Office

Avenue du Tribunal Fédéral 34
CH – 1005 Lausanne
Switzerland

Tel +41 (0)21 510 17 40
Fax +41 (0)21 510 17 01

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