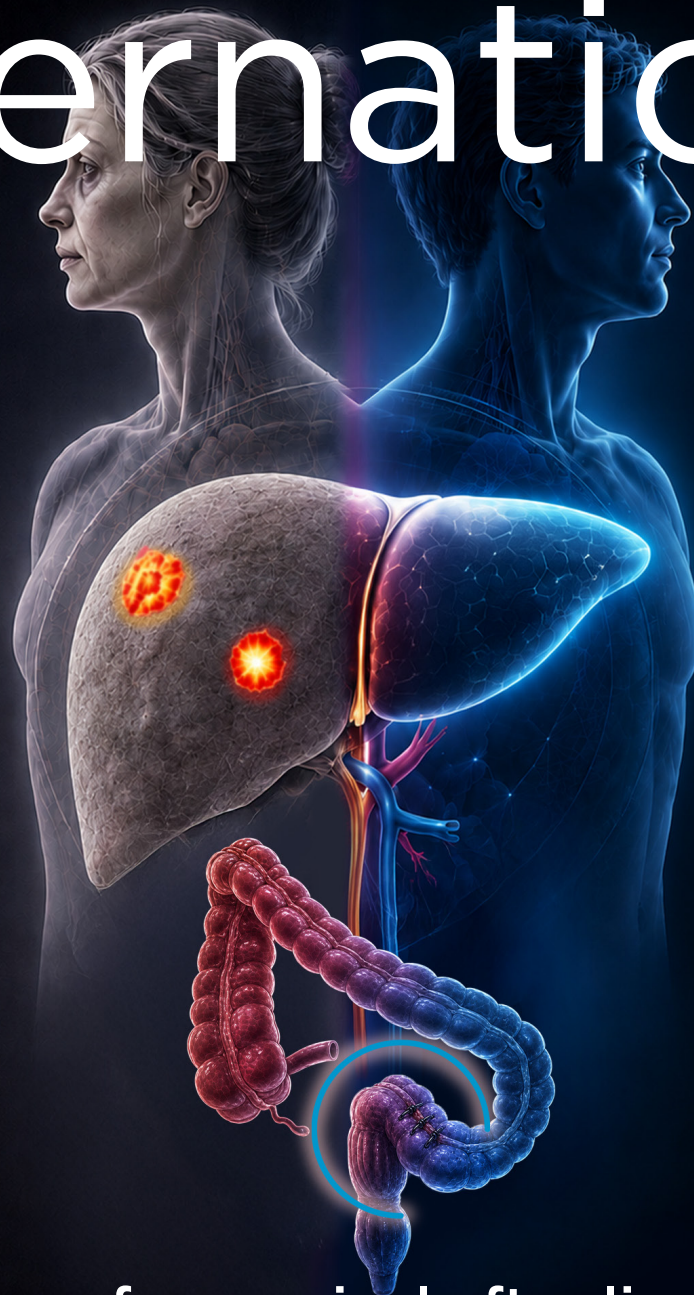


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**Gender matters for survival after liver
transplantation for colorectal metastases**



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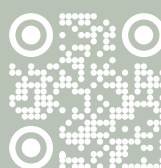
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CD8 regulatory T cell therapy in transplantation: a new path to clinical success?

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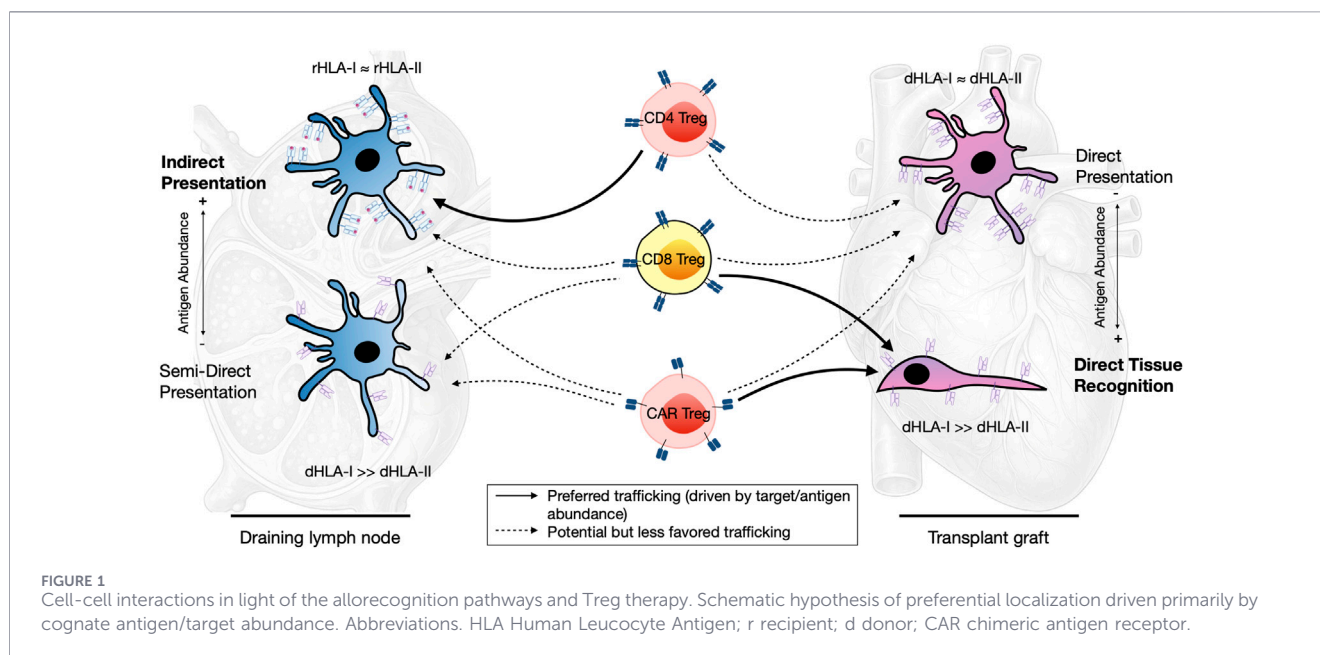
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Since the first success using modified T cells redirected against B cells to treat acute lymphoblastic leukemia and the unexpected, yet durable, remission of cancer observed in Emily Whitehead, cell therapy has emerged as a novel drug masterclass [1]. In particular, the unique capabilities of T cells to actively migrate into tissues and the sites of inflammation where conventional therapies fail have opened novel strategic avenues for treating refractory and severe diseases [2]. In fact, the number of clinical trials involving cell-based therapies in medicine is in an exponential growth phase, commensurate with the expectations and anticipated therapeutic benefits associated with their outcomes [3].

Conceptually, T cells can be harnessed either to selectively deplete specific cellular subpopulations, particularly B cells, or to suppress the licensing of effector T cells. In this context, naturally occurring CD4⁺ regulatory T cells (CD4⁺ Tregs) have attracted considerable attention. To date, more than 30 clinical trials have been registered in the field of transplantation [4]. Thus, since their discovery, awarded with the 2025 Nobel Prize in Physiology or Medicine, CD4⁺ Tregs have generated substantial enthusiasm within the scientific community as they are built with dozens of suppressive mechanisms, enabling Tregs to modulate the immune response in a highly controlled and multifaceted manner [5, 6]. In transplantation, the ONE study, an investigator-led single uncontrolled arm trial performed across eight international centers, could show early safety and promising results with CD4⁺ Tregs on reducing the immunosuppressive treatment in kidney recipients [7]. The TWO study, a phase 2b, is currently ongoing to validate those results.

More recently, the Eight-Treg study is a first-in-human phase I clinical trial designed to evaluate the safety, feasibility, and early signals of efficacy of autologous CD8⁺ Treg therapy in kidney transplantation. CD8⁺ Tregs are defined by a CD8⁺CD45RC^{low/-} phenotype, a subset previously characterized by potent suppressive activity [8–11]. In this protocol, CD8⁺ Tregs are isolated from the peripheral blood of transplant candidates prior to transplantation through cell sorting, and subsequently expanded *ex vivo* under GMP conditions using anti-CD3/anti-CD28 stimulation in the presence of low-dose IL-2, IL-15, and rapamycin, conditions known to promote regulatory stability [12]. The expansion process spans 21 days, enabling the generation of clinically relevant cell numbers while preserving both the phenotypic identity and suppressive function of the cells. At the end of the culture, the cell product exhibits a stable regulatory profile, characterized by homogeneous FOXP3 expression, high GITR levels, and low CD127 expression, consistent with a *bona fide* Treg signature. The expanded cells are reinfused into the recipient the day before transplantation, *in lieu* of conventional induction therapy, and in combination with standard immunosuppressive treatment that may be adjusted according to clinical evolution. The study follows a dose-escalation design primarily aimed at assessing



safety, *in vivo* persistence of the infused cells, and immunological effects, while also exploring preliminary efficacy endpoints, including graft function, incidence of rejection, and markers of immune regulation. Protocol biopsies performed at months 1 and 3 post-transplantation enable the evaluation of graft inflammation, immune cell infiltration, and the presence of regulatory signatures within the tissue. In parallel, longitudinal immunomonitoring is conducted to assess the persistence, phenotype, and functional impact of transferred CD8⁺ Tregs on the recipient immune system.

The rationale for developing CD8⁺ Treg-based therapies is supported by a growing body of experimental and translational evidence demonstrating their potent immunoregulatory capacity in transplantation and autoimmunity [13]. In both rodent and humanized models, CD8⁺ Tregs have been shown to contribute to the maintenance of immune tolerance and to prevent allograft rejection or autoimmune pathology [8, 10, 14, 15]. Mechanistically, these cells suppress pathogenic immune responses through multiple, non-redundant pathways, including IL-2 consumption, modulation of antigen-presenting cell function, and secretion of regulatory cytokines [16]. Notably, CD8⁺ Tregs recognize donor-derived antigens presented by MHC class I molecules, which are ubiquitously expressed on nucleated cells, including graft parenchymal cells [17, 18]. This feature confers upon CD8⁺ Tregs the unique ability to exert regulatory activity directly within transplanted tissues, thereby enabling local control of alloimmune responses at sites of inflammation. In addition, preclinical studies have demonstrated that CD8⁺ Tregs can be efficiently expanded *ex vivo* while maintaining stable suppressive properties [10, 12, 15], supporting their development as a clinically relevant cellular therapeutic product. Collectively, these observations provide a strong mechanistic and translational framework for the clinical evaluation of CD8⁺ Treg adoptive cell therapy as an innovative strategy to promote immune tolerance and improve long-term graft outcomes in organ transplantation.

Key differences arising from ongoing trials testing CD4⁺ and CD8⁺ Tregs may depend on the direct and indirect mechanisms of

antigen presentation and alloantigen recognition pathways [19]. While some evidence suggests that the allogeneic CD8⁺ T cell repertoire is driven by immunodominant, organ-specific peptides rather than conserved regions of non-self major histocompatibility complex (MHC) molecules, the direct pathway is primarily initiated by a cellular, T-cell mediated, allorecognition [20, 21]. Accordingly, CD8⁺ Tregs, through their recognition of HLA class I molecules, are uniquely positioned to interact not only with donor and recipient antigen-presenting cells but also directly with the transplanted tissue itself (Figure 1). In contrast, CD4⁺ Tregs, which primarily target HLA class II molecules, expressed at much lower levels within the graft, are better positioned to inhibit indirect alloantigen recognition pathways. This pathway depends on the processing and presentation of allogeneic peptides by recipient dendritic cells and B cells, ultimately driving chronic antibody-mediated rejection and progressive graft dysfunction [22, 23].

The initiation of clinical trials evaluating CD8⁺ Tregs also echoes the recent enthusiasm surrounding the development of chimeric antigen receptor (CAR) Tregs targeting HLA-A2 molecules, which are expressed in approximately 30% of the general population [24, 25]. By exploiting HLA-A2 mismatches between donor and recipient, Tregs can be engineered to exert potent, antigen-specific suppressive activity in the presence of HLA-A2, even if the selectivity of CAR-Tregs for dendritic cells versus graft tissue or endothelial cells remains still unclear [26, 27]. Ultimately, this strategy is mainly aiming at inducing a highly suppressive local microenvironment within the graft consistent with the well-documented role of Tregs in promoting solid tumor progression and reduced immunotherapeutic efficacy in cancer [28]. It should be noted that peer-reviewed clinical outcomes from HLA-A2 CAR-Treg trials are still missing. Early safety data from the STEADFAST study, a Phase I/II clinical trial initiated by Sangamo Therapeutics, and the LIBERATE trial by Quell Therapeutics, both evaluating HLA-A2 CAR Tregs in kidney and liver transplantation respectively, have been presented at international meetings and correlated with

the migration and persistence of Treg in the transplanted organs. Yet, it remains unclear whether these results will be sufficient to maintain these programs open and ultimately implemented in clinical practice considering the cost and the manufacturing complexity of those cells.

Nevertheless, the field of Treg therapy is receiving encouraging important positive signals from fields outside of SOT. The results from the Phase 3 Precision-T study indicate that donor derived Treg therapies (named Orca-T) can significantly enhance survival free from chronic graft-versus-host disease in patients with acute myeloid leukemia, acute lymphoblastic leukemia, and myelodysplastic syndromes, compared with conventional therapies following allogeneic hematopoietic stem cell transplantation. Thus, Orca-T is currently under Priority Review by the U.S. Food and Drug Administration [29]. Sonoma Biotherapeutics has announced favorable interim results from the ongoing Phase 1 REGULATE-RA study evaluating SBT-77-7101 in patients with refractory rheumatoid arthritis. The data shows encouraging safety profile alongside preliminary evidence of clinical efficacy in this treatment-resistant population.¹

Thus, the initiation of new clinical trials in the field of SOT is not only encouraging but also critically important to maintain scientific dynamism and knowledge gain. We are convinced that such efforts will contribute to the development of more targeted immunosuppressive strategies, ultimately improving long-term patient survival and quality of life. Even if unmodified CD4⁺ or CD8⁺ Treg therapy remains insufficient to induce durable immune tolerance, the expanding repertoire of cellular engineering technologies is an impressive reservoir for future scientific innovation. Thus, Treg antigen-specificity may be easily refined by targeting both direct and indirect presentation of immunodominant allogeneic peptides, including through TCR engineering [30], CD4-to-CD8 co-receptor swapping strategies [31], or by the development of antigen/tissue-specific CARs with optimized signaling domains [32, 33]. The incorporation of supraphysiological functional properties, such as controlled release of Treg pro-survival cytokines [34], is another example that could substantially augment Treg persistence and therapeutic efficacy in the coming years.

References

- Rosenbaum L. Tragedy, perseverance, and chance - the story of CAR-T therapy. *N Engl J Med* (2017) 377:1313–5. doi:10.1056/NEJMp1711886
- Tur C, Eckstein M, Velden J, Rauber S, Bergmann C, Auth J, et al. CD19-CAR T-cell therapy induces deep tissue depletion of B cells. *Ann Rheum Dis* (2025) 84:106–14. doi:10.1136/ard-2024-226142
- Avouac J, Barzel A, Caiati D, Davis RS, Gottschalk S, Grieshaber-Bouyer R, et al. Roads and detours for CAR T cell therapy in autoimmune diseases. *Nat Rev Drug Discov* (2026). 25:290–309. doi:10.1038/s41573-025-01349-4
- Porret R, Lana E, Mancarella A, Guillaume P, Pascual M, Meier RPH, et al. Regulatory T cell therapy for xenotransplantation, what perspectives. *Front Immunol* (2025) 16:1685682. doi:10.3389/fimmu.2025.1685682
- Zuber J, Kaminski H, Scientific COTSFD. The 2025 nobel prize in physiology or medicine honors the immune peacekeepers. *Transpl Int* (2025) 38:15767. doi:10.3389/ti.2025.15767
- Muller YD, Seebach JD, Bühler LH, Pascual M, Golshayan D. Transplantation tolerance: clinical potential of regulatory T cells. *Self Nonself* (2011) 2:26–34. doi:10.4161/self.2.1.15422
- Sawitzki B, Harden PN, Reinke P, Moreau A, Hutchinson JA, Game DS, et al. Regulatory cell therapy in kidney transplantation (the ONE study): a harmonised design and analysis of seven non-randomised, single-arm, phase 1/2A trials. *Lancet* (2020) 395:1627–39. doi:10.1016/S0140-6736(20)30167-7
- Guillonnet C, Hill M, Hubert FX, Chiffolleau E, Hervé C, Li XL, et al. CD40lg treatment results in allograft acceptance mediated by CD8CD45RC T cells, IFN-gamma, and indoleamine 2,3-dioxygenase. *J Clin Invest* (2007) 117:1096–106. doi:10.1172/JCI28801
- Picarda E, Bézie S, Boucault L, Autrusseau E, Kilens S, Meistermann D, et al. Transient antibody targeting of CD45RC induces transplant tolerance and potent antigen-specific regulatory T cells. *JCI Insight* (2017) 2:e90088. doi:10.1172/jci.insight.90088
- Bézie S, Meistermann D, Boucault L, Kilens S, Zoppi J, Autrusseau E, et al. Ex Vivo expanded human non-cytotoxic CD8⁺CD45RC^{low} tregs efficiently delay skin graft

¹ <https://acrabstracts.org/abstract/a-phase-1-study-of-autologous-car-treg-cells-in-refractory-rheumatoid-arthritis-interim-report-of-safety-and-efficacy/>

Data availability statement

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

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rejection and GVHD in humanized mice. *Front Immunol* (2017) 8:2014. doi:10.3389/fimmu.2017.02014

11. Sérizin C, Dugast L, Flippe L, Streitz M, Wendering DJ, Schlickeiser S, et al. Two subsets of regulatory CD8⁺ T cells with differential transcriptome revealed by single cell analysis. *iScience* (2025) 28:113512. doi:10.1016/j.isci.2025.113512

12. Bézie S, Greig J, Salle S, Lasselin J, Dugast L, Tesson L, et al. Stabilization of human CD8⁺ treg in inflammatory environments through FOXP3 expression. *Eur J Immunol* (2026) 56:e70140. doi:10.1002/eji.70140

13. Plaisie C, Bézie S, Guillonnet C. Regulatory T cell therapies: biological foundations, engineering strategies, and clinical translation. *Front Immunol* (2026) 17:1797186. doi:10.3389/fimmu.2026.1797186

14. Benallegue N, Nicol B, Lasselin J, Bézie S, Flippe L, Regue H, et al. Patients with severe multiple sclerosis exhibit functionally altered CD8⁺ regulatory T cells. *Neurol Neuroimmunol Neuroinflamm* (2022) 9:e200016. doi:10.1212/NXI.000000000200016

15. Bézie S, Charreau B, Vimond N, Lasselin J, Gérard N, Nèrrière-Daguin V, et al. Human CD8⁺ tregs expressing a MHC-specific CAR display enhanced suppression of human skin rejection and GVHD in NSG mice. *Blood Adv* (2019) 3:3522–38. doi:10.1182/bloodadvances.2019000411

16. Bézie S, Picarda E, Ossart J, Tesson L, Usal C, Renaudin K, et al. IL-34 is a Treg-specific cytokine and mediates transplant tolerance. *J Clin Invest* (2015) 125:3952–64. doi:10.1172/JCI81227

17. Picarda E, Bézie S, Venturi V, Echasserieau K, Mérieau E, Delhumeau A, et al. MHC-derived alloepitope activates TCR-biased CD8⁺ tregs and suppresses organ rejection. *J Clin Invest* (2014) 124:2497–512. doi:10.1172/JCI71533

18. Picarda E, Bézie S, Usero L, Ossart J, Besnard M, Halim H, et al. Cross-reactive donor-specific CD8⁺ tregs efficiently prevent transplant rejection. *Cell Rep* (2019) 29:4245–55.e6. doi:10.1016/j.celrep.2019.11.106

19. Charmetant X, Pettigrew GJ, Thaunat O. Allorecognition unveiled: integrating recent breakthroughs into the Current paradigm. *Transpl Int* (2024) 37:13523. doi:10.3389/ti.2024.13523

20. Zhang W, Roversi FM, Morris AB, Ortiz K, Zhou G, Hadley A, et al. Major histocompatibility complex and peptide specificity underpin CD8⁺ T cell direct alloresponse. *Am J Transpl* (2025) 25:916–29. doi:10.1016/j.ajt.2024.10.011

21. Pettigrew GJ Direct and indirect allorecognition-not so different after all [editorial]. *Am J Transpl* (2025);25(5):893. doi:10.1016/j.ajt.2025.02.014

22. Willicombe M, Brookes P, Sergeant R, Santos-Nunez E, Steggar C, Galliford J, et al. De novo DQ donor-specific antibodies are associated with a significant risk of antibody-mediated rejection and transplant glomerulopathy. *Transplantation* (2012) 94:172–7. doi:10.1097/TP.0b013e3182543950

23. Béland S, Désy O, El Fekih R, Marcoux M, Thivierge MP, Desgagné JS, et al. Expression of class II human leukocyte antigens on human endothelial cells shows high interindividual and intersubclass heterogeneity. *J Am Soc Nephrol* (2023) 34:846–56. doi:10.1681/ASN.0000000000000095

24. MacDonald KG, Hoeppli RE, Huang Q, Gillies J, Luciani DS, Orban PC, et al. Alloantigen-specific regulatory T cells generated with a chimeric antigen receptor. *J Clin Invest* (2016) 126:1413–24. doi:10.1172/JCI82771

25. Ballou C, Barton F, Payne EH, Berney T, Villard J, Meier RPH, et al. Matching for HLA-DR excluding diabetogenic HLA-DR3 and HLA-DR4 predicts insulin independence after pancreatic islet transplantation. *Front Immunol* (2023) 14:1110544. doi:10.3389/fimmu.2023.1110544

26. Wagner JC, Ronin E, Ho P, Peng Y, Tang Q. Anti-HLA-A2-CAR tregs prolong vascularized mouse heterotopic heart allograft survival. *Am J Transpl* (2022) 22:2237–45. doi:10.1111/ajt.17063

27. Muller YD, Ferreira LMR, Ronin E, Ho P, Nguyen V, Faleo G, et al. Precision engineering of an Anti-HLA-A2 chimeric antigen receptor in regulatory T cells for transplant immune tolerance. *Front Immunol* (2021) 12:686439. doi:10.3389/fimmu.2021.686439

28. Pan Y, Zhou H, Sun Z, Zhu Y, Zhang Z, Han J, et al. Regulatory T cells in solid tumor immunotherapy: effect, mechanism and clinical application. *Cell Death Dis.* (2025) 16:277. doi:10.1038/s41419-025-07544-w

29. Meyer EH, Salhotra A, Gandhi AP, Pantin J, Patel SS, Hoeg RT, et al. Orca-T vs allogeneic hematopoietic stem cell transplantation (Precision-T): a multicenter, randomized phase 3 trial. *Blood* (2026) 147:1168–77. doi:10.1182/blood.2025031313

30. Porret R, Alcaraz-Serna A, Peter B, Bernier-Latmani J, Cecchin R, Alfageme-Abello O, et al. T cell receptor precision editing of regulatory T cells for celiac disease. *Sci Transl Med* (2025) 17:eadr8941. doi:10.1126/scitranslmed.adr8941

31. Porret R, Lebreton F, Pace E, Stefanidis E, Semilietof A, Guillaume P, et al. Dual CD8 and TCR editing in regulatory T cells mediates HLA-A2-restricted tissue-specific homing. *Mol Ther* (2026) (26):00114. doi:10.1016/j.ymthe.2026.02.028

32. Alcaraz-Serna A, Trompette A, Porret R, Lana E, Cecchin R, Shanmuganathan A, et al. Chimeric allergen receptor regulatory T cells suppress birch pollen allergic airway inflammation. *Res Square (Preprint)* (2025). doi:10.21203/rs.3.rs-7301724/v1

33. Lamarthée B, Marchal A, Charbonnier S, Blein T, Leon J, Martin E, et al. Transient mTOR inhibition rescues 4-1BB CAR-tregs from tonic signal-induced dysfunction. *Nat Commun* (2021) 12:6446. doi:10.1038/s41467-021-26844-1

34. Robert J, Feuillolay M, de Temple-Llavero M, Akossi RF, Mhanna V, Cheraï M, et al. Expression of an interleukin-2 partial agonist enhances regulatory T cell persistence and efficacy in mouse autoimmune models. *Nat Commun* (2025) 16:4891. doi:10.1038/s41467-025-60082-z



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Can european law keep pace with biomedical innovation? The reinvention of transplantation regulation

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Europe is rewriting the rules for a generation of therapies that did not exist when the current rules were drafted. Directive 2001/18/EC [1] on genetically modified organisms and Directive 2010/53/EU [2] on human organs were written for a biomedical world in which genetic modification, *ex situ* organ engineering, and cross-species transplantation were theoretical or experimental; in 2024, a patient received a genetically modified pig kidney under compassionate use in the United States [3], and European groups are preparing similar trajectories. The question facing the European Union is therefore not whether to update these instruments, but whether the update will keep pace with the biology or fossilise a step behind it. The answer will determine which patients gain access to emerging therapies, where that innovation is developed, and whether Europe's regulatory architecture remains fit for a field whose boundaries are actively dissolving.

From static oversight to risk-adapted biotechnology governance

European biotechnology governance has historically been defined by the precautionary principle. While this approach has ensured high safety standards, it has also imposed rigidity and slowed the translation of innovation into practice. The current reform trajectory signals a shift in regulatory logic itself: from process-based oversight toward risk-adapted, product-oriented governance, where biological entities are assessed less by how they are made and more by what they do and how they are used (Table 1).

The move toward differentiated risk categories for genetically modified micro-organisms reflects this epistemic transition. Rather than treating genetic modification as a monolithic risk class, the emerging framework acknowledges heterogeneity in design, exposure, and potential clinical or environmental impact. This approach aligns with broader international trends, including European Medicines Agency (EMA) frameworks for gene

TABLE 1 Regulatory evolution in EU biotechnology and transplantation.

	Previous framework	Proposed revision
Regulatory philosophy	- Precautionary, <i>ex ante</i> risk containment - Uniform, process-based oversight with high procedural burden	- Risk-adapted, proportional, lifecycle governance - Differentiated risk tiers, simplified pathways for low-risk procedures
Logic for classification	- Method-based classification (how it is made) - Rigid categories (GMO/non-GMO; organ vs. non-organ) - Segmented regimes (medicines, devices, transplantation)	- Function- and risk-based evaluation (what it does) - Functional, context-dependent classification - Increasing convergence across regulatory domains
Governance model	- Static, rule-based regulation - Sector-specific and fragmented oversight	- Adaptive, data-driven, continuously updated oversight - Enhanced coordination across regulatory bodies
Decision timeline	Pre-authorisation dominant, slow adaptation	Conditional approval with iterative reassessment
Organ regulation	Linear pathway: donation → retrieval → storage → transplantation	Expanded continuum including <i>ex situ</i> organ processing and optimisation
<i>Ex situ</i> interventions	Not explicitly integrated	Explicitly included within regulatory scope

therapy, which increasingly emphasise risk proportionality and context-specific evaluation [4].

Yet this shift introduces new tensions. Concepts such as “low-risk GMMs” or “qualified safety assumptions” create interpretative space that may lead to divergence across Member States. What appears as simplification at the EU level may translate into heterogeneity at implementation level. The core challenge therefore extends beyond scientific classification to maintaining coherence in governance across jurisdictions.

Transplantation redefined: from retrieval to *ex situ* organ engineering

If upstream biotechnology is undergoing regulatory recalibration, transplant medicine is undergoing an even deeper ontological shift. Organ transplantation is no longer confined to retrieval, preservation, and implantation within narrow ischemic limits. Advances in machine perfusion, *ex situ* repair, and functional assessment are transforming the graft into a dynamic biological system—one that can be stabilised, evaluated, and even modified outside the body [5]. Evidence from liver transplantation shows that these technologies can increase organ utilisation and improve outcomes in selected settings [6–8]. More importantly, the imminent horizon holds the potential for interventions giving rise to a fully-fledged discipline of *ex vivo* organ medicine. In such a context both the time window and the conceptual scope of transplantation, will shift it from a discrete surgical act to a continuous process of biological intervention.

The proposed EU framework acknowledges this evolution by explicitly incorporating *ex situ* organ processing (Table 1), recognising that transplantation now spans a spectrum rather than a single event. Yet this expansion brings new regulatory challenges; the growing use of machine perfusion for optimisation or transformation before implantation must be anticipated.

Traditional governance was built around clearly defined stages—donation, allocation, retrieval, implantation. *Ex situ* intervention blurs boundaries, redistributing responsibility across device manufacturers, tissue establishments, clinical teams, and potentially pharmaceutical developers. Classification, in this

context, becomes a determinant not just of compliance, but of whether innovation is feasible.

Formalising authorisation pathways for organ processing by competent authorities offers a pragmatic alternative to default classification as advanced therapy medicinal products, which would impose disproportionate burdens. But proportionality is key: overly complex procedures risk creating bottlenecks, delaying access, and ultimately disadvantaging patients.

A risk-adapted approach—focusing scrutiny -where uncertainty or risk is greatest is essential. At the same time, regulatory simplicity must be preserved for standard graft preservation. Established techniques, even when delivered through machine perfusion, should not face additional constraints that disrupt workflows without clear benefit.

By clarifying roles across transplantation centres, regulators, and other stakeholders, the directive can strengthen governance in an increasingly complex field. The introduction of clinical outcome monitoring in areas of residual uncertainty embeds a more adaptive, evidence-generating model—well suited to a domain defined by small patient populations and rapidly evolving technologies.

Convergence of regulatory domains and fragmented biological reality

The strain that *ex situ* engineering places on transplantation governance is not an isolated problem; it is the first visible symptom of a broader structural tension. Regulating increasingly integrated biomedical systems through historically separate legal categories will inevitably strain the framework. Organs may now be perfused using devices, modified with biologics, assessed via digital biomarkers, and even improved through gene-based interventions. Each falling under different regulatory regimes with distinct evidentiary standards and timelines.

While the directive attempts to define boundaries, biology itself does not adhere to them. The result is growing regulatory ambiguity, particularly in advanced therapies where classification may depend more on framing rather than intrinsic biological differences. Increasingly, regulatory categories do not just evaluate innovation—they shape what innovation is possible.

Evidence maturation drift: a hidden systemic risk

Beyond structural complexity lies a more subtle systemic risk: misalignment between regulatory acceleration and the maturation of clinical evidence. As adaptive and conditional approval pathways expand, market access may precede robust demonstration of clinical benefit.

This “evidence maturation drift” has clear precedents across biomedical fields, where early approval based on surrogate endpoints or technical validity has not consistently translated into meaningful improvements in patient outcomes. The issue is further exacerbated by the fundamentally different regulatory frameworks governing medical devices and pharmaceuticals. Unlike pharmaceuticals, which generally require extensive clinical evidence prior to market authorization, medical devices often enter the market through pathways that emphasize technical performance and substantial equivalence rather than robust demonstration of long-term clinical benefit. Consequently, disparities in evidentiary standards and regulatory oversight may contribute to the premature adoption of technologies whose real-world therapeutic value remains insufficiently validated.

Machine perfusion and organ modification strategies exemplify this tension: they are biologically plausible and technologically sophisticated, yet their long-term comparative effectiveness remains incompletely characterized. Conversely, excessive regulatory pressure may hinder the timely adoption of innovations with the potential to substantially improve clinical outcomes.

Maintaining an appropriate balance between rigorous regulation and biotechnological innovation therefore justifies robust post-authorisation evidence requirements. However, preventing regulatory drift requires more than post-market surveillance alone. It also demands a clear definition of the relevant clinical context, comparative evaluation whenever feasible, and mechanisms for continuous iterative reassessment.

The revised EU framework moves in this direction through strengthened post-authorisation evidence generation, including mandatory national transplant registries and structured data collection on donors, recipients, procedures, and long-term outcomes. Facilitated international data sharing under GDPR further supports this effort. In parallel, ESOT's European transplant registry platform¹ provides a foundation for harmonised, cross-border outcome analysis and continuous evidence generation. Overall, the critical role of scientific societies fostering prospective multicentric networks align with this need for evidence corroboration.

Xenotransplantation: an emerging frontier without a dedicated framework

Perhaps the clearest stress test for the evolving European framework is xenotransplantation. The transplantation of

genetically modified animal organs into humans is no longer theoretical but entering early clinical reality [3]. While feasibility is increasing, major challenges remain, particularly regarding immunological compatibility (impacting on long-term graft function), and infectious risk [9–11].

Regulatory approaches elsewhere, such as those of the U.S. FDA, emphasise lifelong surveillance and stringent zoonotic risk control [10, 11]. In Europe, xenotransplantation has no dedicated legal framework and is only indirectly governed through overlapping regimes on genetic modification, medicinal products, and organ transplantation. This results in a fragmented regulatory landscape, with oversight dispersed across instruments not designed for solid organ xenografts. At EU level, the Organ Transplantation Directive applies only to human organs, while other rules—such as those on xenogeneic cell therapies or medical devices incorporating non-viable animal tissues (Regulation (EU) 2017/745)—do not extend to xenotransplantation. Efforts to classify engineered animal organs as advanced therapy medicinal products remain conceptually unconvincing. Overall, this gap reflects longstanding European caution since the 1990s moratorium and generates significant legal uncertainty.

Furthermore, divergence exists across Member States. Netherlands maintains a *de facto* moratorium through its Special Medical Procedures Act [12], whereas France allows xenotransplantation under strict conditions within authorised research protocols. Established under Law No. 98–535 and Article L. 1127–2 of the Public Health Code, this framework ensures authorisation, traceability, and long-term monitoring, but remains strictly national and does not replace the absence of EU-level harmonisation.

This absence of explicit governance is not merely a technical gap but a structural weakness, as xenotransplantation raises specific ethical and public health risks—particularly cross-species pathogen transmission and the need for long-term recipient surveillance—that cannot be adequately addressed through fragmented regulatory oversight.

Regenerative medicine and the shifting boundaries of organ definition

If xenotransplantation is the stress test the framework faces today, regenerative medicine is the one it will face next. As increasingly complex and functional constructs emerge, regulatory frameworks will need to move beyond traditional definitions based on anatomy alone, and instead account for function, origin, and intended use [13].

Advances in organ engineering and developmental biology further challenge regulatory categorisation. Organoids, chimeric systems, and stem-cell-derived tissues blur the boundary between repair and replacement, raising fundamental questions about what constitutes an “organ”.

This raises questions about whether regulatory frameworks should be anchored in anatomical structure, functional capacity, or developmental origin. As organ engineering progresses, particularly in vascularised and functional constructs, regulatory systems will increasingly need to accommodate entities that do not fit conventional definitions [13].

¹ <https://esot.org/esot-registry/>

Towards adaptive but accountable governance

The ambition of the proposed European framework is clear: to move from static precaution toward adaptive governance capable of keeping pace with biomedical innovation. This reflects a broader recognition that uncertainty is inherent to the life sciences.

The ambition of the proposed European framework is clear: to move from static precautionary regulation toward adaptive governance capable of keeping pace with biomedical innovation. This shift reflects a broader recognition that uncertainty is not an anomaly in life sciences, but a structural condition.

However, adaptability must not come at the expense of evidentiary discipline. The goal is not to trade speed for safety, but to balance them dynamically, to reach this equilibrium scientific and clinical expertise must remain central to decision-making, and regulatory decision-making and real-world clinical data must continuously inform regulatory assessment. Just as importantly, regulatory systems must remain open to revising earlier assumptions as knowledge evolves.

Conclusion

Europe's evolving regulatory architecture for biotechnology and transplantation marks a significant shift-seeking to align governance with the complexity of modern biology a domain where innovation sits at the convergence of genetic engineering, organ manipulation, and regenerative science.

Its success, however, will depend less on legislative intent than on implementation. Without robust mechanisms to align evidence, manage cross-domain convergence, and address emerging fields such as *ex situ* organ manipulation and xenotransplantation, there is a risk that regulatory acceleration will outpace understanding.

Three commitments would turn legislative intent into operational reality:

1. *Ex situ* organ processing should be governed by dedicated competent-authority authorisation pathways rather than by default classification as ATMPs; ATMP designation should apply only to genuinely novel interventions where the risk profile warrants it, so that established machine perfusion and related optimisation techniques are not burdened with disproportionate procedural requirements. Importantly, current EMA guidance for cells and tissues (EMA/681445/2011) already states that products which do not undergo substantial manipulation and are re-administered to fulfil the same essential function will generally not be considered ATMPs. In this regard, machine perfusion procedures clearly fulfil these criteria and should therefore not automatically fall within the ATMP regulatory framework.
2. Xenotransplantation requires an integrated European framework that explicitly bridges GMO, ATMP, and organ regimes, with mandatory cross-border recipient registries, lifetime zoonotic surveillance, and harmonised consent and containment standards — rather than the current dispersal of oversight across instruments not designed for it.

3. Adaptive authorisation must be paired with mandatory post-authorisation evidence infrastructure: national and European registries, structured long-term outcome collection, and pre-specified reassessment thresholds at which conditional approvals are either confirmed, narrowed, or withdrawn. Without this paired commitment, flexibility becomes drift.

The real challenge is not whether Europe can modernise its framework — but whether it can build one that is as adaptive, integrated, and self-correcting as the biology it aims to govern.

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References

1. Directive 2001/18/EC of the European parliament and of the council of 12 march 2001 on the deliberate release into the environment of genetically modified organisms and repealing council directive 90/220/EEC - Commission declaration. (2001). Available online at: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32001L0018&qid=1776180948248> (Accessed May 7, 2026).
2. Directive 2010/53/EU of the european parliament and of the council of 7 July 2010 on standards of quality and safety of human organs intended for transplantation. (2010). Available online at: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02010L0053-20100806&qid=1776180683761> (Accessed May 7, 2026).
3. Kawai T, Williams WW, Elias N, Fishman JA, Crisalli K, Longchamp A, et al. Xenotransplantation of a porcine kidney for end-stage kidney disease. *N Engl J Med* (2025) 392(19):1933–40. doi:10.1056/NEJMoa2412747
4. *Guideline on the Quality, Non-clinical and Clinical Aspects of Gene Therapy Medicinal Products*. (2018). Available online at: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-quality-non-clinical-and-clinical-aspects-gene-therapy-medicinal-products_en.pdf (Accessed May 7, 2026).
5. Niroomand A, Nita GE, Lindstedt S. Machine perfusion and bioengineering strategies in transplantation—beyond the emerging concepts. *Transpl Int* (2024) 37:13215. doi:10.3389/ti.2024.13215
6. for the Consortium for Organ Preservation in Europe Nasralla D, Coussios CC, Mergental H, Akhtar MZ, Butler AJ, et al. A randomized trial of normothermic preservation in liver transplantation. *Nature* (2018) 557(7703):50–6. doi:10.1038/s41586-018-0047-9
7. Tingle SJ, Dobbins JJ, Thompson ER, Figueiredo RS, Mahendran B, Pandanaboyana S, et al. Machine perfusion in liver transplantation. Cochrane hepato-biliary group. *Cochrane Database Syst Rev* (2023) 2023(9). doi:10.1002/14651858.CD014685.pub2
8. Mergental H, Laing RW, Kirkham AJ, Perera MTPR, Boteon YL, Attard J, et al. Transplantation of discarded livers following viability testing with normothermic machine perfusion. *Nat Commun* (2020) 11(1):2939. doi:10.1038/s41467-020-16251-3
9. Ali A, Kurome M, Kessler B, Kemter E, Wolf E. What genetic modifications of source pigs are essential and sufficient for cell, tissue, and organ xenotransplantation? *Transpl Int* (2024) 37:13681. doi:10.3389/ti.2024.13681
10. Ladowski JM, Bazzi N, Kirk AD. Xenotransplantation: promises and perils. *Annu Rev Med* (2026) 77(1):331–45. doi:10.1146/annurev-med-043024-015252
11. Mueller NJ, Scobie L. Potential infectious complications in pig xenograft donors and recipients. *Transpl Int* (2025) 37:13594. doi:10.3389/ti.2024.13594
12. Central Committee on Research Involving Human Subjects (CCMO). *Special Medical Procedures Act (Wet bijzondere Medische Verrichtingen)* (2025). Available online at: <https://english.ccmo.nl> (Accessed May 7, 2026).
13. Berishvili E, Piemonti L, De Koning EJP, Lindstedt S, Scholz H, Scott WE, et al. ESOT roadmap for advanced therapy medicinal products in transplantation: navigating regulatory challenges to enhance access and care. *Transpl Int* (2024) 37:13485. doi:10.3389/ti.2024.13485



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Regulatory approach to manipulated organs in Europe: preserving human organs as non-commercial goods

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We welcome the European Commission's initiative to amend the European Organ Directive within the framework of the proposed Biotech Act, intended to align it with recent scientific and technological advances, particularly organ manipulations aimed at improving transplant outcomes and human health. However, the amendment introduces a definition of organ processing that raises significant regulatory questions, particularly related to the exclusions it contains, which might allow human organs subjected to genetic or enzymatic manipulation to be regulated as medicinal products or as goods under industrial or commercial legal frameworks. The potential consequences of regulating human organs as medicines are reviewed, and awareness is raised of the risk of undermining the altruistic basis of donation and public trust in organ donation and transplantation systems. Moreover, this shift could jeopardise access to this life-saving therapy and threaten the economic sustainability of healthcare systems. Concurrently, potential risks associated with certain forms of manipulation (e.g., genetic modification of organs) must be rigorously assessed before their incorporation into clinical practice. We propose a distinctly European regulatory model that preserves the intrinsic value of human organs, promotes collaboration between medicines and transplant authorities, and enables biomedical innovation through companies providing services and technical solutions to healthcare systems.

KEYWORDS

advanced therapy medicinal products (ATMPs), equitable access, genetically modified human organs, legal framework, organ donation and transplantation, medical devices, *ex vivo* organ perfusion, substances of human origin (SoHO)

Introduction

We are witnessing extraordinary scientific and technological advances in organ transplantation aimed at increasing the availability of organs for clinical use [1] and improving their functionality. Newly developed techniques, such as enzymatic [2] or genetic [3] manipulations, may optimise transplant results and improve health outcomes [4, 5]. Most of these developments are enabled by novel *ex vivo* organ perfusion techniques [6, 7]. Overall, these advances offer new hope for patients in need but also pose significant challenges for healthcare systems and for the current model of organ donation and transplantation.

The transplantation of manipulated human organs presents additional biological risks beyond those associated with standard procedures. Before modified organs can be

incorporated into clinical practice, such risks must be carefully characterised and minimised. Once quality, safety, functionality and efficacy are established, incorporation into healthcare services will require integration within existing organ donation and transplantation programmes, supported by strengthened biovigilance and long-term follow-up systems.

On 16 December 2025, the European Commission (EC) made public its proposal for a Biotech Act [8], aimed at enhancing the competitiveness of European biotechnology companies in the healthcare sector, together with a proposed Directive [9] amending two existing Directives, including Directive 2010/53/EU (hereinafter, the Organ Directive) [10] on the quality and safety of human organs intended for transplantation (xenotransplantation falls outside the scope of the Directive).

We welcome the initiative to amend the Organ Directive, as it enables the legislation to adapt to scientific and technological advances. However, it is concerning that the proposed amendment might allow human organs subjected to specific manipulations to fall outside the scope of the Organ Directive or to be regulated under legal frameworks designed for marketable goods, thereby introducing a significant risk of commodification of human organs, with ethical and social consequences that could undermine organ donation and transplantation systems.

This manuscript analyses the regulatory implications of the proposed amendment to the Organ Directive for modified human organs, as well as its potential consequences, and seeks to contribute to the development of a European model that reconciles biomedical innovation with the protection of the intrinsic value of human organs.

An ambiguous regulatory framework may allow the commodification of human organs

Main amendment introduced by the revision of the Organ Directive

The original scope of the Organ Directive was “the donation, testing, characterisation, procurement, preservation, transport and transplantation of organs intended for transplantation” [10]. In the amended Directive, the term “preservation” is replaced by “processing”, and “organ processing” is defined as “operations involving the handling of organs, including but not limited to preservation, application of chemotherapy and surgery, performed to maintain or improve organ function prior to transplantation”. The definition of processing excludes the following: (1) “the preparatory handling of the organ during the surgical transplantation intervention”; (2) “the repurposing of organs into tissues or cells”; and (3) “the use of a substance with a pharmacological, immunological or metabolic action with the aim to treat or prevent a disease in the recipient to whom the organ will be transplanted, where such use does not constitute processing of the organ”.

Of note, the definition of a medicinal product in Directive 2001/83/EC (which currently regulates medicinal products in the European Union [EU]) [11] is very similar in wording to the third exclusion set out in the definition of organ processing. It is

therefore reasonable to argue that medicinal products may be used in donated organs without this constituting organ processing.

As a consequence, under the proposed amendment, manipulations performed on human organs prior to transplantation through treatment with medicinal products—for example, to induce genetic or enzymatic modifications—would not meet the definition of organ processing and would therefore fall outside the scope of the Organ Directive. In such cases, organs subjected to these interventions might instead be regulated as medicinal products or, more broadly, as commercial or industrial goods.

Moreover, even if the processing of a human organ through the use of a medicinal product or another type of product specifically developed for that purpose were considered to fall within the regulatory framework of the Organ Directive, the proposed amendment to the Organ Directive, which establishes a requirement to authorise such products under their respective regulatory frameworks, would in effect entail subjecting manipulated human organs to regulatory regimes designed for marketable goods.

Understanding the basis for and consequences of this potential regulatory shift requires consideration of the recent activity of the Committee for Advanced Therapies (CAT) of the European Medicines Agency (EMA) immediately preceding the publication of the proposal to amend the Directive. It also requires consideration of the differences between the regulatory pathways governing clinical research and the authorisation of medicines and transplants, and of the paradigm-shifting distinction between access to medicines and organ allocation.

Classification of viral vectors used to genetically modify human organs as medicinal products

On 7 November 2025, the CAT recommended that a viral vector intended to modify human lungs *ex vivo* prior to transplantation be classified as an advanced therapy medicinal product (ATMP), specifically as a gene therapy medicinal product (GTMP). The vector incorporates a sequence to silence HLA antigen expression in donor organs and mitigate immune-mediated rejection in transplant recipients.

The CAT stated that “This classification is without prejudice to the status of the genetically modified organ of human origin and the GMO legislation.” [12] In other words, the CAT refrained from taking a position on the classification of the genetically modified organ itself, since the current European legal framework does not envisage the qualification of human organs as medicinal products [11, 13, 14] or as products distinct from human organs. However, a few weeks after the CAT issued this recommendation, the EC published its proposal to amend the Organ Directive [9] within the context of the proposed Biotech Act [7].

Based on the definition proposed by the EC, the use of a viral vector (classified as a medicinal product) to genetically modify human lungs in order to reduce immunogenicity and prevent graft rejection would not be considered organ processing, and the genetically modified lungs would therefore fall outside the scope of the Organ Directive. We may thus be moving from

speculation to reality, as the amended text opens the door to genetically or enzymatically modified human organs being regulated under alternative legal frameworks.

Regulatory ambiguity

Even though genetically modified organs are not excluded from the scope of the Organ Directive nor expressly regulated as medicinal products, the requirement introduced by the revised Organ Directive to authorise medicinal products under their respective regulatory frameworks prior to the authorisation of the processing of human organs generates regulatory ambiguity and might result in manipulated human organs being regulated under the legal framework for medicines.

The vector used to modify the organ is a relevant example. It has itself been classified as a medicinal product, yet its effects can only be assessed in the transplanted organ. Therefore, evidence of safety and efficacy (essential requirements for marketing authorisation) would necessarily be generated in clinical trials in which the transplantation of the modified organ is evaluated. In practice, this would mean that the modified organ would fall under the regulatory framework applicable to investigational medicinal products. Nevertheless, in order to conduct such clinical trials, altruistically donated human organs used for genetic modification might ultimately be diverted from transplantation to patients on the waiting list. Moreover, the eligibility criteria for patients to participate in the clinical trial would interfere with the allocation criteria established by the competent transplant authorities.

More importantly, if the quality, safety and efficacy of the genetically modified organ have been demonstrated under the medicines framework, on what basis could marketing authorisation for the vector as a medicinal product be granted if it is not intrinsically linked to the modified organ, which constitutes the actual therapy evaluated in clinical trials? This uncertainty extends to approval pathways and competent authorities, since the processes for incorporating transplants and medicinal products into healthcare systems differ substantially and would be at odds with organ donation and transplantation systems, as discussed below.

This outcome would only be avoided if genetically modified organs are maintained within the scope of the Organ Directive, accompanied by a clear regulatory framework under which the authorisation of organ modifications and their clinical evaluation remain within the competence of the relevant transplant authorities.

Consequently, the classification of a viral vector applied *ex vivo* to human organs as a GTMP creates uncertainty regarding the applicable regulatory framework for assessing the quality, safety and efficacy of the therapy, which, in effect, is the genetically modified organ and does not itself qualify as a medicinal product, thereby creating a conflict between regulatory frameworks.

It is possible that the CAT's recommendation to classify the viral vector as a GTMP was influenced by concerns about the biological risks associated with genetic modifications of human organs. At the time of classification, no proposal to amend the Organ Directive—potentially allowing genetically modified organs to be regulated as medicines—had been published. This may have

contributed to classifying the viral vector as a medicinal product to ensure some degree of regulatory oversight of genetically modified organs. Nevertheless, in our view, the viral vector does not fully meet the definition of GTMP.

Some aspects to consider on the classification

On the current EU legal framework [14], “Gene therapy medicinal product means a biological medicinal product which has the following characteristics: (a) it contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence; (b) its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence.”

First, the viral vector is neither used in nor administered to human beings. It is applied to human organs for *ex vivo* modification before transplantation. Administration is therefore indirect, analogous to *ex vivo* gene therapy, where a viral vector modifies human cells before administration to the patient. In such cases, the genetically modified cells (rather than the viral vector itself) constitute the GTMP.

Second, although the CAT states that “its therapeutic effect relates directly to the product of genetic expression of this sequence”, the therapeutic effect derives from the transplanted organ, not from the viral vector, which merely mitigates the risk of rejection. The genetic modification of the organ improves durability rather than constituting the therapy.

While the classification of a vector for *ex vivo* human organ modification as a GTMP could be viewed by some as irrelevant, we take a different view: it implies that genetically modified human organs would fall under the investigational medicinal products framework—thereby interfering with organ donation and transplantation rules—and would open the door to their healthcare provision being delivered by pharmaceutical and biotech companies holding marketing authorisation. This would represent a seismic shift in the way organ transplantation is currently implemented in Europe.

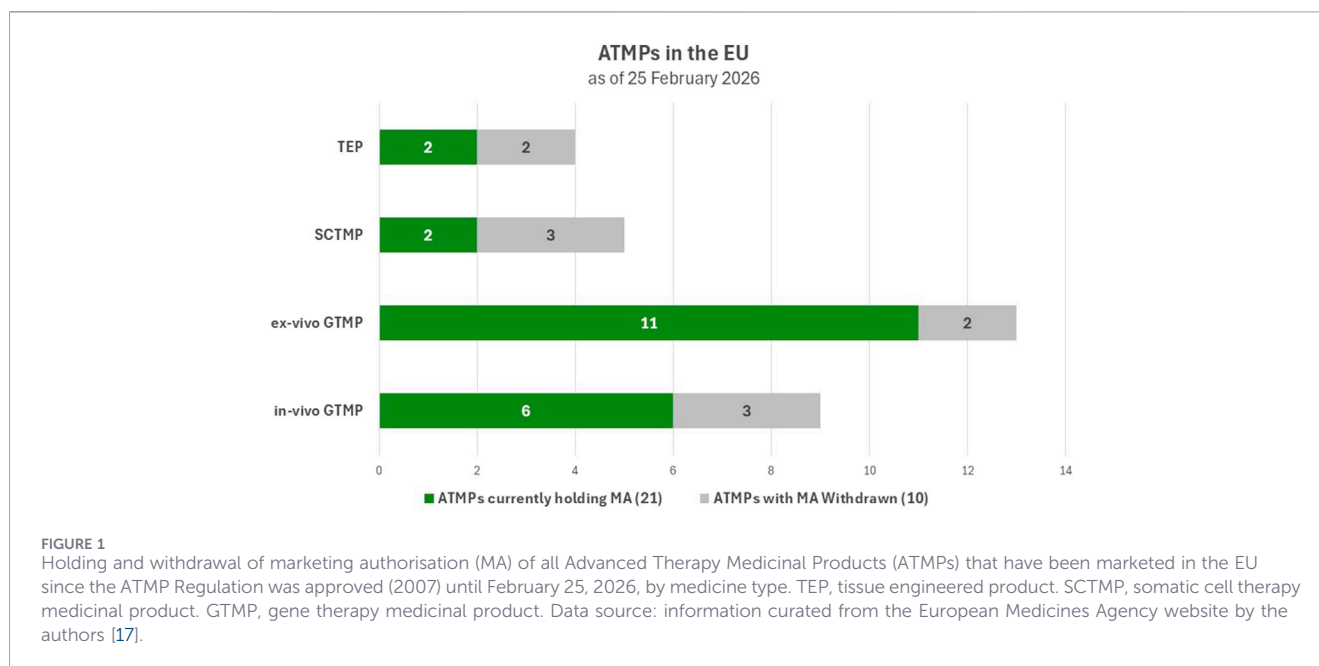
Consequences of regulating human organs as medicinal products

There is no doubt that human organs genetically modified *ex vivo* prior to transplantation pose higher biological risks associated with the genetic modification. Assessment of these risks, as well as evaluation of the modified organ's quality, safety, functionality and effectiveness, must be conducted according to strict standards proportionate to the level of risk. However, classifying the vector as a medicinal product, and potentially manipulated organs as commercial goods, entails significant implications. In particular, it remains unclear how such therapies would be integrated into existing organ donation and transplantation models grounded in altruistic donation, non-remuneration of organs and equity of access.

If organs—or vectors used for their genetic modification—are regulated as ATMPs, the altruistic, not-for-profit model risks

TABLE 1 Advanced therapy medicinal products (ATMPs) holding marketing authorisation (MA) or with MA withdrawn in the European Union as of 25 February 2026.

ATMP type	Currently holding MA	MA withdrawn
Tissue-engineered products	SPHEROX® HOLOCLAR®	MACI® CHONDROCELECT®
Somatic cell therapy medicinal products	ZEMCELPRO® EBVALLO®	ALOFISEL® ZALMOXIS® PROVENGE®
Ex vivo gene therapy medicinal products	WASKYRA® AUCATZYL® CASGEVY® CARVYKTI® BREYANZI® ABECMA® LIBMELDY® TECARTUS® YESCARTA® KYMRIAH® STRIMVELIS®	ZYNTGLO® SKYSONA®
In vivo gene therapy medicinal products	VYJUVEK® HEMGENIX® UPSTAZA® ZOLGENSMA® LUXTURNA® IMLYGIC®	BEQVEZ® GLYBERA® ROCTAVIAN®
Additional information	ATMPs currently holding MA: https://www.ema.europa.eu/en/medicines/human/EPAR/spherox https://www.ema.europa.eu/en/medicines/human/EPAR/holoclar https://www.ema.europa.eu/en/medicines/human/EPAR/zemcelpro https://www.ema.europa.eu/en/medicines/human/EPAR/ebvallo https://www.ema.europa.eu/en/medicines/human/EPAR/waskyra https://www.ema.europa.eu/en/medicines/human/EPAR/aucaatzyl https://www.ema.europa.eu/en/medicines/human/EPAR/casgevvy https://www.ema.europa.eu/en/medicines/human/EPAR/carvykti https://www.ema.europa.eu/en/medicines/human/EPAR/breyanzi https://www.ema.europa.eu/en/medicines/human/EPAR/abecma https://www.ema.europa.eu/en/medicines/human/EPAR/libmeldy https://www.ema.europa.eu/en/medicines/human/EPAR/tecartus https://www.ema.europa.eu/en/medicines/human/EPAR/yescarta https://www.ema.europa.eu/en/medicines/human/EPAR/kymriah https://www.ema.europa.eu/en/medicines/human/EPAR/strimvelis https://www.ema.europa.eu/en/medicines/human/EPAR/vyjuvek https://www.ema.europa.eu/en/medicines/human/EPAR/hemgenix https://www.ema.europa.eu/en/medicines/human/EPAR/upstaza https://www.ema.europa.eu/en/medicines/human/EPAR/zolgensma https://www.ema.europa.eu/en/medicines/human/EPAR/luxturna https://www.ema.europa.eu/en/medicines/human/EPAR/imlygic ATMPs with MA withdrawn: https://www.ema.europa.eu/en/medicines/human/EPAR/maci https://www.ema.europa.eu/en/medicines/human/EPAR/chondroelect https://www.ema.europa.eu/en/medicines/human/EPAR/alofisel https://www.ema.europa.eu/en/medicines/human/EPAR/zalmoxis https://www.ema.europa.eu/en/medicines/human/EPAR/provenge https://www.ema.europa.eu/en/medicines/human/EPAR/zynteglo https://www.ema.europa.eu/en/medicines/human/EPAR/skysona https://www.ema.europa.eu/en/medicines/human/EPAR/beqvez https://www.ema.europa.eu/en/medicines/human/EPAR/glybera https://www.biomarin.com/news/company-statements/biomarin-voluntarily-withdraws-roctavian-from-the-market/	



shifting towards a commodity-based framework in which patient access depends on for-profit entities obtaining marketing approval, followed by price negotiations, reimbursement decisions and commercial strategies. The potential consequences, grouped below into three categories, may compromise altruistic donation and undermine the current organ donation and transplantation systems.

Difficulties in patient accessibility

Experience with the regulatory framework for human cells and tissues provides relevant lessons [7, 15]. Following adoption of Regulation (EC) No 1394/2007 on ATMPs [13], certain therapies previously regulated as transplants migrated to the medicines framework, including artificial skin transplants, limbal cell and chondrocyte cultures. Patient access became conditional upon centralised marketing authorisation or, alternatively, authorisation under the hospital exemption (HE) scheme [16].

Nearly two decades after approval of the ATMP Regulation, no company in the EU has commercialised artificial skin, which represents the last therapeutic option for severe burns. One company obtained centralised marketing authorisation for autologous limbal stem cells (HOLOCLAR®), yet access across Member States has remained limited due to lack of reimbursement, and the company required public rescue funding in 2023 shortly before filing for bankruptcy [17]. Three companies obtained marketing authorisation for autologous cultured chondrocyte based products (CHONDROCELECT®, MACI® and SPHEROX®), but two were subsequently withdrawn from the market [18]. At present, the remaining product has limited availability, restricted to a small number of Member States [19], with even fewer providing public funding [20]. The causes of this situation are multifactorial. Among other factors, cost–utility evaluations have often been unfavourable, since the regulation of these therapies as medicinal products increases costs, while clinical efficacy remains unchanged. Consequently, the

transition from transplant to ATMP legal framework has increased healthcare expenditure and resulted in limited and unequal access to these therapies [21].

More broadly, of the 31 ATMPs granted marketing authorisation in the EU to date, one third have been withdrawn from the market, mainly for commercial reasons following decisions by marketing authorisation holders [18] (Table 1; Figure 1).

Notably, accessibility to these therapies not only depends on marketing authorisation but also on drug availability and public reimbursement. Many ATMPs are available in only a few EU Member States, partly due to companies' limited manufacturing and supply capacities, which lead them to prioritise markets based on commercial strategy, market size, and national pricing and reimbursement systems [19, 20]. Reimbursement for approved indications also varies considerably, with some countries funding only narrower indications than those authorised and delaying decisions to contain pharmaceutical expenditure [22].

The HE scheme, applicable under specific conditions, has also not ensured equitable access across Europe. An EC report [23] showed considerable variability in national implementation. By 2025, over 40% of Member States had not authorised treatments under HE schemes, often due to the absence of national legislation. Among those that had done so, both the number of patients and review timelines varied substantially [24].

In summary, access to ATMPs remains limited and uneven across the EU. This experience suggests that the transition from the transplant to the medicines regulation has not favoured the development of, or equitable access to, these therapies [25].

Risks to the economic sustainability of healthcare systems

The negotiated price of commercial ATMPs ranges from tens or hundreds of thousands of euros to several million euros per dose [19, 20]. Such pricing poses a clear challenge to the sustainability of

publicly funded healthcare systems and contributes to restricted access [7]. Beyond the negotiating leverage of individual countries, the factors underlying drug pricing are complex, depending partially on manufacturing costs and legitimate profit expectations. Additionally, the medicines framework, compared with the transplant framework, entails further costs, including those for obtaining marketing authorisation and fulfilling post-authorisation obligations.

For example, the production of the GTMP STRIMVELIS®—the only curative treatment for ‘bubble children’—initially marketed by two pharmaceutical companies and later ‘rescued’ by a public institution, involves substantial fixed costs, 25% of which are regulatory [26]. Fees payable to the EMA, already burdensome for public institutions, have recently risen [27]. These factors threaten continued market availability, particularly for ultra-rare conditions [28].

Experience with human cells and tissues [25] should therefore serve as a cautionary precedent [29]. Regulating manipulated human organs within the medicines framework may replicate similar challenges for sustainability and access.

Ethical conflicts

Regulating modified human organs as medicines and subjecting them to market forces would entail substantial ethical implications. The donation and transplantation of human organs, given their unique origin, are founded on ethical principles promoted by the World Health Organisation [30–32], the Council of Europe [33–35] and the EU [10, 36]: non-commercialization of the human body, voluntary and unpaid donation, and allocation governed by a transparent and fair system, guided by clinical criteria and ethical norms. These principles underpin public trust and participation in organ donation.

The principle of non-commercialisation of the human body is enshrined in the EU Charter of Fundamental Rights, which prohibits “making the human body and its parts, as such, a source of financial gain” [36]. Regulating modified organs as medicines would give rise to a marketable product capable of generating economic benefit for marketing authorisation holders—profit not subject to regulation beyond market rules—, potentially violating such fundamental principle [37].

Commercialisation may also undermine altruistic donation. Public perception that donated organs generate significant economic benefit for private entities could erode trust in transplant systems [15, 38]. Such commercialisation would pose a serious risk to donation and transplantation programmes in the EU, which rely entirely on altruistic donation and on a uniquely valuable yet highly vulnerable asset: public trust in a system governed by principles fundamentally different from those of the market. Any erosion of that trust could reduce willingness to donate and thereby jeopardise access to life-saving transplantation for thousands of patients.

Furthermore, Europe’s commitment to equitable access requires that modified organs be fully integrated into the same allocation and prioritisation rules as unmodified organs, based solely on clinical criteria to ensure equitable access. The coexistence of dual pathways for organ availability—donated and acquired organs—would threaten such integration.

If modified organs were commercialised and not publicly funded, or reimbursed only for restricted indications—as frequently occurs with ATMPs—this could not only create inequities in access but also undermine donation and transplantation programmes for organs and other substances of human origin (SoHO). Moreover, if wealthy individuals were able to gain access to transplantation of a modified organ by paying for it, this would constitute a direct and irreversible threat, since altruistic donation depends not only on trust but also on the principle of reciprocity. Ultimately, the mere prospect that an altruistically donated, subsequently modified organ could be commercialised and acquired by those with high purchasing power would signal the end of donation and transplantation programmes.

How to promote technological innovation while preserving the ethical principles governing the donation and transplantation of modified organs

Our proposal is grounded in the premise that human organs intended for transplantation—regardless of any manipulation or clinical indication—must remain regulated within the transplant framework and protected from commodification. Table 2 presents a proposal for a governance model to integrate manipulated organs into the current organ donation and transplantation systems and highlights potential ethical issues that may arise.

Industry’s role and organ versus technology ownership

Innovation in organ transplantation requires industry participation. However, such involvement must not compromise the ethical standards governing donation and transplantation [30–36]. Within the medicines framework, companies hold ownership of marketed products. This conflicts with the European transplant model, where organs are altruistically donated to society and allocated through public systems. Companies may legitimately own the technologies used for organ manipulation, but not the organs themselves.

Under the transplant regulatory framework, companies could instead act as service providers for organ modifications, subject to transparent oversight consistent with donation and transplant principles. Although this could seem commercially less attractive than full product ownership, this model would offer advantages: simpler and faster integration into healthcare systems [39], lower regulatory fees and broader uptake. Experience has shown that the high prices of ATMPs lead to restrictions in publicly funded treatments and that, where funding is granted, it is often limited to narrower indications than those authorised.

Collaboration among relevant authorities and distinction between assessment and authorisation procedures

Legitimate concerns regarding biological risks must be addressed through rigorous evaluation. However, assessment and

TABLE 2 Proposed governance model to integrate manipulated organs on the current organ donation and transplantation systems and potential ethical issues arising.

Step	Who does it	Who oversees/ Evaluates it	Who authorizes it	Who owns it	Potential ethical issues
Organ donation (identification, characterization, evaluation, maintenance, consent, authorization, recovery, preservation)	Healthcare systems	Transplant organizations	Transplant authorities	Organs are owned by the society at large	Need to ensure donation remains voluntary and unpaid, to respect human dignity and integrity and to ensure the quality and safety of donated organs
Manipulation of human organs intended for transplantation	Companies act as service providers	Medicines agencies in collaboration with transplant organizations, using standards based on pharmaceutical legislation	Transplant authorities and ethics committees	Companies own the technology	Risk of diversion of transplantable organs if scientific and ethical criteria are not clearly predefined Inequities in access to manipulated organs due to unaffordable service fees
Non-clinical research and quality characterization of manipulated human organs			Ethics committees		
Clinical research and assessment of safety, functionality and effectiveness of manipulated human organs	Healthcare systems in collaboration with companies	Transplant organizations in collaboration with medicines agencies	Transplant authorities and ethics committees	Manipulated organs are owned by the society at large	Differing attributes and clinical outcomes between manipulated and non-manipulated human organs may affect decision-making by transplant organizations and affect prioritization
Allocation of manipulated human organs	Healthcare systems	Transplant organizations, with manipulated organs being integrated into the transplant prioritization system	Transplant authorities		
Transplantation of manipulated human organs and long-term monitoring of outcomes		Transplant organizations, with transplants performed in specifically authorized centres			

authorisation are distinct processes. Authorising genetically modified organs as transplants does not preclude prior evaluation applying standards inspired by the pharmaceutical legislation, nor does it exclude collaboration between medicines agencies and transplant authorities.

In some Member States, such cooperation already exists. For example, medicines authorities may require that transplant organisations assess donor evaluation and organ recovery before authorising clinical trials involving ATMPs derived from human tissues or cells. Similar reciprocal collaborative mechanisms could be established to ensure that manipulated human organs undergo comprehensive assessment of quality, safety, preclinical evidence, patient follow-up and clinical outcomes prior to authorisation under the transplant framework.

Alternatively, transplant organisations could develop strengthened methodologies for evaluating innovation in organ transplantation. Such an approach is already applied to other SoHO [40], in accordance with the relevant European Regulation [41].

Reinforcing the Organ Directive

The draft Biotech Act and the proposed amendment to the Organ Directive represent an opportunity to establish a robust European legal framework governing transplantation of

manipulated organs while preserving ethical principles already enshrined in the current Organ Directive.

However, we strongly advocate the revision of the proposed definition of organ processing to encompass any form of manipulation to which human organs may be subjected. Furthermore, to ensure equitable access, the Organ Directive should also require Member States to maintain transparent allocation systems guided by clinical criteria and ethical standards.

The Organ Directive should strengthen evaluation and biovigilance mechanisms, while ensuring that clinical research on organ modifications and their authorisation remain within the competence of the relevant transplant authorities, in line with the European SoHO Regulation [41], which offers clear advantages over the ATMP Regulation: lower costs; greater flexibility in collecting safety and clinical outcome data based on risk assessment; authorisation of SoHO preparations by national competent authorities under a harmonised EU procedure; a suitable biovigilance system; more equitable and timely access; and a regulatory framework that upholds the principle of non-commercialisation of the human body [39].

Additionally, follow-up registries of living donors and transplant recipients should be explicitly required, as they are essential for assessing the safety and effectiveness of organ transplantation and processing outcomes. Likewise, in the public health interest, competent authorities must have access to appropriate data, in

full compliance with EU data protection rules, to ensure the safety and effectiveness of donation and transplantation processes and to enable international information exchange.

Integration of manipulated organs into donation and transplantation systems

Two key elements are essential for proper integration of manipulated organs into donation and transplantation systems without compromising their fundamental principles.

First, donor selection for organs intended for manipulation must follow scientific and ethical criteria approved by competent transplant authorities. The donation process must remain fully integrated within official programmes, under the responsibility of donor coordinators or procurement organisations, who oversee donor identification, evaluation, and maintenance, as well as coordinate all activities from donation to transplantation, in accordance with protocols approved by transplant authorities.

Second, manipulated organs must be incorporated into the organ allocation system. Accordingly, prioritisation of patients requires that all those eligible for transplantation be registered on the official waiting lists under the supervision of the competent transplant authorities. Both the clinical criteria for inclusion on waiting lists and those governing allocation of different types of organs should be established under the oversight of the competent authorities to ensure equitable access.

Regulating manipulated organs as transplants rather than medicinal products would contribute to healthcare economic sustainability while strengthening the donation model based on altruism, free provision and equitable access. Preserving this model is essential, as it saves thousands of lives annually and improves quality of life for many more.

Conclusion

We advocate a genuinely European model in which biomedical innovation progresses in alignment with the protection of the intrinsic value of human organs, supporting technological developments as services and technical solutions, while ensuring that human organs—regardless of manipulation or therapeutic/preventive use—remain outside any profit-driven commercial framework. This model is consistent with the ESOT position paper on ATMPs in the context of transplantation [7], which offers strategic recommendations to facilitate access to these technologies across Europe and aims to improve transplant

outcomes. Its first recommendation highlights the need to streamline regulatory procedures to expedite approval timelines and reduce associated costs, while maintaining stringent standards of safety and efficacy.

Only under such an approach can scientific and clinical excellence advance without compromising the values that have made Europe's donation and transplantation systems a global benchmark. The Biotech Act and the proposed amendment to the European Organ Directive—provided they preserve the altruistic, non-commercial nature of human organs—offer a strategic opportunity to establish a robust framework for organ manipulation, promoting technical and scientific progress without abandoning the ethical principles underpinning Europe's moral leadership in transplantation.

Author contributions

NC: Conceptualization, Data curation, Visualization, Writing – original draft. AI: Conceptualization, Data curation, Writing – review and editing. BD-G: Conceptualization, Data curation, Writing – review and editing. All authors contributed to the article and approved the submitted version.

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References

- Garreta E, Moya-Rull D, Centeno A, Marco A, Ullate-Agote A, Amato G, et al. Systematic production of human kidney organoids for transplantation in porcine kidneys during *ex vivo* machine perfusion. *Nat Biomed Eng* (2025). doi:10.1038/s41551-025-01542-1
- Wang A, Ribeiro RVP, Ali A, Brambate E, Abdelnour-Berchtold E, Michaelsen V, et al. *Ex vivo* enzymatic treatment converts blood type A donor lungs into universal blood type lungs. *Sci Transl Med* (2022) 14:eabm7190. doi:10.1126/scitranslmed.abm7190
- Nykanen AI, Keshavjee S, Liu M. Creating superior lungs for transplantation with next-generation gene therapy during *ex vivo* lung perfusion. *J Heart Lung Transpl* (2024) 43(5):838–48. doi:10.1016/j.healun.2024.01.016
- Montgomery RA, Stern JM, Lonze BE, Tatapudi VS, Mangiola M, Wu M, et al. Results of two cases of pig-to-human kidney xenotransplantation. *N Engl J Med* (2022) 386(20):1889–98. doi:10.1056/NEJMoa2120238
- He J, Shi J, Yang C, Peng G, Ju C, Zhao Y, et al. Pig-to-human lung xenotransplantation into a brain-dead recipient. *Nat Med* (2025) 31(10):3388–93. doi:10.1038/s41591-025-03861-x
- Nakata K, Alderete IS, Hughes BA, Hartwig MG. *Ex vivo* lung perfusion: recent advancements and future directions. *Front Immunol* (2025) 16:1513546. doi:10.3389/fimmu.2025.1513546

7. Berishvili E, Piemonti L, de Koning EJP, Lindstedt S, Scholz H, Scott WE, et al. ESOT roadmap for advanced therapy medicinal products in transplantation: navigating regulatory challenges to enhance access and care. *Transpl Int* (2024) 37:13485. doi:10.3389/ti.2024.13485
8. COM. 1022: proposal for a REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on establishing a framework of measures for strengthening Union's biotechnology and biomanufacturing sectors particularly in the area of health and amending regulations (EC) no 178/2002, (EC) no 1394/2007, (EU) no 536/2014, (EU) 2019/6, (EU) 2024/795 and (EU) 2024/1938 (european biotech act) (2025). Available online at: [https://www.europarl.europa.eu/RegData/docs_autres_institutions/commission_europeenne/com/2025/1022/COM_COM\(2025\)1022_EN.pdf](https://www.europarl.europa.eu/RegData/docs_autres_institutions/commission_europeenne/com/2025/1022/COM_COM(2025)1022_EN.pdf) (Accessed February 25, 2026).
9. COM. 1031: proposal for a DIRECTIVE OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL amending directives 2001/18/EC and 2010/53/EU as regards the placing on the market of genetically modified micro-organisms and the processing of organs (2025). Available online at: <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52025PC1031> (Accessed February 25, 2026).
10. European Parliament and Council of the European Union. Directive 2010/53/EU of 7 July 2010 on standards of quality and safety of human organs intended for transplantation. *Official J Euro Union [Internet]* (2010) L207: 14–29. Available online at: <https://eur-lex.europa.eu/eli/dir/2010/53/oj/eng> (Accessed May 19, 2026).
11. European Parliament and Council of the European Union. Directive 2001/83/EC of 6 November 2001 on the Community code relating to medicinal products for human use. *Official J Euro Union [Internet]* L311: pp. 67–128. Available online at: <https://eur-lex.europa.eu/eli/dir/2001/83/oj/eng> (Accessed May 19, 2026).
12. European Medicines Agency. *Scientific Recommendations on Classification of Advanced Therapy Medicinal Products*. Amsterdam: EMA (2025). Available online at: <https://www.ema.europa.eu/en/human-regulatory-overview/marketing-authorisation/advanced-therapies-marketing-authorisation/scientific-recommendations-classification-advanced-therapy-medicinal-products> (Accessed February 25, 2026).
13. European Parliament and Council of the European Union. Regulation (EC) No 1394/2007 of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004. *Official J Euro Union [Internet]* (2007) L324: 121–37. Available online at: <https://eur-lex.europa.eu/eli/reg/2007/1394/oj/eng> (Accessed May 19, 2026).
14. European Commission. Commission Directive 2009/120/EC of 14 September 2009 amending Directive 2001/83/EC on the Community code relating to medicinal products for human use as regards advanced therapy medicinal products. *Official J Euro Union [Internet]* (2009) L242: 3–12. Available online at: <https://eur-lex.europa.eu/eli/dir/2009/120/oj/eng> (Accessed May 19, 2026).
15. Cuende N, Vilarrodona A, Vuelta E, Marazuela R, Herrera C, Querol S, et al. Addressing risks derived from the commodification of substances of human origin: a European proposal applicable worldwide. *Transplantation* (2023) 107(4):867–77. doi:10.1097/TP.00000000000004527
16. Cuende N, Ciccocioppo R, Forte M, Galipeau J, Ikonomou L, Levine BL, et al. Patient access to and ethical considerations of the application of the european union hospital exemption rule for advanced therapy medicinal products. *Cytotherapy* (2022) 24(7): 686–90. doi:10.1016/j.jcyt.2022.03.007
17. Paterlini M. Stem-cell therapy company saved by public funds. *Nat Italy News* (2023). doi:10.1038/d43978-023-00183-9
18. European Medicines Agency. Committee for advanced therapies. CAT quarterly highlights and approved ATMPs. March 2026. Amsterdam: EMA. Available online at: https://www.ema.europa.eu/en/documents/committee-report/cat-quarterly-highlights-approved-atmps-march-2026_en.pdf (Accessed April 19, 2026).
19. Čechová Z, Kubátová J, Bártová A, Jamárik J, Samek J. Beyond reimbursement status: availability of advanced therapy medicinal products across the European Union. *Ther Innov Regul Sci* (2025) 59(4):728–36. doi:10.1007/s43441-025-00769-z
20. Iglesias-López C, Agustí A, Vallano A, Obach M. Financing and reimbursement of approved advanced therapies in several European countries. *Value Health* (2023) 26: 841–53. doi:10.1016/j.jval.2022.12.014
21. Verbeke G, Convents L, Delmotte N, Draye JP, Jennes S, Vanderkelen A, et al. Current state-of-play of the EU advanced therapy medicinal product (ATMP) field, with an emphasis on Belgian human cell and tissue products. *Wound Repair Regen* (2025) 33: e70039. doi:10.1111/wrr.70039
22. Rejon-Parrilla JC, Espin J, Garner S, Kniazkov S, Epstein D. Pricing and reimbursement mechanisms for advanced therapy medicinal products in 20 countries. *Front Pharmacology* (2023) 14(28 Nov):1199500. doi:10.3389/fphar.2023.1199500
23. European Commission. European health and digital executive agency. In: *Study on the Hospital Exemption: Final Report*. Brussels: Publications Office of the European Union (2025). Available online at: <https://op.europa.eu/en/publication-detail/-/publication/d4ddbc57-9db8-11f0-97c8-01aa75ed71a1> (Accessed February 25, 2026).
24. European Commission. *European Health and Digital Executive Agency. Study on the Hospital Exemption: Member State Reports. Annex 1 to the Final Report*. Brussels: Publications Office of the European Union (2025). Available online at: <https://op.europa.eu/en/publication-detail/-/publication/b89eb680-7730-11f0-9af8-01aa75ed71a1> (Accessed February 25, 2026).
25. Izeta A, Cuende N. Regulation of advanced therapies in Europe: are we on the right track? *Cell Stem Cell* (2023) 30(8):1013–6. doi:10.1016/j.stem.2023.07.004
26. Benvenuti S. Fondazione telethon current strategies and future perspectives on patient access to gene therapy for ultra-rare disease. In: *En: 32nd Congress of the European Society of Gene and Cell Therapy*. Sevilla (2025).
27. European Parliament and Council of the European Union. Regulation (EU). 2024/ 568 of 7 February 2024 on fees and charges payable to the European Medicines Agency, amending Regulations (EU) 2017/745 and (EU) 2022/123 and repealing Regulation (EU) No 658/2014 and Council Regulation (EC) No 297/95. *Official J Euro Union [Internet]* (2024). Available online at: <https://eur-lex.europa.eu/eli/reg/2024/568/oj/eng> (Accessed May 19, 2026).
28. Fox TA, Booth C. Improving access to gene therapy for rare diseases. *Dis Model Mech* (2024) 17(6):dmm050623. doi:10.1242/dmm.050623
29. Cuende N, Tullius SG, Izeta A, Plattner V, Börgel Msc M, Ciccocioppo R, et al. Promoting equitable and affordable patient access to safe and effective innovations in donation and transplantation of substances of human origin and derived therapies. *Transplantation* (2025) 109(1):36–47. doi:10.1097/TP.0000000000005169
30. World Health Assembly. *Resolution 63.22 on Human Organ and Tissue Transplantation*. Geneva: World Health Organization (2010). Available online at: https://apps.who.int/gb/ebwha/pdf_files/WHA63/A63_R22-en.pdf (Accessed February 25, 2026).
31. World Health Assembly. *Resolution 77.4. increasing availability, ethical access and oversight of transplantation of human cells, tissues and organs*. Geneva: WHO (2024). Available online at: https://apps.who.int/gb/ebwha/pdf_files/WHA77/A77_R4-en.pdf (Accessed February 25, 2026).
32. WHO. *WHO Guiding Principles on Human Cell, Tissue and Organ Transplantation*. Geneva: World Health Organization (2010). Available online at: <https://apps.who.int/iris/bitstream/handle/10665/341814/WHO-HTP-EHT-CPR-2010.01-eng.pdf?sequence=1&isAllowed=y> (Accessed February 25, 2026).
33. Convention. *Convention for the Protection of Human Rights and Dignity of the Human Being with Regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine*. Strasbourg: Council of Europe (1997). Available online at: <https://www.coe.int/en/web/conventions/full-list/-/conventions/treaty/164> (Accessed February 25, 2026).
34. Additional Protocol to the Convention. *Additional Protocol to the Convention on Human Rights and Biomedicine Concerning Transplantation of Organs and Tissues of Human Origin*. Strasbourg: Council of Europe (2002). Available online at: <https://www.coe.int/en/web/conventions/full-list/-/conventions/treaty/186> (Accessed February 25, 2026).
35. Guide-financial-gain. *Guide for the Implementation of the Principle of Prohibition of Financial Gain with Respect to the Human Body and its Parts from Living or Deceased Donors*. Strasbourg: Council of Europe Committee on Bioethics (2018). Available online at: <https://rm.coe.int/guide-financial-gain/16807bfc9a> (Accessed February 25, 2026).
36. European Union. Charter of fundamental rights of the european union (2026). Available online at: https://www.europarl.europa.eu/charter/pdf/text_en.pdf (Accessed February 25, 2026).
37. Council of Europe. *European Committee on Organ Transplantation. Position Statement on Risk of Commodification of Substances of Human Origin*. Strasbourg: PA/PH/TO (22) 11 DEF. Available online at: <https://www.edqm.eu/en/areas-of-work-transplantation> (Accessed February 25, 2026).
38. Pirnay JP, Baudoux E, Cornu O, Delforge A, Delloye C, Guns J, et al. Access to human tissues for research and product development: from EU regulation to alarming legal developments in Belgium. *EMBO Rep* (2015) 16(5):557–62. doi:10.15252/embr.201540070
39. Cuende N, Domínguez-Gil B. Reframing the future of SoHo-derived medicines: opportunity cost. *Transplantation* (2026) 110(2):e285–e287. doi:10.1097/TP.0000000000005549
40. GAPP. Good practice guideline to authorization on preparation processes in blood, tissue and cells establishments (2022). Available online at: https://gapp-pro.eu/wp-content/uploads/2024/05/GAPP_Guideline-and-Technical-Annexes.pdf (Accessed February 25, 2026).
41. European Parliament and Council of the European Union. Regulation (EU) 2024/ 1938 of 13 June 2024 on standards of quality and safety for substances of human origin intended for human application and repealing Directives 2002/98/EC and 2004/23/EC. *Official J Euro Union [Internet]*. (2024) L1938:1–80. Available online at: <https://eur-lex.europa.eu/eli/reg/2024/1938/oj/eng> (Accessed May 19, 2026).



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Sex-related differences in survival based on [¹⁸F]FDG PET/CT metabolic tumor volume after liver transplantation for colorectal liver metastases

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[¹⁸F]FDG PET/CT-derived metabolic tumor volume (MTV) is a strong prognostic biomarker in patients undergoing liver transplantation (LT) for non-resectable colorectal liver metastases (nCRLM). This study aimed to evaluate whether MTV-based survival outcomes after LT for nCRLM differ between males and females. Patients who underwent LT for nCRLM between 2006 and 2022 were included (n = 45). Baseline characteristics were compared using the Mann–Whitney U test and Fisher's exact test. Overall survival (OS), disease-free survival (DFS), and survival after relapse (SAR) were estimated using Kaplan–Meier analysis with the log-rank test. Cox regression analyses including sex, dichotomized MTV, and an interaction term between sex and MTV were performed. Female patients had significantly shorter OS compared with male patients (median 58 vs. 92 months, *p* = 0.047). Low MTV was associated with significantly longer OS, DFS and SAR in both sexes. Among patients with high MTV, females had significantly shorter OS than males (median 25 vs. 59 months, *p* < 0.001). Females had more non-pulmonary recurrences than males (*p* = 0.006) and shorter median SAR than males (17 vs. 46 months, *p* < 0.001) with high MTV. A significant interaction between sex and MTV was observed, indicating a particularly adverse prognostic impact of high MTV in female patients.

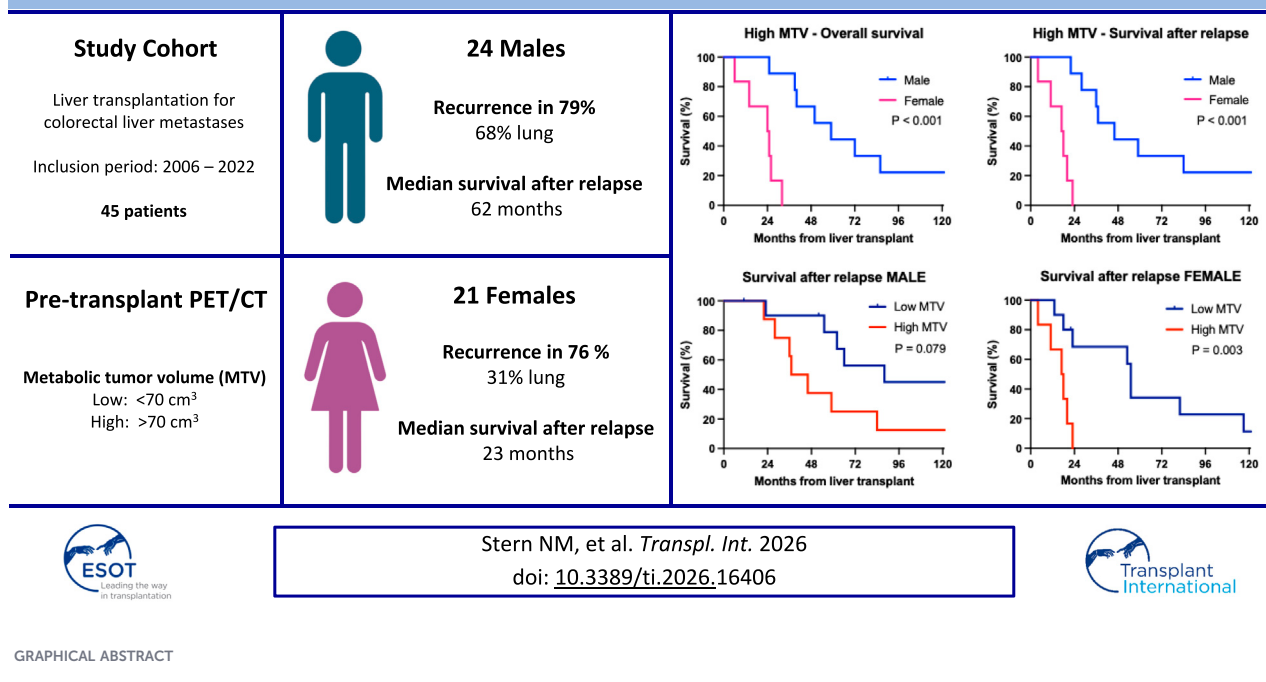
KEYWORDS

[¹⁸F]FDG PET/CT, colorectal liver metastases, liver transplantation, metabolic tumor volume, sex-related differences

Introduction

Liver transplantation (LT) for non-resectable colorectal liver metastases (nCRLM) has evolved from an experimental approach to a potentially curative treatment in a highly selected group of patients with liver-limited disease [1]. The pioneering SECA study from Oslo University Hospital demonstrated long-term overall survival (OS) in patients transplanted beyond traditional resection criteria, thereby establishing LT as a viable oncological surgical strategy [2, 3]. Comparable outcomes have subsequently been

Sex-related differences in survival based on [18F]FDG PET/CT metabolic tumor volume after liver transplantation for colorectal liver metastases



reported from other centers, including the Rochester protocol in the United States [4], demonstrating the reproducibility of this concept in independent cohorts.

Accurate patient selection remains critical, as disease recurrence occurs in most cases. Conventional morphologic and biochemical markers alone provide only an indirect approximation of tumor biology or behaviour, as they rely on surrogate measures such as carcinoembryonic antigen levels, tumor growth rate, response to chemotherapy, and the presence or absence of extrahepatic disease.

[18F]FDG PET/CT provides quantitative information on tumor glucose metabolism, which may reflect tumor aggressiveness in addition to providing the well-established volumetric parameter, metabolic tumor volume (MTV), for quantifying metabolic burden and predicting prognosis in colorectal cancer [5]. In a seminal study, Grut et al. [6] demonstrated that pre-transplant MTV was a strong predictor of survival after LT in nCRLM. These findings were subsequently confirmed in a larger national cohort [7] and validated externally by Wehrle et al. [8], thereby establishing MTV as a reproducible prognostic biomarker in transplant oncology [9].

Recently, the TRANSMET trial [10] demonstrated that patients randomized to LT plus chemotherapy against chemotherapy alone obtained a substantial transplant benefit thereby providing level 1 evidence for LT in nCRLM. In a recent multicentre study, Vitale et al. identified female sex as an independent predictor of inferior survival after LT for nCRLM, despite otherwise comparable clinicopathologic features [11]. MTV was not part of the risk stratification in this study.

The aim of this study was therefore to explore potential sex-related differences in the prognostic value of pre-transplant MTV measured by [18F]FDG PET/CT.

Materials and methods

Patient selection

This retrospective cohort included consecutive patients who underwent LT for nonresectable liver-only CRLM at Oslo University Hospital, Norway, between 2006 and 2022 (SECA 1 and 2 studies). All patients had a histologically confirmed colorectal adenocarcinoma that had been surgically resected and were evaluated by a multidisciplinary hepatobiliary tumor board prior to inclusion. Eligibility criteria included R0 resection of the primary tumor, nCRLM confined to the liver, absence of extrahepatic disease on CT, MRI or [18F]FDG PET/CT, an Eastern Cooperative Oncology Group performance status of 0–1, body mass index <30 and having received pre-transplant chemotherapy.

[18F]FDG PET/CT procedure and MTV analysis

All SECA patients underwent a standard routine whole-body PET/CT examination prior to LT. Any chemotherapy treatment was paused for 4–6 weeks before the PET/CT scan. Patients fasted for at least 6 h, and plasma glucose levels were measured before intravenous injection of [18F]FDG. Imaging was performed approximately 60 min post-injection with image acquisition from the skull base to mid-thigh, on hybrid PET/CT scanners (Siemens Biograph 64/16, or GE Healthcare Discovery MI), using iterative reconstruction with attenuation and scatter correction. For anatomical correlation and attenuation correction, a low-dose CT without intravenous contrast enhancement was performed. Metabolic tumor volumes were delineated semi-automatically using a fixed 40% SUV_{max} threshold

with Siemens syngo.via software. Total MTV for each patient was calculated as the sum of all hepatic MTVs.

Statistical analysis

Continuous variables were reported as medians and compared using the Mann–Whitney U-test while categorical variables were compared using the Fisher's exact test. Overall survival (OS), disease-free survival (DFS), and survival after relapse (SAR) were estimated using Kaplan–Meier method and compared using the log-rank test. DFS was defined as the time from LT to detection of metastatic lesions, local relapse identified by CT/MRI or PET, detection of new CRC primary, or other malignant disease. OS was defined as the time from LT to death or end of follow-up. SAR was defined as the time from recurrence to death. MTV was analysed as a dichotomized variable using a predefined cut-off of 70 cm³ (low ≤70 cm³, high >70 cm³). Cox proportional hazards regression analyses were performed for OS including sex, dichotomized MTV, and an interaction term between sex and MTV to assess whether the prognostic impact of high MTV differed according to sex. The interaction term specifically evaluated whether female patients with high MTV had a different risk profile than expected from the independent effects of sex and MTV alone. Optimal MTV cut-off values for predicting 5-year OS in male and female patients were determined using receiver operating characteristic (ROC) analysis. All statistical analyses were performed using IBM SPSS Statistics version 29 (IBM Corp., Armonk, NY, USA). A P-value <0.05 was considered statistically significant. Kaplan–Meier survival plots were generated using GraphPad Prism version 10.

Ethics

The study complied with the Declaration of Helsinki, was approved by the Regional Committee for Medical and Health Research Ethics and was registered at [ClinicalTrials.gov](https://clinicaltrials.gov) (NCT01311453 for SECA-1 and NCT01479608 for SECA-2). Written informed consent had been obtained from all participants prior to inclusion in the original studies.

Results

Baseline characteristics

The study included 45 patients: 24 (53%) male and 21 (47%) female. Baseline characteristics stratified by sex are summarised in [Table 1](#). Male and female patients had similar age, primary tumor location, MTV, number and size of liver metastases, T and N stage, carcinoembryonic antigen levels, lines of chemotherapy, time of detection of liver metastases and number of patients with progression at LT.

Survival analysis

Overall survival

Median OS was 92 months in male patients and 58 months in female patients ($p = 0.047$, log-rank test). Males with low MTV had a

median OS of 166 months compared to 59 months in males with high MTV ($p = 0.004$). Females with low MTV had an OS of 87 months compared to 24 months in females with high MTV ($p < 0.001$) ([Figure 1](#)). Overall survival did not differ significantly between males and females with low MTV (median 166 vs. 87 months, $p = 0.053$). Among patients with high MTV, males had significantly longer OS than females (median 59 months vs. 24 months, $p < 0.001$) ([Figure 2](#)).

In Cox regression analyses for OS, high MTV was associated with increased risk of death in both univariable (HR 4.64, 95% CI 2.15–10.01, $p < 0.001$) and multivariable analyses (HR 4.68, 95% CI 1.55–14.15, $p = 0.006$). Sex showed a non-significant association with OS in univariable analysis (HR 2.13, 95% CI 0.99–4.58, $p = 0.053$) and multivariable analysis (HR 2.43, 95% CI 0.85–6.95, $p = 0.098$). A significant interaction between sex and MTV was observed (HR 8.20, 95% CI 1.12–60.11, $p = 0.038$) ([Table 2](#)). This interaction term reflects that female patients with high MTV had particularly poor survival beyond the independent effects of sex and MTV alone.

Receiver operating characteristic (ROC) analysis identified optimal MTV cut-off values for predicting 5-year OS of 76 cm³ for females and 66 cm³ for males. The area under the curve was 0.74 for both sexes.

Disease-free survival

Among male patients with low MTV, median DFS was not reached. In contrast, males with high MTV had median DFS of 12 months ($p < 0.001$). Female patients with low MTV had an estimated DFS of 22 months compared with 3 months in those with high MTV ($p = 0.006$) ([Figure 1](#)). All patients with high MTV experienced recurrence, however, no significant difference in DFS between sexes (median 11 vs. 3 months, $p = 0.683$) ([Figure 2](#)). Female patients had significantly more non-pulmonary relapses than males ($p = 0.006$).

Survival after relapse

Male patients with low MTV had a median SAR of 88 months compared with 46 months in those with high MTV ($p = 0.079$). Female patients with low MTV had a median SAR of 55 months compared with 17 months in those with high MTV ($p = 0.003$) ([Figure 1](#)). Among patients with high MTV, males had significantly longer SAR than females (median 46 vs. 17 months, $p < 0.001$) ([Figure 2](#)).

Discussion

This study confirms that [¹⁸F] FDG PET/CT-derived metabolic tumor volume is a robust predictor of survival following LT for nCRLM and demonstrates a significant interaction between sex and MTV, indicating that the adverse prognostic impact of high MTV (>70 cm³) differs between males and females. Female recipients appear to have a lower tolerance for high metabolic tumor burden, with more frequent non-pulmonary recurrences, resulting in poorer outcomes despite otherwise similar baseline characteristics.

The prognostic importance of MTV has been consistently demonstrated in prior work from our group [8, 9] and subsequently validated internationally [10]. The present findings

TABLE 1 Comparison of baseline characteristics.

Characteristics	Men	Women	P-value
Number of patients	24	21	
Age at LT, years*	56 (34–71)	58 (44–66)	0.802
Site of primary tumor			0.658
Left sigmoid and rectum	21	18	
Right	2	3	
Metabolic tumor volume, cm ³ *	27.54 (0–397.10)	14.44 (0–874.12)	0.475
Number of metastases at LT*	7.5 (1–40)	7 (2–53)	0.464
Size of the largest metastasis at LT, mm*	35.5 (7–130)	28 (3–120)	0.241
CEA (ng/mL)*	7.25 (1–2002)	2 (1–404)	0.070
Time of liver metastases			1.000
Synchronous	21 (88)	19 (91)	
Metachronous	3 (12)	2 (9)	
Progression at LT			1.000
Yes	9 (37)	7 (33)	
No	15 (63)	14 (67)	
Lines of chemotherapy			0.197
1	11 (46)	11 (52)	
2	9 (38)	10 (48)	
3	4 (16)	0	
First site relapse, n (%)			0.006
Lungs	13 (68)	5 (31)	
Non-lung	6 (32)	11 (69)	
T stage, n (%)			0.177
(y)pT0	0	3 (14)	
(y)pT1	0	1 (5)	
(y)pT2	3 (13)	2 (8)	
(y)pT3	20 (83)	15 (71)	
(y)pT4	1 (4)	0	
N Stage, n (%)			0.675
(y)pN0	12 (50)	9 (43)	
(y)pN1	7 (29)	9 (43)	
(y)pN2	5 (21)	3 (14)	
KRAS status			0.890
Wild type	17 (71)	14 (67)	
Mutated	6 (25)	5 (24)	
Unknown	1 (4)	2 (9)	

*Median (range).

*Increased size of liver metastases on CT, and/or increased carcinoembryonic antigen (CEA).

LT, liver transplantation.

KRAS, kirsten rat sarcoma viral oncogene homolog.

expand this evidence by formally demonstrating a sex-dependent interaction between MTV and survival in multivariable analyses. In a recent multicentre cohort, not including PET-derived MTV data, Vitale et al. reported that female sex was independently associated with poorer outcomes after LT [11].

Sexual dimorphism in cancer is based on the observation that differences in the incidence and mortality of non sex-related cancers cannot be explained solely by sociobehavioral (gender related) factors, but may instead be related to biological differences, including sex chromosomes [12]. Variations in health-seeking

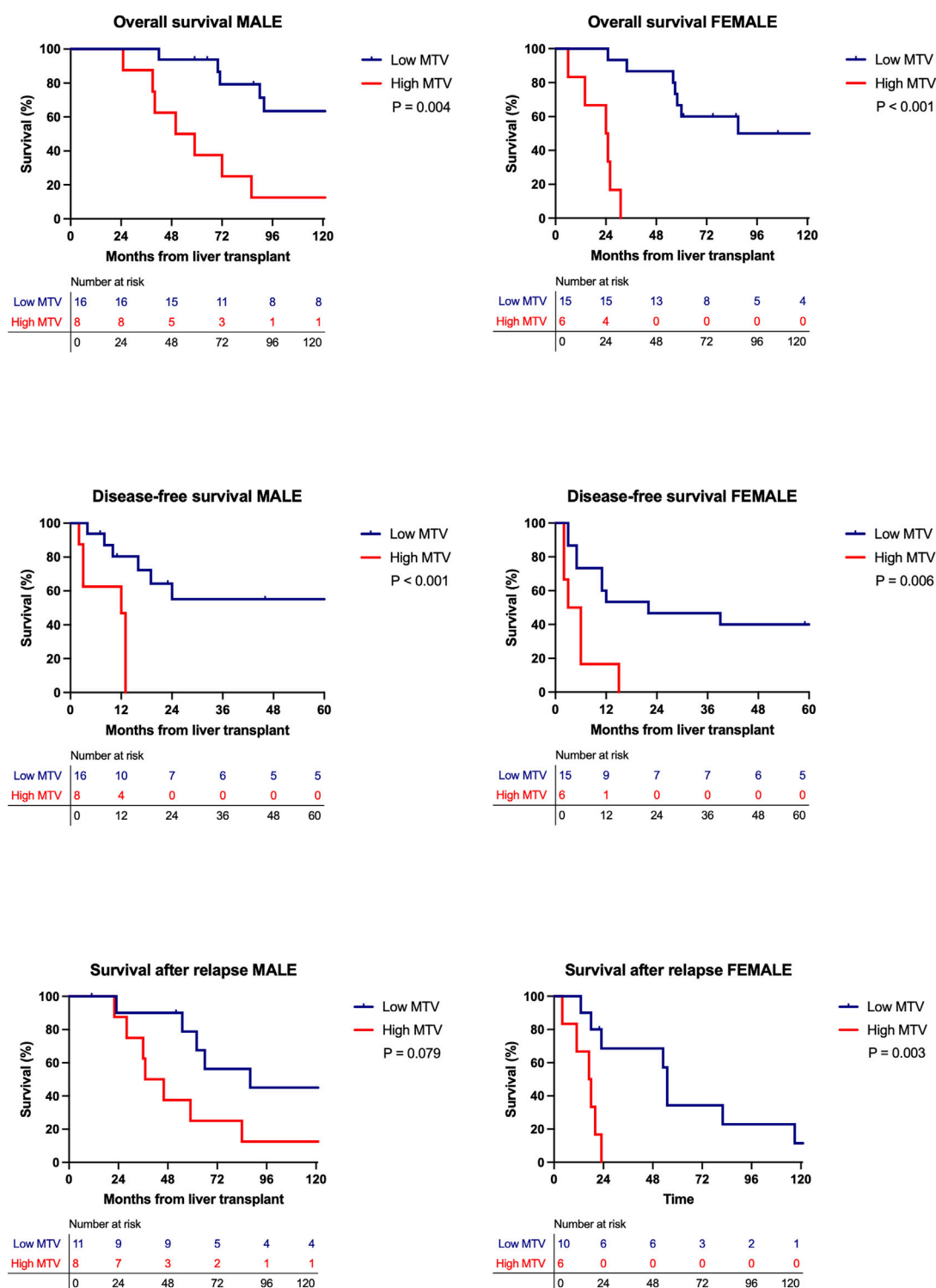


FIGURE 1
Kaplan-Meier survival curves for patients with high versus low metabolic tumor volume for males and females. Patients with low metabolic tumor volume had significantly longer overall survival and disease-free survival in both sexes and female patients had significantly shorter survival after relapse.

behavior, lifestyle factors, and environmental exposures may contribute to these disparities, but are insufficient to explain the full extent of sex-related differences observed in cancer epidemiology [13]. For most cancers unrelated to reproductive

function, both incidence and prevalence tend to be modestly higher in males than in females, with the exceptions of thyroid and gallbladder cancer. Similarly, cancer-related mortality is generally higher in males than in females [13, 14].

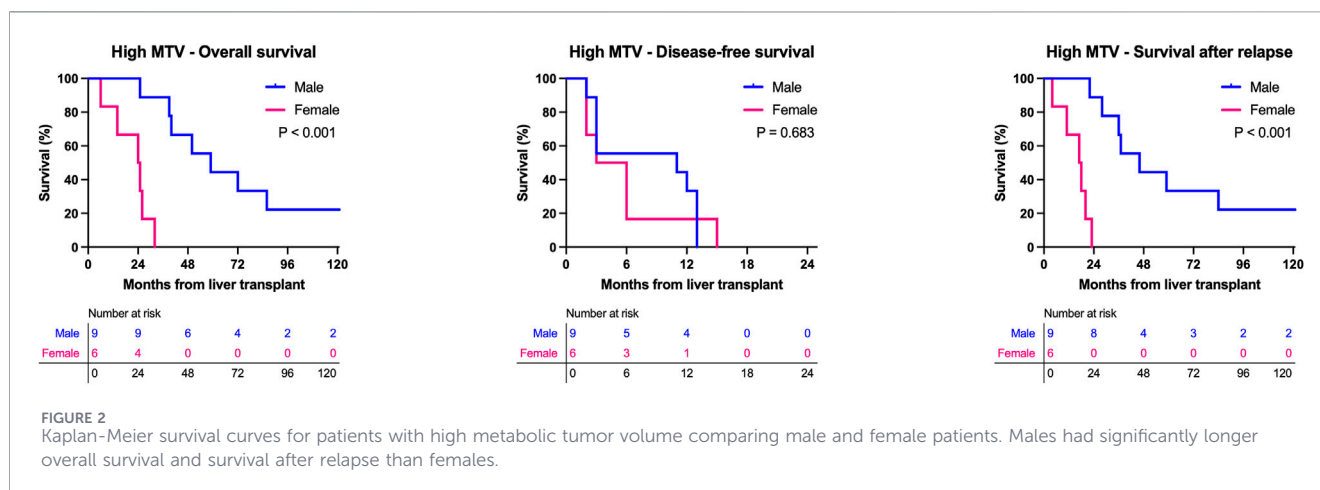


TABLE 2 Cox regression analyses for overall survival.

Variable	Univariable HR (95% CI)	P-value	Multivariable HR (95% CI)	P-value
Sex (male/female)	2.13 (0.99–4.58)	0.053	2.43 (0.85–6.95)	0.098
MTV (high/low)	4.64 (2.15–10.01)	<0.001	4.68 (1.55–14.15)	0.006
Interaction (MTV × sex)	-	-	8.20 (1.12–60.11)	0.038

Sex hormones may play a role in the initiation and progression of CRC, with age-dependent effects. While premenopausal females generally exhibit superior survival compared with age-matched males, post-menopausal females often experience poorer outcomes, potentially due to diagnosis at more advanced stages or intrinsically more aggressive tumor characteristics, such as right-sided tumor location and BRAF mutations [11, 15]. Sex-specific patterns of adipose distribution and lipid metabolism may further influence cancer risk. Visceral fat-associated inflammation is a recognized driver of carcinogenesis and may contribute to the increased incidence of several cancers after menopause, when females tend to shift towards greater visceral adiposity [13].

The female immune system appears to have a higher activation threshold for T-cell mediated immune responses, partly driven by estrogen, potentially reflecting immunological adaptations related to pregnancy [16]. Sex-related differences regarding immune system responses have been shown to influence disease onset, symptom presentation, patterns of progression and treatment outcomes across a range of conditions, including autoimmune disorders, infectious diseases, cancers and vaccine efficacy [17]. Also, the liver is an estrogen-sensitive organ [18], and experimental data suggest that estrogen may modulate the microenvironment of liver metastases in a sex-specific manner [19]. However, these proposed mechanisms remain speculative and are not directly supported by data in the present study.

Interestingly, population studies have shown that females are about 25% less likely to undergo surgery for liver metastases, and in this cohort, the all-cause mortality is significantly higher in females compared to males. Since the LT patients all had nCRLM, this may indicate that the gender related effects on

outcome are related to biological differences in tumor biological behavior [20].

Given a limited availability of liver grafts, optimizing patient selection is of clear clinical relevance. In the present study, males more frequently presented with pulmonary metastases (two-thirds of recurrences), which are known to have relatively indolent course and are often amenable to pulmonary resection with prolonged SAR [21, 22]. In contrast, approximately two-thirds of females experienced non-pulmonary recurrence, a pattern associated with poorer survival outcomes [22].

The current analysis shows that males with high MTV had an acceptable median OS of 59 months. Thus, males who otherwise demonstrate favorable characteristics during pre-transplant evaluation may still be considered for inclusion despite high MTV (>70 cm³), although this should be interpreted with caution given the limited sample size. The ROC analysis identified optimal MTV cut-off values for predicting 5-year OS of 76 cm³ in females and 66 cm³ in males. Accordingly, the currently used, non-sex-specific cut-off value of 70 cm³ appears appropriate, with no clear indication at present for separate MTV thresholds for males and females.

Several limitations merit consideration. This was a retrospective study with a limited sample size, reflecting the rarity of LT for nCRLM; consequently, the statistical power for sex-stratified analyses should be regarded as modest. Exact information on pre- and postmenopausal status among female patients was unavailable. However, given the median age of the female patients was 58 years and that all patients received chemotherapy, it is likely that a majority were postmenopausal at the time of LT. PET/CT acquisition protocols and scanner technology evolved during the inclusion period, which may have introduced variability in SUV quantification, although this is

unlikely to have substantially affected MTV measurements. The 70 cm³ MTV threshold is empirically derived and should not be considered as an absolute. Minor variations in preoperative chemotherapy and immunosuppressive regimens may also have influenced outcomes. Finally, as with all observational studies, unmeasured confounding factors cannot be excluded. Nonetheless, the use of uniform national selection criteria and long-term follow-up adds robustness to the observed associations.

Introducing different inclusion and exclusion criteria based on sex would be premature and is not the intention of this study. Some recent papers, however, indicate that relevant gender specific differences are clinically relevant [20] and the findings from the current study underscore that biological sex should be considered as one of several factors when planning and individualizing treatment strategies aimed at achieving the best possible outcomes.

In summary, pre-transplant [¹⁸F]FDG PET/CT-derived MTV remains a robust prognostic biomarker after LT for nCRLM. Female patients exhibit poorer OS and SAR at high MTV levels. These findings suggest that sex may modify the prognostic impact of MTV but should be considered hypothesis-generating and require validation in larger cohorts. The underlying mechanisms remain to be elucidated.

Conclusion

Female patients had shorter OS than males, including when the analysis was restricted to patients with high MTV. This may be explained by the post-transplant recurrence patterns, with females developing more unfavourable non-pulmonary recurrences. These findings are hypothesis-generating and should be interpreted with caution and require validation in larger cohorts before clinical implications can be drawn.

Data availability statement

The datasets presented in this article are not readily available because of regulatory restrictions on data sharing but are available from the corresponding author upon reasonable request. Requests to access the datasets should be directed to Harald Grut, harald.grut@gmail.com.

References

1. Dueland S, Smedman TM, Syversveen T, Grut H, Hagness M, Line PD. Long-term survival, prognostic factors, and selection of patients with colorectal cancer for liver transplant: a nonrandomized controlled trial. *JAMA Surg* (2023) 158:e232932. doi:10.1001/jamasurg.2023.2932
2. Hagness M, Foss A, Line PD, Scholz T, Jørgensen PF, Fosby B, et al. Liver transplantation for nonresectable liver metastases from colorectal cancer. *Ann Surg* (2013) 257(5):800–6. doi:10.1097/SLA.0b013e3182823957
3. Dueland S, Syversveen T, Solheim JM, Solberg S, Grut H, Bjornbeth BA, et al. Survival following liver transplantation for patients with nonresectable liver-only colorectal metastases. *Ann Surg* (2019) 271:212–8. doi:10.1097/sla.0000000000003404
4. Byrne MM, Chávez-Villa M, Ruffolo LI, Loria A, Endo Y, Niewiemiński A, et al. The Rochester protocol for living donor liver transplantation of unresectable colorectal liver metastasis: a 5-year report on selection, approval, and outcomes. *Am J Transpl* (2025) 25(4):780–92. doi:10.1016/j.ajt.2024.09.027
5. Kido H, Kato S, Funahashi K, Shibuya K, Sasaki Y, Urita Y, et al. The metabolic parameters based on volume in PET/CT are associated with clinicopathological N stage

Ethics statement

The studies involving humans were approved by the Regional Committee for Medical and Health Research Ethics and was registered at ClinicalTrials.gov with (NCT01311453 for SECA-1 and NCT01479608 for SECA-2). 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

HG and NM designed the study and drafted the manuscript. All authors contributed to data collection and analysis. All authors contributed to the article and approved the submitted version.

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of colorectal cancer and can predict prognosis. *EJNMMI Res* (2021) 11(1):87. doi:10.1186/s13550-021-00831-5

6. Grut H, Dueland S, Line PD, Revheim ME. The prognostic value of 18F-FDG PET/CT prior to liver transplantation for nonresectable colorectal liver metastases. *Eur J Nucl Med Mol Imaging* (2017) 45:1–8. doi:10.1007/s00259-017-3843-9

7. Grut H, Line PD, Syversveen T, Dueland S. Metabolic tumor volume predicts long-term survival after transplantation for unresectable colorectal liver metastases: 15 years of experience from the SECA study. *Ann Nucl Med* (2022) 36:1073–81. doi:10.1007/s12149-022-01796-8

8. Wehrle CJ, Chávez-Villa M, Byrne M, Kusakabe J, Gross A, Mahajan P, et al. Pretransplant metabolic tumor volume predicts recurrence following liver transplantation for colorectal metastasis: a multicenter study. *Liver Transpl* (2025) 31(3):298–310. doi:10.1097/lt.0000000000000535

9. Bonney GK, Chew CA, Lodge P, Hubbard J, Halazun KJ, Trunecka P, et al. Liver transplantation for non-resectable colorectal liver metastases: the international hepatopancreato-biliary association consensus guidelines. *Lancet Gastroenterol Hepatol* (2021) 6(11):933–46. doi:10.1016/s2468-1253(21)00219-3

10. Adam R, Piedvache C, Chiche L, Adam JP, Salamé E, Bucur P, et al. Liver transplantation plus chemotherapy *versus* chemotherapy alone in patients with permanently unresectable colorectal liver metastases (TransMet): results from a multicentre, open-label, prospective, randomised controlled trial. *Lancet* (2024) 404(10458):1107–18. doi:10.1016/s0140-6736(24)01595-2
11. Vitale A, Lanari J, Cillo U, Billato I, Rovetta A, Mansournia MA, et al. Sex-based differences in survival after liver transplantation for colorectal cancer liver metastases: a multivariable analysis. *JHEP Rep* (2025) 7(10):101505. doi:10.1016/j.jhepr.2025.101505
12. Clocchiatti A, Cora E, Zhang Y, Dotto GP. Sexual dimorphism in cancer. *Nat Rev Cancer* (2016) 16(5):330–9. doi:10.1038/nrc.2016.30
13. Bernardez B, Higuera O, Martinez-Callejo V, Cardeña-Gutiérrez A, Marcos Rodríguez JA, Santaballa Bertrán A, et al. Sex and gender differences in cancer pathogenesis and pharmacology. *Clin Translational Oncol* (2025) 27(10):3837–48. doi:10.1007/s12094-025-03894-1
14. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2024) 74(3):229–63. doi:10.3322/caac.21834
15. Schmuck R, Gerken M, Teegen EM, Krebs I, Klinkhammer-Schalke M, Aigner F, et al. Gender comparison of clinical, histopathological, therapeutic and outcome factors in 185,967 colon cancer patients. *Langenbecks Arch Surg* (2020) 405(1):71–80. doi:10.1007/s00423-019-01850-6
16. Rackaityte E, Halkias J. Mechanisms of fetal T cell tolerance and immune regulation. *Front Immunol* (2020) 11:588. doi:10.3389/fimmu.2020.00588
17. Klein SL, Morgan R. The impact of sex and gender on immunotherapy outcomes. *Biol Sex Differ* (2020) 11(1):24. doi:10.1186/s13293-020-00301-y
18. Kasarinaite A, Sinton M, Saunders PTK, Hay DC. The influence of sex hormones in liver function and disease. *Cells* (2023) 12(12):1604. doi:10.3390/cells12121604
19. Milette S, Hashimoto M, Perrino S, Qi S, Chen M, Ham B, et al. Sexual dimorphism and the role of estrogen in the immune microenvironment of liver metastases. *Nat Commun* (2019) 10(1):5745. doi:10.1038/s41467-019-13571-x
20. Ljunggren M, Weibull CE, Palmer G, Osterlund E, Glimelius B, Martling A, et al. Sex differences in metastatic surgery following diagnosis of synchronous metastatic colorectal cancer. *Int J Cancer* (2023) 152(3):363–73. doi:10.1002/ijc.34255
21. Grut H, Solberg S, Seierstad T, Revheim ME, Egge TS, Larsen SG, et al. Growth rates of pulmonary metastases after liver transplantation for unresectable colorectal liver metastases. *Br J Surg* (2017) 105:295–301. doi:10.1002/bjs.10651
22. Hagness M, Foss A, Egge TS, Dueland S. Patterns of recurrence after liver transplantation for nonresectable liver metastases from colorectal cancer. *Ann Surg Oncol* (2014) 21(4):1323–9. doi:10.1245/s10434-013-3449-9



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Establishing a core outcome measure for cancer in trials in kidney transplantation: a standardized outcomes in nephrology-kidney transplantation consensus workshop report

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Cancer is a critically important outcome for kidney transplant recipients that inconsistently and infrequently reported in trials. We convened a consensus workshop to establish a core outcome measure for cancer for trials in kidney transplant recipients. Workshop attendees included 21 transplant recipients, 2 caregivers and 46 health professionals from 12 countries. Transcripts were analyzed thematically. Three themes were identified. "Fear of cancer occurrence due to immunosuppression" reflected the psychological burden

and uncertainties when balancing suppression with long-term cancer risk. “*Capturing the details of type, stage and recurrence*”, encompassed recognizing the differential consequences of cancer types, delineating the stage of cancer to convey severity, and distinguishing recurrent from *de novo* cancer. “*Recognizing the challenges of capturing cancer events*” included under-reporting and incomplete documentation of longer-term outcomes, variability in cancer screening practices, and absence of coordinated trial networks to support harmonization and aggregation of cancer outcomes across studies. Participants agreed that occurrence, with stratification by cancer type, stage, and recurrence, where possible, would be a practical and meaningful core outcome measure for cancer. Consistent reporting of cancer using a standardized core outcome measure may help to improve the consistency and relevance of trial findings and assist in shared decision-making in kidney transplantation.

KEYWORDS

cancer, chronic kidney disease, core outcome measure, kidney transplantation, patient-centered outcomes

Introduction

Cancer is a leading cause of mortality and morbidity in kidney transplant recipients, who have a 2-3-fold increased risk of developing cancer and cancer-related death compared to the age- and sex-matched general population [1, 2]. Such elevated risk is primarily attributed to the effects of immunosuppression, which impair immune surveillance, disrupts normal tumor elimination mechanisms and thus increases susceptibility to virus and non-virus-related cancers [3–5]. With advancements in kidney transplantation leading to improved long-term graft survival, cancer is an increasing problem for patients and the health system, representing a significant barrier to long-term survival in kidney transplant recipients.

The Standardized Outcomes in Nephrology-Transplantation (SONG-Tx) international consensus process involving patients, caregivers and health professionals has identified cancer as a critically important core outcome to be reported in all trials in kidney transplant recipients, along with graft health, cardiovascular disease, infection, life participation and mortality [6, 7]. Despite this, cancer is infrequently reported in trials, with highly variable definitions, terminology, metrics and time points used. Of 819 trials in kidney transplantation published between 2000 and 2021, only 84 (10%) reported a cancer outcome, with 72 different cancer outcome measures used [8]. These limitations in how cancer is reported in trials restrict the ability to compare or aggregate data between trials in kidney transplantation, and thus impair decision-making about interventions to address cancer in this population.

A core outcome measure can help to ensure consistent reporting of cancer in trials to improve the comparability, relevance and use of evidence [9, 10]. To inform this work, an international SONG-Tx survey was conducted with 403 patients, caregivers and health professionals from 55 countries to prioritize cancer outcomes identified through a systematic review and consultation with the expert working group [11]. Stakeholders consistently identified the occurrence of cancer, death from cancer and impact of cancer on

graft function as the most critical cancer domains for trials in kidney transplant recipients. As mortality and graft health are also core outcomes identified by SONG-Tx, cancer occurrence is the sole remaining, highest priority outcome not captured in the core outcome set [7]. To advance this work, an international workshop was convened to establish consensus on defining and implementing cancer occurrence as a core outcome measure for cancer in trials in kidney transplantation. We aimed to summarize the perspectives of patients, caregivers, and health professionals shared during the workshop and present the key recommendations for the development and implementation of a core cancer outcome and relevant metric in trials of kidney transplant recipients.

SONG-Tx Cancer workshop

Context and scope

The one-hour SONG-Tx Cancer Consensus Workshop was held using Zoom videoconference on May 21, 2024. The workshop was informed by a systematic review of cancer outcomes reported in trials in kidney transplant recipients [8], and an international survey in which patient, caregivers and health professionals prioritized cancer outcomes for trials in kidney transplantation [11]. The aim was to bring together insights from patients, caregivers and health professionals and discuss the identification and implementation of a core outcome measure for cancer in clinical trials. The study was approved by the human research ethics committee of University of Sydney (2015-228).

Workshop attendees and contributors

Sixty-nine stakeholders attended the workshop, including 21 transplant recipients, 2 caregivers and 46 health professionals (adult and pediatric nephrologists, other physicians, surgeons, nurses, and researchers). The attendees were from 12 countries, with 31 from Australia, 24 from the United States of America, 3 from Canada, 2 from each of Hong Kong and the United Kingdom, and one participant from each of France, Indonesia, Italy, Malaysia,

Abbreviations: SONG-Tx, Standardized Outcomes in Nephrology-Transplantation.

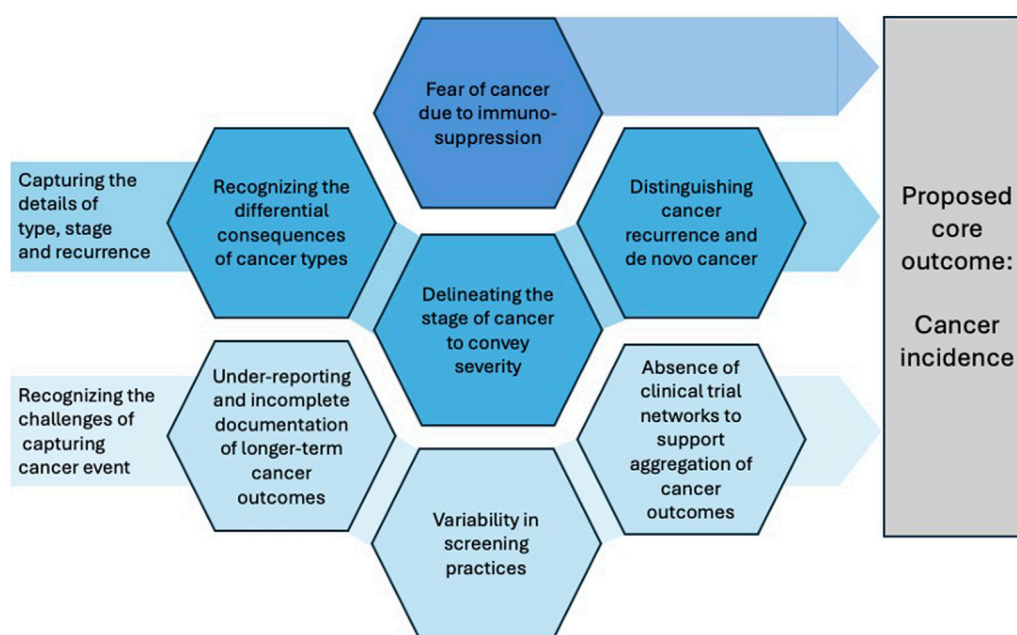


FIGURE 1
Schema of the themes derived from the consensus workshop.

Mexico, Switzerland and Turkey. We sent targeted invitations to health professionals with clinical expertise in cancer in kidney transplantation (identified by the SONG-Tx expert working group and through previous SONG activities). Patients and caregivers were invited by members of the SONG-Tx Cancer expert working group. 133 individuals were invited to participate. The full list of SONG-Tx Cancer workshop attendees and contributors is provided in the [Supplementary Table S1](#).

Workshop program and materials

Background materials and the workshop program were sent to all facilitators and co-facilitators 1 week before the workshop. The question guide for the workshop facilitators and definitions for cancer outcomes were included in the [Supplementary Tables S2, S3](#). The workshop commenced with a brief presentation introducing the SONG Initiative and core outcomes, the results from the systematic review and survey on cancer in kidney transplantation, and the proposed outcome measure with justification [8, 11]. The presentation was followed by facilitated discussions in pre-allocated breakout groups, selected to maximize diverse discussions and broad knowledge exchange among different stakeholders. There were seven breakout groups, each comprising 9–11 members, with at least one SONG-Tx Cancer expert working group member and three patients or caregivers in each group. Each group was led by a facilitator (G.W., D.S., N.S.R., A.V., E.D., J.C. and A.v.Z.) who prompted the discussion using a question guide developed by the SONG-Tx Cancer expert working group and investigators ([Supplementary Table S2](#)). The questions covered: should the occurrence of cancer be the core outcome measure, are there any other important aspects that should be included in a core outcome measure, and are there any suggestions to

encourage and support the implementation of this measure in trials? The breakout groups reconvened in the final plenary session, where a volunteer from each group provided a brief summary of their discussion. The Chair (G.W.) moderated this session and provided a summary of the key points from all groups and closing remarks.

All plenary and breakout discussions were recorded and transcribed verbatim. The transcripts were then imported into HyperRESEARCH (ResearchWare Inc. United States; Version 4.5.7) software for coding and thematic analysis of the data. First author (E.D.) read and coded each transcript line by line to identify themes and subthemes related to determining the core outcome measure for cancer. The themes were reviewed by A.v.Z, G.W. and A.J. A draft workshop report was distributed to all participants and non-attending contributors and feedback was invited within a 2-week timeframe. Additional comments were integrated into the final report to ensure that the findings reflected participants' perspectives and opinions. The recommendations for implementation table were generated from the content of the workshop as well as from discussion and review by the expert working group.

Synthesis of workshop discussion

We identified three themes that reflected the range of perspectives on establishing a core outcome measure for cancer: 1) fear of cancer occurrence due to immunosuppression, 2) capturing the details of type, stage and recurrence, and 3) recognizing the challenges of capturing cancer events. The themes are schematically represented in [Figure 1](#), and described in detail below. Selected quotations for each theme are provided in [Table 1](#). A summary of recommendations that arose from the workshop are presented in [Table 2](#).

TABLE 1 Themes and illustrative quotations.

Theme	Quotations
Fear of cancer occurrence due to immunosuppression	“It’s always been something from a patient’s perspective that I’ve watched, that perhaps my kidney graft might last, but other things like cancer might get me.” P1 “I think it’s very important to the patients. Particularly, we have several here who have very long-term function, which is wonderful. And that [cancer] becomes the enemy more so than the organ function.” H1 “Now that I’m in a longer-term stage of just maintaining the transplant that I have, there’s a bit more focus on managing increased cancer risk. I Have the colorectal screening kit sitting on my desk right now. It’s really relevant for the patient perspective, because cancer is such a big scary monster in the lay population” P2 “Particularly if you’ve gone out further and further with your transplant... cancer is now the thing that you fear most rather than the kidney because the kidney is just going to carry on... if I was to get colorectal cancer now, I’d have to come off my immunosuppression, probably may lose the kidney... therefore it’s like a death sentence... it is a big worry, the cancer side of transplantation. And I think it’ll only get bigger and bigger as people’s transplants go out further and further.” P5 “The emphasis is on not losing the kidney, which is important, but I lost my father, my uncle, my brother, it’s that cancer is not addressed as aggressively as keeping the kidney and the cancer has caused the premature death of almost all of them.” P7 “Having been immunosuppressed for 9 years now, I think that the cancer occurrence should definitely be the core outcome measure, because you cannot address an illness until you know it exists, plain and simple.” P4
Capturing the details of type, stage and recurrence	Recognizing the differential consequences of cancer types “I actually really like the fact that we’re actually looking at all types of cancer in a kidney transplant recipient... it’s actually about trying to capture the information about all of the different types of cancers that our patient community gets affected by.” P1 “The type of cancer is extremely important... because different types of cancers have different outcomes and the risks that patients may be willing to accept will be determined on the type of cancer and the certain therapy that might be associated with.” H7 “Knowing whether this disease or if this treatment is associated with skin cancer versus brain cancer that’s a very different conversation with patients.” H7 “Definitely, the cancers are different. They have different pathologies, prognosis, different treatment patterns. I think for sure they need to be separate.” H3 “The occurrence of cancer should be the measure and [I] support more depth of information because I think from a patient carer perspective, it’s the so what? Like does it mean? There’s a tick against cancer. Well, what does that actually mean for the patient, for the carer? More information leads to improved advocacy for things like screening. And also the power is then perhaps more with the patient and the carer to initiate conversations about cancer. The nature of the cancer, the site of the cancer, whether it’s new, recurrent, I understand you’re looking for a measure, but depth of measure I think is really important as much as possible” P7 Distinguishing cancer recurrence and <i>de novo</i> cancer “We’ll often be transplanting folks who’ve got a past history of cancer. That’s where the recurrence question may come in. Have they been cancer-free long enough?” H6 “I think it’s important to make a distinction between whether it is a <i>de novo</i> malignancy or whether it’s a recurrence of a malignancy that existed prior to transplant because they’re definitively separate entities.” H5 “It’s important because the <i>de novo</i> lesions are primarily driven by virally mediated cancers. And in the pre-transplant cancer population, there is a much lesser proportion of cancers that are driven by viral infection. It’s the immunosuppression that makes the difference between the two... In terms of good high quality epidemiologic data, it is far less robust dealing with preexisting malignancies than it is with a <i>de novo</i> malignancy. So, they’re markedly different types of cancers, groups of cancers that invite different questions and present significantly different epidemiologic challenges.” H5
	Delineating the stage of cancer to convey severity “What does this mean for the patient’s life? What extra treatment are they going to require as a result of this cancer? Ultimately, practical things. Practical from a layperson’s perspective anyway.” P2 “We may not be able to capture all of the specifics related to a particular tumour. Still, if we are capturing the cancer category or site, that may be reasonable also to capture the cancer stage or whether cancer is metastatic or not at the time of diagnosis. I think is important considering the severity and potential treatment patients may undergo following cancer occurrence.” H5 “I think what you want to know is whether it’s metastatic. It does create difficulties for cancers like the PTLD and other haematological malignancies. But if you could just have localised or metastatic as a staging thing” H3

(Continued)

TABLE 1 Continued

Theme	Quotations
Recognizing the challenges of capturing cancer events	Under-reporting and incomplete documentation of longer-term cancer outcomes
	“Typically, trials are relatively short because of funding limitations and cancer may not be identified during that window of time. While I definitely support the fact that cancer, as a whole, is now being considered as an outcome measure, I would strongly encourage some element of extended follow-up for these patients, even if it’s beyond the trial period wherever possible.” H4 “I do not know that companies and other things are going to fund it for the 20 years that it would take. But if there’s a way to somehow mandate that it be linked to registries that already collect those data, that might be a way to get the answer.” H1 “I favour having this as a core outcome measure primarily because cancer is a late outcome and often not captured in other clinical trials. The longest it will go up to two to three years, so it’s really a vexing issue that does need to be tackled and is very important to the patients.” H3 “I like the idea of linking cancer databases to follow up these patients in the longer term, but for example, in low- and middle-income countries, there are no good cancer registries. . . And the participation of patients in clinical trials in lower and middle income countries may not be possible, [as well as] linking to a solid database to follow up the cancer.” H1 “That there’s a trial period, we have that period of time, but then for the next period of time they have the ability to pull the patient’s records. And how we go about doing that might be different in every country, as well.” P1 “Sometimes cancer is underreported. It is really not well recognised, or it is hard to keep track of. Large transplant centres may not be able to keep track properly of some of those diagnoses, whether or not they’re diagnosed years after transplant. . . whether or not registries would be useful just to keep track and make sure we’re not underscoring the diagnosis of cancer. It’s just hard to keep track sometimes, especially when you deal with a large population. And then we now have patients that are keeping the organs for longer time” H3
	Variability in cancer screening practices
	“It depends on the health system, whether you screen for the cancer regularly, for example, the common cancers, you do a mammogram, or you do a pap smear, or you do a colonoscopy. If you do not do it, then your incidence of cancer will be quite low. And if it’s a skin cancer, whether you send the patient to a dermatologist every year for screening” H7 “There’s an issue of the screening as well as the pace of the cancer that may not develop during that short period of time. So, I see that several challenges in reporting.” H7 “With skin and melanoma, for the most part, with good surveillance, you can manage it pretty well, speaking to someone who’s had a lot of cuts on his body. But what about those types of cancers that are not going to occur as common, and would not be picked up in testing?” P2
	Absence of clinical trial consortia to support integration, harmonization and aggregation of cancer outcomes across studies
	“We really should bring together a group together, before we even start doing a trial to think about aggregating data at the beginning, so we can actually have information down the track. Because it is such a rare outcome with cancer, even though it is such an important outcome.” H1 “It is so difficult to power a trial to cancer. . . what are thoughts about bringing trialists together, researchers and patients, to a big group like this and actually design an outcome that we could actually combine all outcomes, later on down the track when the trials is finished?” H1 “Perhaps each trial will not have enough numbers to really make any difference. But then if there’s some mechanism where they can easily share data on this particular question, that may then provide what patients really care about” H2 “But I think it’s sort of like to me the two biggest goals. . . we want people to capture cancer and we want people to capture it uniformly so there’s the opportunity for comparison. And the easiest way to do that is to push people towards specific outcome measures that they do not have to reinvent.” H5

H, health professional, P, patient, number indicated refers to group ID from the workshop (1-7).

Fear of cancer occurrence due to immunosuppression

Patients feared cancer, which was described as a “big scary monster”. Some patients were not initially aware of the increased risk of cancer prior to receiving a transplant, but over time gained an understanding that their cancer risk increased with the

duration, load and intensity of immunosuppression. Both patients and health professionals expressed that it was difficult navigating the balance between minimizing the risk of cancer (through reducing immunosuppression) and the fear of graft loss from rejection (through insufficient immunosuppression) – “perhaps my kidney graft might last, but other things like cancer might get me” (patient).

Furthermore, there was a fear of being diagnosed with cancer as it could necessitate a reduction in immunosuppression and pose a threat to graft function, or the treatments for cancer may directly impair graft function. Both health professionals and patients were uncertain about how and when to screen for cancer due to a lack of consistent guidelines and some patients felt that they had to self-advocate to access screening for cancer.

Capturing the details of type, stage and recurrence

Recognizing the differential consequences of cancer types

Cancer was identified to have “different pathologies, prognosis, different treatment patterns” (health professional). Therefore, patients and health professionals believed it was informative to know about the type of cancer given that the prognosis and treatment of a cancer is specific to its type – “it’s skin cancer versus brain cancer, that’s a very different conversation with patients” (health professional).

Delineating the stage of cancer to convey severity

Participants noted that knowing the stage of cancer was necessary to inform decisions about the treatments possible and the patient’s prognosis – “it is important considering the severity and potential treatment patients may undergo following cancer occurrence” (health professional). Patients considered the stage of cancer to reveal “practical things” – “What does this mean for the patient’s life? What extra treatment are they going to require as a result of this cancer?” (patient). However, it was acknowledged that collecting information on cancer stage could be challenging as different cancers have different staging systems. Both patients and health professionals however, supported the inclusion of staging, potentially as a binary descriptor: localized/regional versus metastatic.

Distinguishing cancer recurrence and *de novo* cancer

Health professionals emphasized the importance of collecting information on cancer recurrence in trials to help inform clinical practice, for example, to provide data to justify and support the minimum duration a patient must be cancer free to be considered for a transplant – “we’ll often be transplanting folks who’ve got a past history of cancer. That’s where the recurrence question comes in. Have they been cancer-free long enough?” (health professional). Health professionals emphasized that first time occurring (*de novo*) cancer after transplantation could be associated with viral-mediated cancers and immunosuppression. Thus, cancer recurrence and *de novo* cancer were seen as two “groups of cancers that invite different questions and present significantly different epidemiologic challenges” (health professional) and should be distinguished when reported in trials.

Recognizing the challenges of capturing cancer events

Under-reporting and incomplete documentation of longer-term cancer outcomes

Participants recognized that cancer was typically a long-term outcome that could take many years to develop, be detected and may not occur during the period of a trial, which is usually conducted over a short period of time – “the trial period is limited. And the consequences [cancer] can occur in a long run” (patient). Participants supported the use of cancer occurrence as a core outcome measure, to enforce data on cancer occurrence being collected in trials – “I favor having [cancer occurrence] as a core outcomes measure primarily because cancer is a late outcome and often not captured in other clinical trials” (health professional). Patients and health professionals discussed and supported the long-term follow up of patients to capture the occurrence of cancer after trial completion through registries or data linkage – “if there’s a way to somehow mandate that [trials] be linked to registries that already collect [cancer] data, that might be a way to get an answer” (health professional). However, challenges in conducting long-term follow-up for cancer occurrence through registries were identified, including the absence of registries in some regions, particularly low- and middle-income countries, and the challenges of tracking patients between centers and over decades of follow-up.

Variability in cancer screening practices

The lack of standardized screening protocols and access to screening were discussed as issues that may lead to variations in the reporting of cancer occurrence in different settings. Participants highlighted that there are variable screening practices based on location, local risk factors and resource availability; for example, healthcare systems with fewer resources may have reduced capacity to offer colonoscopies for colorectal cancer screening. Patients identified screening as an essential aspect of their care, because early cancer diagnosis through screening can enable timely interventions that improve patient outcomes – “with skin and melanoma, for the most part, with good surveillance, you can manage it pretty well, speaking as someone who’s had a lot of cuts on his body” (patient). However, it was also acknowledged that early cancer detection through screening may initially lead to an increased incidence, as cancers that would have been diagnosed later are being detected earlier. More importantly, the concerns of overdiagnosis were raised as screening can detect slow-growing or indolent cancers that would never have caused symptoms, morbidity or death during the person’s lifetime, causing unnecessary worry and stress.

Absence of clinical trial networks to support integration, harmonization and aggregation of cancer outcomes across studies

Participants acknowledged that cancer events, particularly when distinguished into types, occur in small numbers in clinical trials – “[cancer] is such a rare outcome...even though it is such an

TABLE 2 Recommendations from the consensus workshop on implementing a core outcome measure for cancer in kidney transplant trials.

Overarching principle
- Ensure a balance between a simple measure that is easy to implement, and a measure that includes additional details to capture aspects important to stakeholders
For health professionals
- Monitor, record and report cancer in transplant recipients to registries and trialists
- Advocate for core outcomes, such as cancer occurrence, to be included in trials
For trialists/funders/pharmaceutical companies
- Adopt standardised core cancer outcome measures in trials
- Support the development of and invest in registries
- Partner with registries to facilitate the long-term collection of cancer outcomes
For researchers
- Develop clear and precise definitions of the core outcome and its components in collaboration with patients, health professionals, trialists and registry organisations
- Work with journals, guideline organizations and regulatory agencies to secure endorsement and use of core cancer outcome measures
- Implement core outcome sets in appropriate research settings, ensuring they are responsive to patient values
- Continue to champion the inclusion of patient-centred outcomes, such as cancer occurrence (with details of cancer types, stage, and whether the malignancy is recurrent or <i>de novo</i>), in transplant research agendas
For consumers
- Continue to advocate for patient-centred outcomes and reporting in clinical research and trials

important outcome” (health professional). The small number of events in individual trials makes it difficult to draw meaningful conclusions about patient outcomes without the ability to harmonize and aggregate data between trials. Combined with the long latency periods as discussed above, the small number of events led participants to advocate for the development of consortia to collate data across trials - “perhaps each trial will not have enough numbers to make any difference. But then if there’s some mechanism where they can easily share data on this particular question, that may then provide what patients really care about” (health professional). Furthermore, participants supported identifying a core outcome measure for cancer as it would further facilitate the aggregation and harmonization of data across trials - “we want people to capture cancer, and we want people to capture it uniformly so there’s the opportunity for comparison. And the easiest way to do that is to push people towards specific outcome measures that they do not have to reinvent” (health professional).

Discussion

In this workshop, participants reached consensus that cancer occurrence should be established as the core outcome measure for cancer in kidney transplant trials. The workshop participants also acknowledged the inherent tension between minimizing immunosuppression to reduce cancer risk and maintaining sufficient immunosuppression to prevent acute rejection and graft loss. Details of cancer type, stage, and disease recurrence were identified as key factors that influence clinical outcomes and should be included. Cancer occurrence represents a significant event in a patient’s health journey and should be clearly defined and recorded. Yet, despite its relevance and importance, cancer is not uniformly and

routinely captured in clinical trials and registries. There is an urgent need to harmonize and integrate long-term cancer data capture through registry-embedded trials and collaborative platforms. This will ensure consistent reporting, facilitate meaningful comparisons between trials, and support evidence-based shared decision-making to improve post-transplant outcomes.

While cancer occurrence was agreed upon as the core outcome measure, both patients and health professionals sought further details in the measure to improve its relevance and applicability. Health professionals identified that cancer encompasses a wide spectrum of pathologies, with the type of cancer and stage informing potential treatments required and prognosis. Furthermore, collecting information on whether a cancer is recurrent or *de novo* could help inform transplant practices. Patients and caregivers supported the addition of these details to enhance the utility and meaningfulness of the outcome, given the direct implications of these factors on treatment options, monitoring and prognosis. Therefore, the proposed core outcome was cancer occurrence, with specification of cancer type/site, stage as regional/localized versus metastatic and whether it is a recurrence or *de novo* cancer. It was acknowledged that an ideal core outcome measure should be simple to support its implementation and interpretation by patients and caregivers.

Although there was consensus that cancer occurrence is a critically important outcome to report in all kidney transplantation trials, a number of challenges to implementation were acknowledged that require action from clinicians, trialists and registry organizations (summarized in Table 2). Kidney transplantation trials are typically short in duration, while cancers usually take many months to years to develop and be detected. Therefore, trials in immunosuppression or other interventions that may impact cancer risk are potentially unlikely to demonstrate a difference in the effect of an intervention on cancer occurrence in short-term follow up. Furthermore, although kidney

transplant recipients have a 2-3-fold increased risk of developing cancer compared to age- and sex-matched peers [1, 2], the absolute rates are initially small, with kidney transplant recipients 10, 20 and 30 years post-transplantation having cancer rates of 4.4%, 14.6% and 33.2%, respectively [12]. These challenges highlight the value of cancer occurrence as a core outcome, to allow the aggregation and harmonization of data across individual studies and populations. All stakeholders (consumers, health professionals and researchers) should continue to advocate for the inclusion of patient-centred and clinically relevant core outcomes, such as cancer incidence, in clinical trials. Attendees proposed strategies to assist the collection of data on cancer occurrence in kidney transplant recipients, including funding and expansion of registries to collect longer-term cancer outcomes and extend the follow-up of trial participants. Post-trial registry-based studies may enable clearer understanding of the risks and outcomes of cancer, compared to the findings of currently predominately retrospective studies. Challenges related to the availability and resources of registries across different settings and the reliance on health professionals for the recording and reporting of these outcomes were acknowledged. Similarly, participants felt that developing clinical trial consortia and implementing a core outcome measure for cancer occurrence would facilitate the harmonization and aggregation of results. Endorsement from journals, guideline organizations and regulatory agencies will be critical in encouraging the inclusion and reporting of core outcome measures.

In this workshop, we elicited detailed discussions regarding the development of a core outcome measure for cancer, identifying features that are critically important to patients, caregivers and health professionals. We recorded and transcribed the discussions and coded the data systematically to ensure that all ideas and experiences were captured. The preliminary findings were shared with all attendees to ensure that the reported details accurately reflected the diversity and depth of the discussions. However, this workshop has several limitations. Although we aimed to include a diverse range of stakeholders, the number of participants was relatively small, which may limit the breadth of perspectives captured. Although attendees came from 12 countries, including Indonesia, Mexico and Turkey, the majority were from Australia. However, there was substantial overlap in the themes expressed by the Australian and non-Australian participants. Furthermore, the participants were largely comprised of nephrologists, with comparatively limited representation from other disciplines such as oncology, nursing, and allied health. This may have influenced the prioritisation and framing of outcomes, particularly given the multidisciplinary nature of cancer care in transplant recipients. The workshop was conducted in English, which may have restricted participation from non-English-speaking individuals. In addition, the online format may have introduced participation bias, as patients and caregivers required access to technology, digital literacy, and sufficient resources to engage, potentially favouring individuals from higher socioeconomic settings or patients with fewer comorbidities. Consequently, the views represented may be more reflective of higher-resource contexts and may not fully capture the experiences and priorities of patients and healthcare providers in lower-resource settings.

Recommendations from the workshop represent the collective input from patients, caregivers and health professionals and will inform the development of a core outcome measure for cancer that is

relevant and meaningful to all stakeholders. Establishing a meaningful and feasible outcome for cancer will support efforts to improve the frequency and consistency of cancer reporting in all kidney transplantation trials. Implementation of this core outcome measure will require support and actions from consumers, nephrologists, oncologists, hematologists and other stakeholders, including trialists, registries, guideline organizations, researchers and journals. Improved reporting of this high-priority outcome is needed to support effective shared decision-making about interventions to reduce the burden of cancer in kidney transplant recipients.

Data availability statement

The datasets presented in this article are not readily available. The de-identified coded dataset may be requested. Requests to access the datasets should be directed to Ellen Dobrijevic, ellen.dobrijevic@health.nsw.gov.au.

Author contributions

ED, AZ, GW and AJ were responsible for the conceptualisation of the project. ED read and coded each transcript. The themes were reviewed by AZ, GW and AJ. The themes and manuscript were reviewed and approved by all authors. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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This article has been corrected with minor changes. These changes do not impact the scientific content of the article.

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References

1. Au EH, Chapman JR, Craig JC, Lim WH, Teixeira-Pinto A, Ullah S, et al. Overall and site-specific cancer mortality in patients on dialysis and after kidney transplant. *J Am Soc Nephrol* (2019) 30(3):471–80. doi:10.1681/asn.2018090906
2. Ietto G, Gritti M, Pettinato G, Carcano G, Gasperina DD. Tumors after kidney transplantation: a population study. *World J Surg Oncol* (2023) 21(1):18. doi:10.1186/s12957-023-02892-3
3. Manickavasagar R, Thuraisingham R. Post renal-transplant malignancy surveillance. *Clin Med (Lond)* (2020) 20(2):142–5. doi:10.7861/clinmed.2019-0423
4. Al-Adra D, Al-Qaoud T, Fowler K, Wong G. *De novo* Malignancies after Kidney Transplantation. *Clin J Am Soc Nephrol* (2022) 17(3):434–43. doi:10.2215/cjn.14570920
5. Au E, Wong G, Chapman JR. Cancer in kidney transplant recipients. *Nat Rev Nephrol* (2018/08/01 2018) 14(8):508–20. doi:10.1038/s41581-018-0022-6
6. Sautenet B, Tong A, Manera KE, Chapman JR, Warrens AN, Rosenbloom D, et al. Developing consensus-based priority outcome domains for trials in kidney transplantation: a multinational Delphi survey with patients, caregivers, and health professionals. *Transplantation* (2017) 101(8):1875–86. doi:10.1097/tp.0000000000001776
7. Tong A, Gill J, Budde K, Marson L, Reese PP, Rosenbloom D, et al. Toward establishing core outcome domains for trials in kidney transplantation: report of the standardized outcomes in nephrology-kidney transplantation consensus workshops. *Transplantation* (2017) 101(8):1887–96. doi:10.1097/tp.0000000000001774
8. Au EH, Wong G, Tong A, Teixeira-Pinto A, van Zwielen A, Dobrijevic E, et al. Scope and consistency of cancer outcomes reported in randomized trials in kidney transplant recipients. *Kidney Int Rep* (2023) 8:274–281. doi:10.1016/j.ekir.2022.10.032
9. Dobrijevic E, Scholes-Robertson N, Guha C, Howell M, Jauré A, Wong G, et al. Patient-centered research and outcomes in cancer and kidney transplantation. *Semin Nephrol* (2024) 44(1):151499. doi:10.1016/j.semnephrol.2024.151499
10. Tong A, Budde K, Gill J, Josephson MA, Marson L, Pruett TL, et al. Standardized outcomes in nephrology-transplantation: a global initiative to develop a core outcome set for trials in kidney transplantation. *Transpl Direct* (2016) 2(6):e79. doi:10.1097/txd.0000000000000593
11. Dobrijevic E, van Zwielen A, Jaure A, von Huben A, Howell M, Teixeira-Pinto A, et al. Critically important cancer outcomes to report in kidney transplant trials. *Kidney Int Rep* (2025) 10:3664–9. doi:10.1016/j.ekir.2025.07.006
12. Fuhrmann JD, Valkova K, von Moos S, Wüthrich RP, Müller TF, Schachtner T. Cancer among kidney transplant recipients >20 years after transplantation: post-transplant lymphoproliferative disorder remains the most common cancer type in the ultra long-term. *Clin Kidney J* (2022) 15(6):1152–9. doi:10.1093/ckj/sfac013

Supplementary material

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Implementation of a controlled DCD program in Western Austria – key considerations and insights

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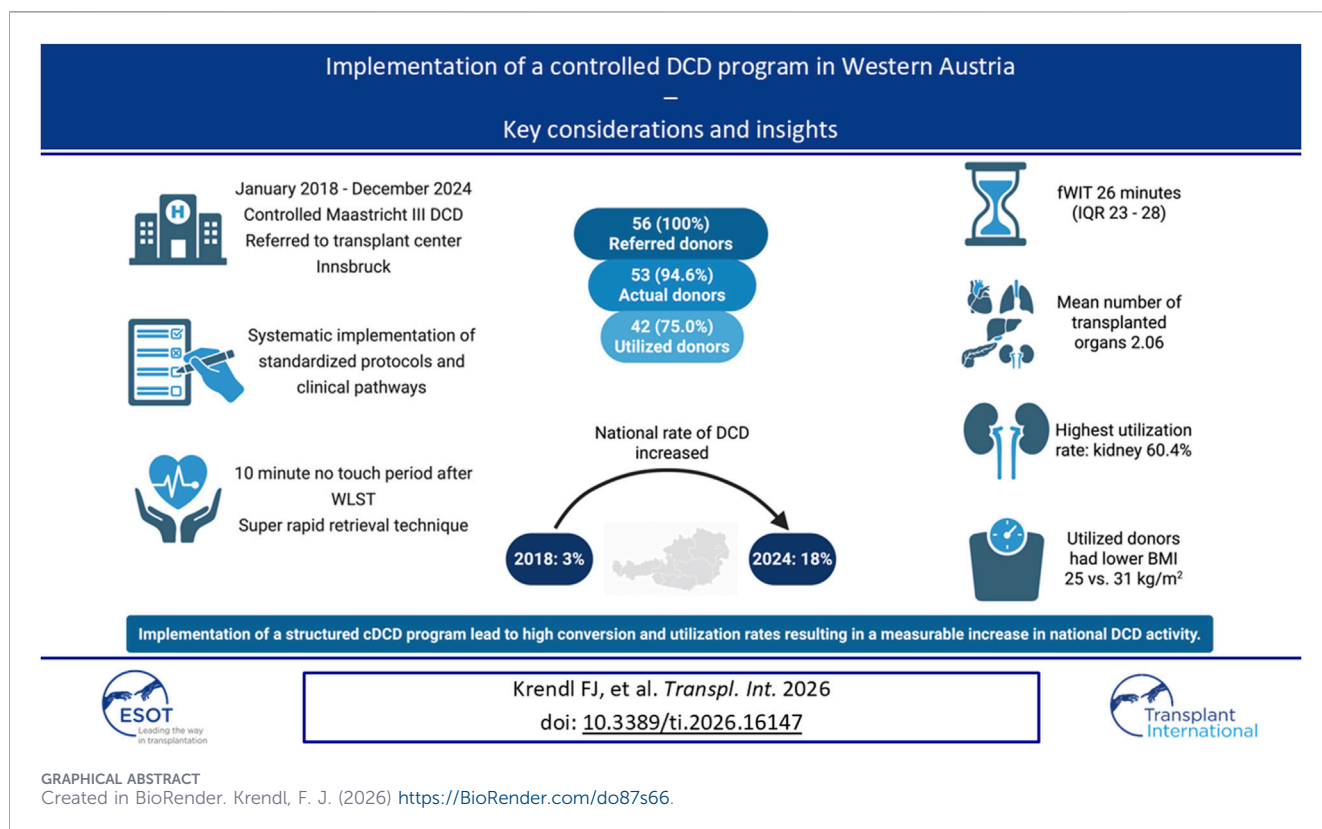
Controlled donation after circulatory death (cDCD) offers an opportunity to expand the deceased donor pool, yet implementation remains limited in many countries. We conducted a retrospective single-center analysis of all cDCD donors (Maastricht category III) referred to the Transplant Center at the Medical University of Innsbruck between January 1, 2018, and December 31, 2024. Donor characteristics, ischemia times, organ utilization, and program-level trends were analyzed. In addition, key steps and protocols essential for establishing a cDCD program were systematically evaluated. Of 56 referred cDCD donors, 53 (94.6%) proceeded to organ recovery (i.e., actual donors), and 42 (75.0%) resulted in the transplantation of at least one organ (i.e., utilized donors). Utilized donors had significantly lower BMI than non-utilized donors (25 vs. 31 kg/m², $p = 0.003$). The median functional warm ischemia time was 26 min (IQR 23–28). The mean number of transplanted organs per donor was 2.06. Organ utilization rates were highest for kidneys (60.4%). Nationwide DCD activity increased from 3% to 18% following the implementation of a structured cDCD program in Western Austria. In summary, we have outlined steps and protocols required to successfully implement a cDCD program, resulting in high utilization rates and a measurable impact on national cDCD activity.

KEYWORDS

DCD (donation after circulatory death), donation, NRP, organ procurement, program development

Introduction

Before the Harvard brain death criteria had been published in 1968 [1], all deceased donors were declared dead using circulatory arrest criteria [2]. After the legal framework for the diagnosis of brain death according to neurologic criteria had been established, most countries almost exclusively relied on BD (brain-dead) donors [2]. However, due to the



increasing shortage of suitable donor organs, transplant organizations have explored different strategies to expand the donor pool. One such strategy is the implementation of donation after circulatory determination of death (DCD) [3].

Outcomes following transplantation of DCD organs have historically been inferior compared to transplantation of DBD organs [4, 5], prompting many centers to be cautious in accepting DCD organs for transplantation. Yet, with improved preservation strategies entering routine clinical practice [6–8] and better donor-recipient matching [9], outcomes following DCD transplantations are beginning to equal those of DBD transplantation [10–14]. Furthermore, the utilization of DCD organs has led to decreased waitlist times and waitlist mortality rates [15–18]. Thus, in an era of continued organ supply-demand mismatch, increased recovery and utilization of DCD organs should be encouraged [16]. Globally, DCD accounts for approximately 20% of all deceased donors used for transplantation [19]. In Europe and even within the Eurotransplant network, the DCD landscape is heterogeneous. While in Germany DCD is currently prohibited by law, the Netherlands and Belgium run very successful DCD programs with DCD rates accounting for up to 50% of all deceased donors [19]. In Austria, the third Eurotransplant member country to implement an active DCD program, DCD accounted for 18% of utilized deceased donors in the year 2024 – a significant increase from 2018, when a controlled DCD (cDCD) program was initiated in the western region of the country. We herein, report on our experience with the initiation of a cDCD program with focus on donor utilization as well as key aspects required for the successfully implementation of such a program.

Materials and methods

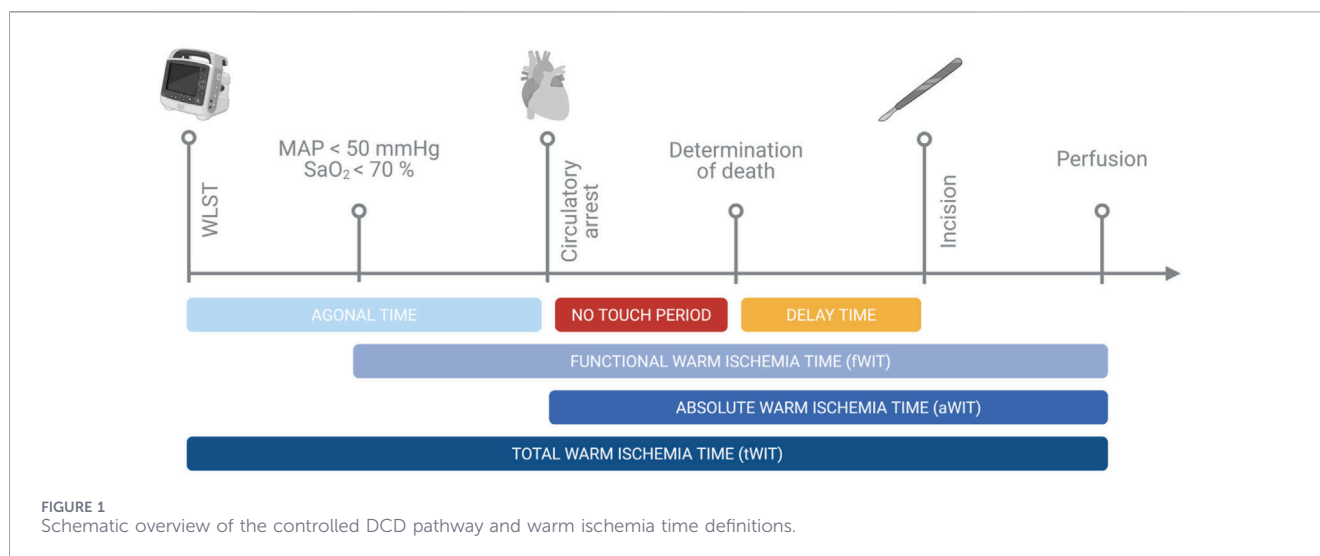
From January 1st, 2018 to December 31st, 2024 all DCD donors referred to the Transplant Center at the Medical University of Innsbruck (MUI) were included in this analysis. Donor data as well as periprocedural data were recorded and analyzed. All included donors corresponded to category III according to modified Maastricht criteria [20].

Terms and definitions

DCD donor

A DCD donor is any donor in whom organ donation occurs after determination of death by circulatory criteria. In clinical practice withdrawal of life-sustaining treatment (WLST) is followed by a variable period of progressive hypoxia and hypotension (functional WIT) until circulatory arrest occurs (Figure 1). In Austria circulatory arrest is determined either through invasive arterial blood pressure measurement or by echocardiography. ECG monitoring is not an effective modality to determine circulatory arrest as electrical activity (i.e., pulseless electrical activity) may still occur despite absence of mechanical activity of the heart.

Circulatory arrest marks the beginning of a mandatory stand-off period, during which no interventions must take place (no-touch period), to ensure no return of spontaneous circulatory activity. The length of the no-touch period differs among countries. In Austria the duration of the no-touch period is 10 min, after which death is confirmed by a standardized clinical examination [19]. This is



performed by two fully licensed physicians who are independent of the donation process following a two-person verification principle. In the planning of a DCD procedure, it should be ensured that two physicians are present and available for the determination of death.

Potential, referred, actual and utilized DCD donor

Any patient potentially suitable for cDCD is termed a potential donor. Any potential donor that is reported to the organ procuring transplant center following initial screening for medical and legal contraindications is defined as a referred donor. An actual donor is a donor in which the procurement operation is started, while a utilized donor is a donor from whom at least one organ has been transplanted [21].

Warm ischemia time (WIT)

Total WIT (tWIT) is defined as the time from WLST to initiation of perfusion. tWIT can be further divided into agonal WIT, functional WIT and absolute (asystolic) WIT. Agonal WIT is the time from WLST to circulatory arrest. Absolute or asystolic WIT is the time from circulatory arrest to the start of perfusion (Figure 1) [22, 23]. The definition of donor WIT (dWIT) or functional WIT (fWIT) varies, even amongst centers within the same country. We defined fWIT as the time-point when the mean arterial pressure (MAP) decreases below 50 mmHg or oxygen saturation (SaO₂) falls below 70% until the start of perfusion.

Legal and ethical framework

In Austria organ donation including DCD is regulated by national law. Any critical care physician, who is treating a suitable potential donor is authorized, encouraged and even mandated to report the potential donor–DBD or DCD–to the regional transplant center. However, no standardized referral criteria exist, and referral is left at the discretion of the primary care team. Austria has an opt-out system in place where the potential donors would have needed to opt-out during their lifetime to avoid becoming a donor. Thus, for every potential donor, the national objection registry is consulted prior to initiating the formal donor

evaluation process. Even though from a legal standpoint organ donation may proceed in the absence of a registered objection, the family is always approached for consent and may also stop the DCD process at any time. Families are approached by the primary care team of the ICU and may be supported by the local transplant coordinator if needed.

Alongside the legal framework, ethical considerations remain central to the DCD process, particularly with regard to antemortem interventions, the separation of end-of-life care from transplant decision-making as well as the maintenance of transparency and public trust [24].

Antemortem interventions

There is consensus in Austria that antemortem interventions are only allowed under two conditions:

1. The comfort of the patient is not compromised.
2. The intervention or diagnostic measure serves to improve the transplantation outcome.

The following interventions can currently be performed under these two perspectives:

- Imaging procedures, including advanced imaging such as CT scans
- Bronchoscopy/transesophageal echocardiography under adequate analgesia and sedation
- Premortem administration of heparin
- Premortem administration of cortisone
- Preparing and draping the patient
- Placement of arterial and venous catheters

Protocol development and implementation

Communication and education as starting point

Clear, effective and open communication and education of the public, medical professionals, support staff and donor families are

key elements for a successful DCD program. All involved professional groups (intensive care nurses, intensive care physicians, anesthesia nursing staff, anesthesiologists, operating room nursing and support staff) and hospital management should be briefed and informed about the DCD procedure in advance to the start of the program. Regional transplantation coordinators should organize on-site training or support local authorities in conducting such events. Further to this, briefings as well as de-briefings are essential components of an effective communication strategy, ensuring that all stakeholders are aligned. When a DCD procedure is planned, sufficient time for an on-site briefing for the involved physicians, nursing team and operating room support staff should be planned at the donor hospital. During the briefing of the procurement teams, tasks must be clearly defined and assigned. This includes who will be responsible for diagnosing and confirming death (two-person verification principle), who will accompany the relatives of the donor during their stay in the hospital (family discussion, guiding them to the operating room, accompanying them back to the intensive care unit, et cetera), who will inform the retrieval team about the ongoing process and expected end time of the no-touch period as well as briefing of the entire team involved in the organ procurement (e.g., surgeons, nurses, perfusionists, anesthesiologists, etc.).

Even individuals who are only peripherally involved with the presumptive organ donor, such as the patient porters, should not be overlooked in the information dissemination process.

Following the DCD procedure, a de-briefing should take place with all persons of the donor hospital who were involved in the DCD process, in order to address any potential questions, clarify misunderstandings, and identify potential issues for further improvement. The primary care team of the patient bears the responsibility for the communication process with the donor's relatives. If necessary, the regional or in-house transplantation coordinator can support the primary care team. Continuity of care and communication before, during and after the donation process is helpful and highly appreciated by the relatives. Before a planned DCD procedure, important aspects that differ from DBD donation should be discussed with the patient's relatives. These include the following points:

- Explaining the concept of changing treatment goals, palliative analgesation, WLST and the dying process
- Explaining the DCD procedure
- Clarifying whether the relatives wish to be present during the process of dying
- Explaining that in exceptional cases, if the dying process takes too long, organ donation may not be feasible due to the unavoidable ischemic damage of the organs
- Explaining that investigations are carried out beforehand to assess the suitability of the organs for donation, but ultimately, it can only be determined after organ retrieval whether transplantation is possible
- Introducing the rules of conduct in the operating room (e.g., which parts of the patient may be touched)
- Clarifying if there is a wish for psychological or spiritual support of the family and if the relatives wish another farewell after organ donation, and if so, organization of the appropriate facilities

Additional questions from relatives may arise during the process of dying. These may concern medication administration or monitoring. A member of the primary care team should conduct another meeting with the relatives after organ donation and offer further support if needed. The discussion regarding a possible objection to organ donation by the potential organ donor is conducted after informing the relatives about the unfavorable prognosis of the patient and the planned WLST.

Cooperation and coordination between donor hospital and organ procuring transplant center

Once a potential donor is identified, early contact with the procuring transplant center is recommended, and the following topics should be discussed in advance:

- Required medical examinations of the potential donor
- Evaluation of the assumed quality of the potential retrieved organs
- Modality and extent of life-sustaining therapies
- Planned location for WLST (ICU vs. OR)
- Planned presence of relatives of the potential donor at the time of WLST
- Necessity for social or psychological support for the family of the potential donor
- Requirements for potential machine perfusion of the retrieved organs in the OR
- Timing and dosing of cortisone and heparin administration
- Necessity of tracheal re-intubation after determination of death

Documentation

The transplant center requires the documentation and transmission of specific time points during the DCD procedure (WLST, desaturation below 70%, drop of blood pressure, cessation of circulation, etc.) which need to be passed on to Eurotransplant and which are also important for the hospital's internal documentation. The DCD protocol remains in the patient's medical record. A copy should be provided to the organ procurement team. Until the patient died in the OR, either the ICU documentation is continued, or an anesthesia documentation protocol must be generated. A documentation of the operation is required for the retrieval procedure with a final sign off on the protocol by the retrieval surgeons after final on-site organ assessment and referral of the documentation to the transplant center.

Clinical pathway for controlled DCD

Planning and preparation

Upon identifying a patient as a potential donor, the donor hospital in cooperation with the local transplant coordinator contacts the regional transplant center. The transplant center evaluates the suitability of the potential donor for organ donation based on the available medical findings and by consulting the national registry to exclude any objection against organ donation. Once medical and legal contraindications to organ donation have

been excluded, the potential donor is considered a referred donor. The ICU team at the donor hospital together with the transplant coordinators of the regional transplant center—which also deploys the procurement team—then determines a suitable time for WLST and the procurement procedure. At the donor hospital an internal briefing is held as described above.

Once the organ procurement team arrives at the donor hospital an external briefing is convened. The external briefing should cover topics such as donor identification as well as a detailed discussion of the whole donation process to make sure everyone is informed about the individual tasks. Furthermore, maximum tolerable ischemia times specific for each organ are determined based on the donor's risk profile (pre-existing conditions, age, current organ function, etc.). Throughout the donation procedure a person who is not member of the procurement team, with knowledge and understanding of DCD procedures should be present.

WLST and determination of death

The decision to change treatment goals to palliative care and WLST is made, documented and implemented by the primary care team independent from any considerations of organ donation. This decision is carried out according to local medical and ethical protocols. Once the decision has been made, treatment becomes symptom-oriented (e.g., analgesia and sedation) aiming to ensure best supportive care until death occurs. From the time of the decision to change treatment goals until WLST, measures to maintain organ quality are often necessary (circulatory support, volume resuscitation, anticoagulation, potential donor preparation for surgery, etc.). The location of WLST may vary between the donor hospitals, depending on the local conditions. WLST can occur either in the ICU or in the OR. However, it has been proven to be advantageous to withdraw life-sustaining therapy in the OR. In any case, efforts should be made to ensure the shortest possible WIT. Once the patient has been transferred to the OR pre-mortem interventions such as administration of heparin and cortisone as well as prepping and draping of the patient are carried out. Before WLST a modified Team Time Out is performed. The organ procurement team is then asked to step outside the OR and the surgical instruments are covered before WLST commences. The relatives of the patient may be present during the dying process if so desired.

The timepoints of WLST, O_2 -desaturation $<70\%$, and the drop of the MAP ≤ 50 mmHg, as well as the time of circulatory arrest are documented. The 10-min no-touch period following the onset of circulatory arrest together with the time until organ perfusion is initiated constitutes the asystolic/absolute WIT (Figure 1). Following the no-touch period, death is diagnosed and certified by two physicians using the four-eye principle according to the recommendations of the Austrian Supreme Medical Council. Immediately after determination of death, surgical measures for organ retrieval and preservation are commenced.

Surgical procedure

We have implemented a modification of the super-rapid technique first described by the Pittsburgh group in 1995 [25], which has become the standard for abdominal DCD organ retrieval.

The anterior abdominal wall is elevated with clamps, and a midline laparotomy from the xyphoid process down to the symphysis pubis using a scalpel is performed. The cut is deepened down to the linea alba and preperitoneal fat. The peritoneal cavity is entered bluntly just above the umbilicus. The small bowel is placed in a lab pad and retracted towards the donor's left shoulder. The right iliac common artery or distal aorta is incised and cannulated. The aorta is either cross-clamped right below the diaphragm or intrathoracically via a median sternotomy, and the inferior vena cava (IVC) is vented intrapericardially. The abdominal viscera are cooled with sterile ice until perfusion with cold preservation solution is completed.

For heart donation from a cDCD donor, the cardiac surgical team prepares two separate instrument tables to ensure procedural independence from the abdominal retrieval team and the primary scrub nurse. At our institution, a direct procurement and perfusion strategy utilizing normothermic *ex situ* heart perfusion (ESHP) is currently employed. Sharp dissection through the skin and subcutaneous tissue down to the sternum and a median sternotomy are performed. The pericardium is widely opened. A venous cannula is then inserted into the right atrium to collect blood for priming of the ESHP circuit. To ensure adequate priming volume, typically at least 1.2 L, the donor is placed in Trendelenburg position to optimize venous return. Subsequently, a perfusion cannula is inserted into the ascending aorta, followed by intrathoracic aortic cross-clamping. Cold cardioplegic solution is administered into the aortic root and cold saline is intermittently poured over the epicardial surface to maintain topical cooling. From this point onward, the surgical steps mirror those of standard DBD procurement, including myocardial cooling, cardiectomy, and back-table preparation of the graft for *ex situ* perfusion and subsequent transport.

For lung donation from a DCD donor, organ removal is performed in close temporal conjunction with super-rapid abdominal and heart removal, with priority given to minimizing warm ischemia. Following median sternotomy and further opening of the pericardium, the pulmonary trunk is then exposed and cannulated. The left atrium is opened or vented via the left atrial appendage region to relieve the heart. After intrathoracic aortic clamping, a cold perfusion solution in combination with Prostaglandin E (e.g., 250 μ g per 3000 mL bag) is applied antegrade via the pulmonary artery, while the lungs are cooled topically with sterile ice or slush. Controlled ventilation is maintained to prevent atelectasis. After complete perfusion and sufficient cooling, the following steps mimic standard DBD procurement steps. Depending on the protocol, back-table procedure includes retrograde irrigation via the pulmonary veins and preparation for transport or subsequent *ex-situ* lung perfusion.

Ischemia times and aborted donation

If the agonal WIT (Figure 1) exceeds 120 min the cardiothoracic team stands down, while the abdominal retrieval team will wait a total of 240 min before standing down. Typically, the duration of the agonal phase is significantly shorter. To estimate the expected agonal WIT, the Wisconsin Score can be used [26]. Should a prolonged agonal WIT preclude organ retrieval, at least one member of the treatment team (along with the family) remains in the operating room and awaits the patient's passing. If, unexpectedly, the dying

TABLE 1 Donor characteristics.

Donor factors	Referred (n = 56)	Actual (n = 53)	Non-utilized (n = 11)	Utilized (n = 42)	P-value
Age (years)	57 (50–65)	58 (51–65)	60 (52–69)	57 (49–65)	0.278
Sex					0.697
- Female	12 (21.4)	12 (22.6)	3 (27.3)	9 (21.4)	
- Male	44 (78.6)	41 (77.4)	8 (72.7)	33 (78.6)	
Blood group					0.664
- O	19 (33.9)	19 (35.8)	5 (45.5)	14 (33.3)	
- A	23 (41.1)	22 (41.5)	5 (45.5)	17 (40.5)	
- B	13 (23.2)	11 (20.8)	1 (9.1)	10 (23.8)	
- AB	1 (1.8)	1 (1.9)	0	1 (2.4)	
BMI (kg/m ²)	26 (24–30)	26 (24–29)	31 (27–32)	25 (23–28)	0.003
ECMO		3 (5.7)			
ICU stay (days)	5 (3–7)	5 (3–7)	4 (3–7)	5 (4–7)	0.019
Warm ischemia time (minutes)					
- tWIT		27 (24–30)	23 (20–29)	27 (25–30)	0.271
- Agonal WIT		12 (9–15)	10 (9–13)	13 (10–15)	0.155
- Asystolic WIT		14 (12–15)	14 (13–16)	14 (12–15)	0.664
- fWIT		25 (21–28)	22 (19–28)	26 (23–28)	0.363
Time to cannulation		3 (2–5)	4 (3–6)	3 (2–5)	0.368

Overview over referred, actual, non-utilized and utilized donor characteristics. A higher donor BMI was associated non-utilization. (31 kg/m² vs. 25 kg/m², $p = 0.003$). Significant P-values are shown in bold.

process extends over several hours, a return to the intensive care unit may be considered. If the fWIT exceeds 60 min, the liver and pancreas are not retrieved. Kidney and lung retrieval is aborted if the fWIT surpasses 120 min. In heart transplantation a fWIT longer than 30 min, precludes organ utilization.

Members of the transplant centers, as well as of the donor hospitals need to be aware that there is a possibility that the dying process may last longer than it is acceptable for organ donation or that other reasons may prevent organ retrieval. This, however, should not deter intensive care physicians from reporting potential organ donors.

Results

Donors

A total of 56 Maastricht category III potential donors were referred during the study period. The median donor age was 57 years (range 15–76), 44 donors were male (78.6%) and 12 female (21.4%).

Of the 56 referred donors, 53 (94.6%) became actual donors. In one referred donor a renal cell carcinoma was suspected based on further workup which ultimately precluded donation. As for the two other cases, in one case the referred donor did not progress to circulatory arrest and was transferred back to the ICU, while in the

other case, no suitable recipient could be identified within the Eurotransplant region.

Overall, in 42 cases, cDCD donation resulted in successful transplantation of at least one organ corresponding to a utilization rate of 75.0%. Table 1 shows donor characteristics according to potential, actual, non-utilized and utilized donors. Utilized donors had lower BMIs compared to non-utilized donors (25 kg/m², IQR 23–28 vs. 31 kg/m², IQR 27–32; $p = 0.003$).

For utilized donors the median agonal, absolute and functional WITs were 13 min (IQR 10–15), 14 min (IQR 12–15) and 26 (IQR 23–28) minutes respectively. The median time to cannulation (time from skin incision to start of cold perfusion) was 3 min (IQR 2–5, range). Four donors (7.1%) were on ECMO before WLST. Normothermic regional perfusion (NRP) was employed in one case during the seventh and final year of the study period as part of the donation process according to Italian law, which requires a 20-min no-touch period. In this case the fWIT was 66 min. Liver and left kidney of this donor were successfully transplanted.

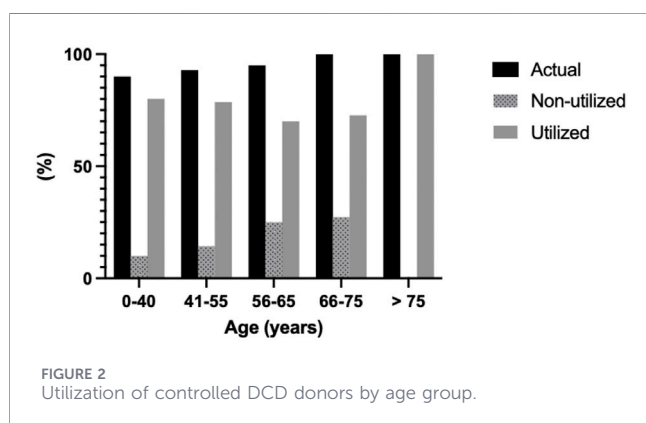
Transplanted organs

The mean number of organs transplanted per actual donor was 2.06. Twenty-two donors (41.5%) were single-organ donors, and of those, ten (45.5%) were kidney-only donors. Utilization rates were

TABLE 2 Offered, procured and transplanted organs.

Donor status	Number of donors, n and (%)							
Referred donors	56 (100)							
Actual donors	53 (94.6)							
Utilized donors	42 (75.0)							
Organ disposition	Heart	Left lung	Right lung	Liver	Pancreas	Left kidney	Right kidney	Intestine
Offered	11	23	23	56	6	48	48	2
Procured	5	15	15	45	3	39	39	0
Transplanted	5	11	11	23	3	29	27	0
Utilization rate (%)	45.5	47.8	47.8	41.1	50.0	60.4	56.3	0

Values are presented as absolute numbers and percentages in parentheses. Utilization rates were highest for kidneys.



highest for kidney (60.4%), pancreas (50.0%), lung (47.8%), heart (45.5%) and liver (41.1%).

Table 2 provides an overview of all reported, procured and transplanted organs. Figure 2 depicts numbers and percentages of potential, actual, non-utilized and utilized donors according to age groups.

Influence on donation rates

Following the introduction of the cDCD program in Western Austria, the proportion of DCD donors among all deceased donors in Austria increased from 3% in 2018 to 18% in 2024, despite a concurrent decline in overall deceased donor volume. This decline was largely attributable to the lack of ICU capacities during the COVID-19 pandemic, reflecting global trends observed between 2020 and 2022.

Discussion

Initiating a DCD program is a multidimensional process requiring (1) the development and implementation of protocols, (2) educating members of the medical community to avoid misconceptions and foster trust and (3) training the medical professionals involved in the DCD procedure. We have outlined

the process required to implement and establish such a program in a European setting and highlighted key factors for its success. Furthermore, we report on local protocols and workflows which have been implemented to ensure a standardized pre-, intra- and postprocedural approach.

Implementing a cDCD program has the potential to increase the overall donor volume or at least compensate for static DBD donation rates [27, 28]. A concern that has been voiced is that the implementation of DCD donation programs could negatively impact DBD donation rates [29], which would be undesirable for multiple reasons. One reason is the reduced number of transplanted organs per donor, which, on average is significantly lower for DCD donors compared to DBD donors [30]. Another reason is the additional WIT, which is unavoidably incurred as part of the DCD donation process. Thus, it is important to make sure that implementing a cDCD program does not lead to a shift from DBD to DCD donation but rather an increase in overall deceased donor donation rates. Austrian registry data show overall stable organ donation rates across all regions that have implemented active DCD programs, taking into account an overall decline in organ donation during the COVID-19 pandemic due to a lack of ICU capacities [31]. Since the cDCD program in Innsbruck - covering the Western region of Austria - has been established, the proportion of DCD donation in Austria has increased from 3% to 18%. In the years prior to 2018 there was only a small number of utilized DCD donors in Austria (2017: n = 7, 0.8 PMP; 2016: n = 5, 0.6 PMP; 2015: n = 6, 0.7 PMP; 2014: n = 6, 0.7 PMP; 2013: n = 3, 0.4 PMP.). Almost all these DCD donors were Maastricht category I and were performed by only one Austrian transplant center. Following the implementation of the cDCD program in Innsbruck in 2018, increasing awareness and acceptance of organs from cDCD has resulted in cDCD programs being started in the remaining three Austrian transplant centers covering the Eastern, Central and Southern Region of Austria.

In countries with long-established DCD programs, such as the United States, Spain, the United Kingdom, and the Netherlands, DCD nowadays accounts for up to 50% of all deceased donor organ donation [32]. Steps to further increase DCD activity in Austria and moving towards similarly high rates include continued efforts to raise awareness among all relevant stakeholders, the implementation of

nationwide protocols and structured algorithms for the identification and referral of potential DCD donors in ICUs, and the standardization of donor screening processes across the country. In addition, strengthening collaboration between regional transplant centers and local donor hospitals represents an important step toward optimizing the DCD pathway [33]. Additionally, raising public awareness and educating the population on the subject of organ donation and its process through targeted informational initiatives is of crucial importance to foster a supportive environment for organ donation and transplantation.

Key performance metrics in the DCD donation process are the donor conversion (number of actual donors divided by number of referred donors) and utilization rates. In our cohort, the donor conversion and utilization rates were as high as 95% and 75% respectively. An important reason for a referred donor not to become an actual donor is an unacceptably long agonal WIT. The agonal WIT can be highly variable and might be unpredictable. The uncertainty regarding the agonal WIT poses logistical challenges for the donor hospital, the retrieval team as well as the recipient center and may ultimately lead to the abortion of the cDCD procedure which imposes a financial strain and human resource burden on the healthcare system and emotional distress on the donor family and the intensive care team. Thus, different scores and machine learning based models have been developed to predict agonal WIT, identify suitable donors and reduce futile procurements [26, 34–37].

Besides the use of the Wisconsin score [26], an individual evaluation of every potential cDCD donor by an experienced ICU physician of the transplant center allowed us to allocate resources to the donation process only if a high likelihood of donor suitability had been ascertained. This approach has resulted in a median agonal WIT of 12 min (range 1–112 min) and a high donor conversion rate of 94.6%. The consistently high utilization rates across all age categories (Figure 2), along with higher rates in older donors compared to previous studies [21], further underscore the nuanced donor selection process. We consider this a crucial element when establishing a DCD program. High conversion and utilization rates foster trust and confidence in the “new” process among all stakeholders. On the other hand, keeping the selection process too narrow may lead to a significant number of missed referred donors and thus potentially transplantable organs. Therefore, striking a balance between maximizing donor referral and the negative resource and emotional implications of an aborted cDCD procedure outlined above is key—especially in the early phase of implementation.

Current guidelines recommend that cardiothoracic retrieval teams wait for a minimum of two hours following WLST, while for abdominal teams a waiting time of three to four hours is recommended before standing down [38, 39]. These recommendations strike a balance of waiting long enough to not miss out on an actual donor while keeping the logistical aspects somewhat reasonable. The “stand down” process can be further adjusted for individual organs in conjunction with the recipient centers [38]. Within this context, it is important to note that a prolonged agonal WIT does not correlate with an increased fWIT and a prolonged agonal WIT alone is not an independent factor for unfavorable outcomes [40–42]. Rather than agonal or total WIT, fWIT appears to be the key determining factor for posttransplant outcomes in DCD organ transplantation [9].

Unfortunately, fWIT is not uniformly defined complicating comparisons across different countries and sometimes even across different centers within the same country [22, 23, 43]. Thus, an international consensus on fWIT would be desirable. Furthermore, acceptable fWIT times differ for different countries as well as different organs. For liver grafts most countries - except for Italy, where the no-touch period is 20 min long - define a fWIT cutoff of 30 min [29]. For lung and kidney grafts longer fWIT may be tolerated. In current practice, most lung transplant centers consider a fWIT of 60 min or more as a contraindication to lung transplantation [43]. However, fWIT cutoffs continue to be a matter of controversy, and based on ISHLT DCD registry data, the upper limit of tolerable fWIT for lung grafts may even extend 60 min [42]. The longer tolerable fWIT for lung grafts has been attributed to the local oxygen storage capacity in the alveoli as well as the relatively low metabolic demands compared to that of other organs [44]. For kidney grafts fWIT may be even up to 120 min long [45]. Besides fWIT definitions and cutoffs, graft acceptance and utilization rates also vary significantly across organs and countries. DCD utilization rates as low as 5% have been reported for lung DCD grafts in the US [46], with utilization rates approaching 20% in the current era of DCD transplantation [47].

Eden et al. have shown that for liver grafts DCD utilization rates range from 18.9% to 74.2% [29]. Utilization rates were higher at centers and in countries with active machine perfusion programs [29]. The reason for this observation appears to be twofold: (1) normothermic machine perfusion (NMP) allows for viability assessment of the graft, providing a more objective way to decide which organs qualify for transplantation; (2) hypothermic oxygenated machine perfusion (HOPE) offers the possibility for graft reconditioning, leading to more grafts fulfilling predefined viability criteria. Combining a period of graft reconditioning (during HOPE) with controlled rewarming (COR) followed by viability assessment (during NMP) combines the advantages of both technologies, facilitating increased graft utilization rates without causing increased posttransplant complication rates [48, 49]. Similar observations have been made for other organs. DCD heart transplantation was essentially non-existent until a few years ago and has only become possible through the clinical application of NMP [50, 51]. In the context of lung transplantation, *ex vivo* lung perfusion (EVLVP) has the potential to elevate the previously low utilization rates to up to 60% [52]. With the increasing use of *ex-situ* machine perfusion preservation, relying on absolute fWIT cutoffs or other donor factors for risk stratification will probably become obsolete as more objective, standardized ways to assess organs become available. This is expected to lead to an increase in DCD graft utilization.

Historically, half of DCD donors were single-organ donors. In 96% of these single-organ donors the kidneys were the only organs that were procured [38]. In our study, 41.5% of actual donors were single-organ donors, with kidney-only donors accounting for 45% of these cases. The mean number of organs transplanted per actual donor was 2.06, consistent with previously reported reference ranges (0.9–2.1) [21, 30]. While *ex-situ* machine perfusion following organ retrieval can lead to improved utilization rates, the possibility of performing normothermic regional perfusion (NRP) before the retrieval process results in both more organs being recovered per donor and more of the recovered organs being utilized for transplantation [53–55]. Since NRP has the potential to enhance

organ quality and thus not only leads to increased utilization rates but also improved posttransplant outcomes approaching those of DBD transplantation [56, 57].

Combining the benefits of NRP and *ex-situ* machine perfusion allows to further expand the safe use of extended criteria DCD donors, previously thought to be unsuitable for DCD, thus increasing the overall DCD donor pool [58].

It seems that the clinical implementation of new technologies will help tap into the underused potential of DCD transplantation and that the number of organs transplanted per DCD donor will increase and approach those of DBD donors.

In summary, we have outlined steps and protocols required to successfully implement a cDCD program. Following the implementation, an increase in the overall cDCD volume in Austria has been observed. A survey amongst European countries has shown that many countries without active DCD programs are planning to establish a cDCD program [19]. This is to be commended as one strategy to expand the donor pool. While we acknowledge that the circumstances and legal frameworks might differ from country to country, we hope that our shared experience can be a helpful resource for others who intend to implement a cDCD program. Technological advances in terms of organ recovery, organ reconditioning as well as organ preservation and viability assessment will ultimately result in an increased DCD donor pool and lead to improved utilization rates without jeopardizing outcomes, unlocking the full potential of DCD. Due to the high-quality evidence now available in favor of NRP [55, 59–61], we are currently in the process of establishing and transitioning to an NRP-DCD program.

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 Ethikkommission der Medizinischen Universität Innsbruck. 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

Designed the study: FK, BC, and SE. Collected the data: FK, BC, CS, WL, RB, and SE. Performed statistical analysis: FK, FP, and SO. Wrote and revised the manuscript: FK, SO, FP, FM, SSd, MM, AW, TR, RO, LS, JD, CS, WL, RB, BC, SSb, SE. Approved the final version of the manuscript: all authors. All authors contributed to the article and approved the submitted version.

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References

1. A definition of irreversible coma. Report of the ad hoc committee of the harvard medical school to examine the definition of brain death. *Jama* (1968) 205(6):337–40.
2. Croome KP, Taner CB. The changing landscapes in DCD liver transplantation. *Curr Transplant Rep* (2020/09/01 2020) 7(3):194–204. doi:10.1007/s40472-020-00283-1
3. Sonnenberg EM, Hsu JY, Reese PP, Goldberg DS, Abt PL. Wide variation in the percentage of donation after circulatory death donors across donor service areas: a potential target for improvement. *Transplantation* (2020) 104(8):1668–74. doi:10.1097/tp.0000000000003019
4. de Kok MJC, Schaapherder AFM, Alwayn IPJ, Bemelman FJ, van de Wetering J, van Zuilen AD, et al. Improving outcomes for donation after circulatory death kidney transplantation: science of the times. *PLoS One* (2020) 15(7):e0236662. doi:10.1371/journal.pone.0236662
5. Croome KP, Lee DD, Perry DK, Burns JM, Nguyen JH, Keaveny AP, et al. Comparison of longterm outcomes and quality of life in recipients of donation after cardiac death liver grafts with a propensity-matched cohort. *Liver Transplantation* (2017) 23(3):342–51. doi:10.1002/lt.24713
6. van Rijn R, Schurink IJ, de Vries Y, van den Berg AP, Cortes Cerisuelo M, Darwish Murad S, et al. Hypothermic machine perfusion in liver transplantation - a randomized trial. *N Engl J Med* (2021) 384(15):1391–401. doi:10.1056/NEJMoa2031532
7. Tingle SJ, Figueiredo RS, Moir JA, Goodfellow M, Talbot D, Wilson CH. Machine perfusion preservation versus static cold storage for deceased donor kidney transplantation. *Cochrane Database Syst Rev* (2019) 3(3):Cd011671. doi:10.1002/14651858.CD011671.pub2
8. Gouchie DA, Sanchez PG, D'Cunha J, Bermudez C. A, Daneshmand M. A., Davis R. D, et al. *Ex Vivo* lung perfusion in donation after circulatory death: a post-hoc analysis of the NOVEL trial. *J Thorac Cardiovasc Surg* (2024). 168 (3), 724–734. doi:10.1016/j.jtcvs.2024.03.011
9. Schlegel A, Kalisvaart M, Scalera I, Laing RW, Mergental H, Mirza DF, et al. The UK DCD risk score: a new proposal to define futility in donation-after-circulatory-death liver transplantation. *J Hepatol* (2018) 68(3):456–64. doi:10.1016/j.jhep.2017.10.034
10. Wallace D, Cowling TE, Suddle A, Gimson A, Rowe I, Callaghan C, et al. National time trends in mortality and graft survival following liver transplantation from circulatory death or brainstem death donors. *The Br Journal Surgery* (2021) 109(1): 79–88. doi:10.1093/bjs/zna347
11. Rijkse E, Ceuppens S, Qi H, Jnm IJ, Hesselink DA, Minnee RC. Implementation of donation after circulatory death kidney transplantation can safely enlarge the donor

pool: a systematic review and meta-analysis. *Int J Surg* (2021) 92:106021. doi:10.1016/j.ijsu.2021.106021

12. Van Raemdonck D, Keshavjee S, Levvey B, Cheriak WS, Snell G, Erasmus M, et al. Donation after circulatory death in lung transplantation-five-year follow-up from ISHLT registry. *J Heart Lung Transpl* (2019) 38(12):1235–45. doi:10.1016/j.healun.2019.09.007

13. Shahrestani S, Webster AC, Lam VW, Yuen L, Ryan B, Pleass HCC, et al. Outcomes from pancreatic transplantation in donation after cardiac death: a systematic review and meta-analysis. *Transplantation* (2017) 101(1):122–30. doi:10.1097/tp.0000000000001084

14. Messer S, Rushton S, Simmonds L, Macklam D, Husain M, Jothidasan A, et al. A national pilot of donation after circulatory death (DCD) heart transplantation within the United Kingdom. *J Heart Lung Transpl* (2023) 42(8):1120–30. doi:10.1016/j.healun.2023.03.006

15. Miyake K, Chau LC, Trudeau S, Kitajima T, Wickramaratne N, Shimada S, et al. Improved waitlist outcomes in liver transplant patients with Mid-MELD-Na scores listed in centers receptive to use of organs donated after circulatory death. *Transplantation* (2024) 108:1943–53. doi:10.1097/tp.0000000000004955

16. Ishaque T, Eagleson MA, Bowring MG, Motter JD, Yu S, Luo X, et al. Transplant candidate outcomes after declining a DCD liver in the United States. *Transplantation* (2023) 107(12):e339–e347. doi:10.1097/tp.0000000000004777

17. Ahmed HF, Kulshrestha K, Kennedy JT, Gomez-Guzman A, Greenberg JW, Hossain MM, et al. Donation after circulatory death significantly reduces waitlist times while not changing post-heart transplant outcomes: a united network for organ sharing analysis. *J Heart Lung Transpl* (2024) 43(3):461–70. doi:10.1016/j.healun.2023.10.013

18. Mirshekar-Syahkal B, Summers D, Bradbury LL, Aly M, Bardsley V, Berry M, et al. Local expansion of donation after circulatory death kidney transplant activity improves waitlisted outcomes and addresses inequities of access to transplantation. *Am Journal Transplantation* (2017) 17(2):390–400. doi:10.1111/ajt.13968

19. Lomero M, Gardiner D, Coll E, Haase-Kromwijk B, Procaccio F, Immer F, et al. Donation after circulatory death today: an updated overview of the European landscape. *Transpl Int* (2020) 33(1):76–88. doi:10.1111/tri.13506

20. Thuong M, Ruiz A, Evrard P, Kuiper M, Boffa C, Akhtar MZ, et al. New classification of donation after circulatory death donors definitions and terminology. *Transpl Int* (2016) 29(7):749–59. doi:10.1111/tri.12776

21. Leiden H, Haase-Kromwijk B, Hoitsma A, Jansen N. Controlled donation after circulatory death in the Netherlands: more organs, more efforts. *Neth J Med* (2016) 74(7):285–91.

22. Kubal C, Roll GR, Ekser B, Muiesan P. Donation after circulatory death liver transplantation: what are the limits for an acceptable DCD graft? *Int J Surg* (2020) 82s: 36–43. doi:10.1016/j.ijsu.2020.04.064

23. Kalisvaart M, Croome KP, Hernandez-Alejandro R, Pirenne J, Cortés-Cerisuelo M, Miñambres E, et al. Donor warm ischemia time in DCD liver transplantation-working group report from the ILTS DCD, liver preservation, and machine perfusion consensus conference. *Transplantation* (2021) 105(6):1156–64. doi:10.1097/tp.0000000000003819

24. Georgiou A, Tan W, Ionescu MI, Kuhn IL, Fritz Z. Ethical issues in uncontrolled donation after circulatory determination of death: a scoping review to reveal areas of broad consensus, and those for future research. *Transpl Int* (2025) 38:13992. doi:10.3389/ti.2025.13992

25. Casavilla A, Ramirez C, Shapiro R, Nghiem D, Miracle K, Bronsther O, et al. Experience with liver and kidney allografts from non-heart-beating donors. *Transplantation* (1995) 59(2):197–203. doi:10.1097/00007890-199501000-00008

26. Lewis J, Peltier J, Nelson H, Snyder W, Schneider K, Steinberger D, et al. Development of the university of Wisconsin donation after cardiac death evaluation tool. *Prog Transpl* (2003) 13(4):265–73. doi:10.1177/152692480301300405

27. Rao V, Dhanani S, MacLean J, Payne C, Paltser E, Humar A, et al. Effect of organ donation after circulatory determination of death on number of organ transplants from donors with neurologic determination of death. *Cmaj* (2017) 189(38):E1206–e1211. doi:10.1503/cmaj.161043

28. Le Dinh H, Monard J, Delbouille MH, Hans MF, Weekers L, Bonvoisin C, et al. A more than 20% increase in deceased-donor organ procurement and transplantation activity after the use of donation after circulatory death. *Transpl Proc* (2014) 46(1):9–13. doi:10.1016/j.transproceed.2013.10.012

29. Eden J, Sousa Da Silva R, Cortes-Cerisuelo M, Croome K, De Carlis R, Hessheimer AJ, et al. Utilization of livers donated after circulatory death for transplantation - an international comparison. *J Hepatol* (2023) 78(5):1007–16. doi:10.1016/j.jhep.2023.01.025

30. Hodgson R, Young AL, Attia MA, Lodge JPA. Impact of a national controlled donation after circulatory death (DCD) program on organ donation in the United Kingdom: a 10-Year study. *Am Journal Transplantation* (2017) 17(12): 3172–82. doi:10.1111/ajt.14374

31. Transplant Ö. Transplant-jahresbericht (2024). Available online at: <https://transplant.goeg.at/Jahresbericht2024> (Accessed March 26, 2026).

32. Martin F, Carmona M, Mahillo B, Alvarez M, Luengo A, Chatzixiros E, et al. Organ donation and transplantation worldwide: the global observatory on donation and

transplantation 2024 report. *Transplantation* (2026) 110(3):e655–e669. doi:10.1097/tp.0000000000000567

33. Becker F, Roberts KJ, Nadal M, Zink M, Stiegler P, Pemberger S, et al. Optimizing organ donation: expert opinion from Austria, Germany, Spain and the U.K. *Ann Transplant* (2020) 25:e921727. doi:10.12659/aot.921727

34. Davila D, Ciria R, Jassem W, Briceño J, Littlejohn W, Vilca-Meléndez H, et al. Prediction models of donor arrest and graft utilization in liver transplantation from maastricht-3 donors after circulatory death. *Am Journal Transplantation* (2012) 12(12): 3414–24. doi:10.1111/j.1600-6143.2012.04242.x

35. Brieva J, Coleman N, Lacey J, Harrigan P, Lewin TJ, Carter GL. Prediction of death in less than 60 minutes after withdrawal of cardiorespiratory support in potential organ donors after circulatory death. *Transplantation* (2014) 98(10):1112–8. doi:10.1097/tp.0000000000000186

36. Scales NB, Herry CL, van Beinun A, Hogue ML, Hornby L, Shahin J, et al. Predicting time to death after withdrawal of life-sustaining measures using vital sign variability: derivation and validation. *Crit Care Explor* (2022) 4(4):e0675. doi:10.1097/cce.0000000000000675

37. Yanagawa R, Iwadoh K, Nakayama T, Firl DJ, Wehrle CJ, Bekki Y, et al. Development and validation of a machine-learning model to reduce futile procurements in donations after circulatory death in liver transplantation in the USA: a multicentre study. *Lancet Digit Health* (2025) 7(10):100918. doi:10.1016/j.landig.2025.100918

38. British Transplant Society. UK guidelines on transplantation from deceased donors after circulatory death. Available online at: <https://bts.org.uk/transplantation-from-deceased-donors-after-circulatory-death> (Accessed April 05, 2024).

39. van Heurn LW, Talbot D, Nicholson ML, Akhtar MZ, Sanchez-Fructuoso AI, Weekers L, et al. Recommendations for donation after circulatory death kidney transplantation in Europe. *Transpl Int* (2016) 29(7):780–9. doi:10.1111/tri.12682

40. Law J, Hornby K, Payne C, Sener A, Luke PP. Missed opportunities for DCD kidney donors: evaluation of warm ischemic time and associated functional warm ischemic time. *Clin Transpl* (2019) 33(11):e13724. doi:10.1111/ctr.13724

41. Malik AK, Tingle SJ, Chung N, Owen R, Mahendran B, Counter C, et al. The impact of time to death in donors after circulatory death on recipient outcome in simultaneous pancreas-kidney transplantation. *Am Journal Transplantation* (2024) 24:1247–56. doi:10.1016/j.ajt.2024.02.008

42. Levvey B, Keshavjee S, Cypel M, Robinson A, Erasmus M, Glanville A, et al. Influence of lung donor agonal and warm ischemic times on early mortality: analyses from the ISHLT DCD lung transplant registry. *J Heart Lung Transpl* (2019) 38(1):26–34. doi:10.1016/j.healun.2018.08.006

43. Mora V, Ballesteros MA, Naranjo S, Sánchez L, Suberviola B, Iturbe D, et al. Lung transplantation from controlled donation after circulatory death using simultaneous abdominal normothermic regional perfusion: a single center experience. *Am Journal Transplantation* (2022) 22(7):1852–60. doi:10.1111/ajt.17057

44. Ceulemans LJ, Inci I, Van Raemdonck D. Lung donation after circulatory death. *Curr Opin Organ Transpl* (2019) 24(3):288–96. doi:10.1097/mot.0000000000000627

45. Manara AR, Murphy PG, O'Callaghan G. Donation after circulatory death. *Br J Anaesth* (2012) 108(Suppl. 1):i108–21. doi:10.1093/bja/aer357

46. Bobba CM, Whitson BA, Henn MC, Mokadam NA, Keller BC, Rosenheck J, et al. Trends in donation after circulatory death in lung transplantation in the United States: impact of era. *Transpl Int* (2022) 35:10172. doi:10.3389/ti.2022.10172

47. Alderete IS, Pontula A, Halpern SE, Patel KJ, Klapper JA, Hartwig MG. Thoracoabdominal normothermic regional perfusion and donation after circulatory death lung use. *JAMA Netw Open* (2025) 8(2):e2460033. doi:10.1001/jamanetworkopen.2024.60033

48. van Leeuwen OB, Bodewes SB, Lantinga VA, Haring MPD, Thorne AM, Brüggewirth IMA, et al. Sequential hypothermic and normothermic machine perfusion enables safe transplantation of high-risk donor livers. *Am Journal Transplantation* (2022) 22:1658–70. doi:10.1111/ajt.17022

49. van Leeuwen OB, de Vries Y, Fujiyoshi M, Nijsten MWN, Ubbink R, Pelgrim GJ, et al. Transplantation of high-risk donor livers after *Ex Situ* resuscitation and assessment using combined Hypo- and normothermic machine perfusion: a prospective clinical trial. *Ann Surg* (2019) 270(5):906–14. doi:10.1097/sla.0000000000003540

50. Feizpour CA, Gauntt K, Patel MS, Carrico B, Vagefi PA, Klassen D, et al. The impact of machine perfusion of the heart on warm ischemia time and organ yield in donation after circulatory death. *Am Journal Transplantation* (2022) 22(5):1451–8. doi:10.1111/ajt.16952

51. Dhital KK, Iyer A, Connellan M, Chew HC, Gao L, Doyle A, et al. Adult heart transplantation with distant procurement and *ex-vivo* preservation of donor hearts after circulatory death: a case series. *Lancet* (2015) 385(9987):2585–91. doi:10.1016/s0140-6736(15)60038-1

52. Cypel M, Yeung JC, Donahoe L, Chen M, Zamel R, Hoetzenecker K, et al. Normothermic *ex vivo* lung perfusion: does the indication impact organ utilization and patient outcomes after transplantation? *J Thorac Cardiovasc Surg* (2020) 159(1): 346–55.e1. doi:10.1016/j.jtcvs.2019.06.123

53. Bakhtiyar SS, Maksimuk TE, Gutowski J, Park SY, Cain MT, Rove JY, et al. Association of procurement technique with organ yield and cost following donation after circulatory death. *Am Journal Transplantation* (2024) 24:1803–15. doi:10.1016/j.ajt.2024.03.027
54. Bekki Y, Croome KP, Myers B, Sasaki K, Tomiyama K. Normothermic regional perfusion can improve both utilization and outcomes in DCD liver, kidney, and pancreas transplantation. *Transpl Direct* (2023) 9(3):e1450. doi:10.1097/txd.0000000000001450
55. Oniscu GC, Mehew J, Butler AJ, Sutherland A, Gaurav R, Hogg R, et al. Improved organ utilization and better transplant outcomes with *in situ* normothermic regional perfusion in controlled donation after circulatory death. *Transplantation* (2023) 107(2):438–48. doi:10.1097/tp.0000000000004280
56. Mastrovangelis C, Frost C, Hort A, Laurence J, Pang T, Pleass H. Normothermic regional perfusion in controlled donation after circulatory death liver transplantation: a systematic review and meta-analysis. *Transpl Int* (2024) 37:13263. doi:10.3389/ti.2024.13263
57. Moreno P, González-García J, Ruiz-López E, Alvarez A. Lung transplantation in controlled donation after circulatory-determination-of-death using normothermic abdominal perfusion. *Transpl Int* (2024) 37:12659. doi:10.3389/ti.2024.12659
58. Torri F, Balzano E, Melandro F, Maremmanni P, Bertini P, Lo Pane P, et al. Sequential normothermic regional perfusion and end-ischemic *Ex Situ* machine perfusion allow the safe use of very old DCD donors in liver transplantation. *Transplantation* (2024) 108:1394–402. doi:10.1097/tp.0000000000004963
59. Gaurav R, Butler AJ, Kosmoliaptsis V, Mumford L, Fear C, Swift L, et al. Liver transplantation outcomes from controlled circulatory death donors: SCS vs *in situ* NRP vs *ex situ* NMP. *Ann Surg* (2022) 275(6):1156–64. doi:10.1097/sla.0000000000005428
60. Hessheimer AJ, Coll E, Torres F, Ruiz P, Gastaca M, Rivas JI, et al. Normothermic regional perfusion vs. super-rapid recovery in controlled donation after circulatory death liver transplantation. *J Hepatol* (2019) 70(4):658–65. doi:10.1016/j.jhep.2018.12.013
61. Sellers MT, Grandas J, Warhooover MT, Poland JD, Clapper DC. Normothermic regional perfusion performed by a United States organ procurement organization for nonthoracic organ donors. *Am Journal Transplantation* (2025) 25:1677–84. doi:10.1016/j.ajt.2025.04.005



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Serum α -Klotho and SIRT1 - Relationship with graft function, inflammation and hospitalization rates in kidney transplant recipients

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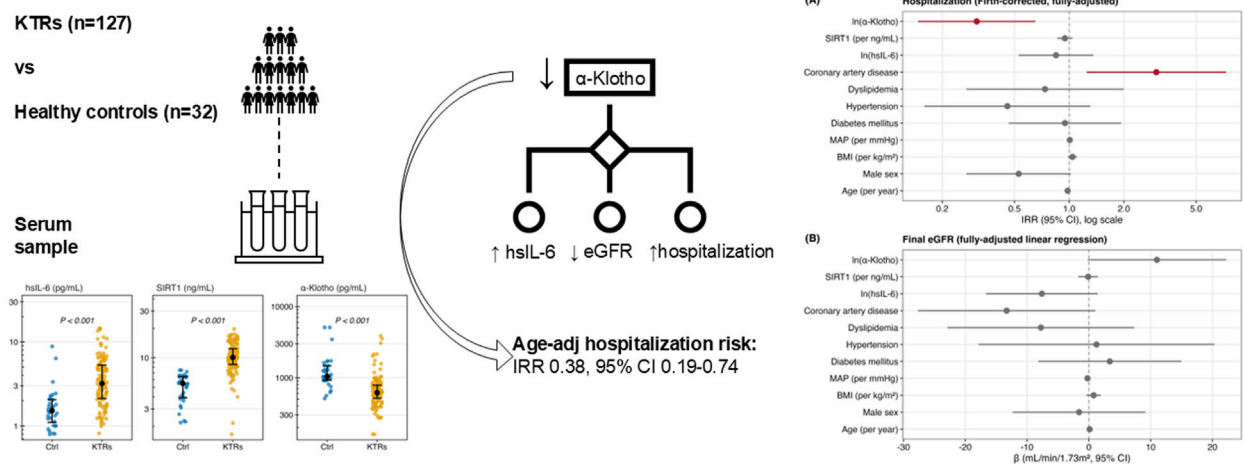
Emerging evidence indicates kidney transplant recipients (KTRs) suffer from accelerated cellular aging, low-grade inflammation and variable degrees of allograft dysfunction. These pathobiological processes shape chronic kidney disease in the graft, for which α -Klotho is being explored as a candidate peptide marker for risk stratification. In this observational cohort study, we recruited 127 KTRs in outpatient care at the University Hospital in Kraków, Poland between September 2016 and June 2019. Serum α -Klotho, sirtuin-1 (SIRT1), and high-sensitivity interleukin-6 (hsIL-6) were assayed using ELISA kits in KTRs and 32 healthy controls (HCs). Thereafter, we utilized crude, age-adjusted, and fully adjusted Firth Poisson regression models with robust standard errors to evaluate predictors of all-cause hospitalization. We observed that circulating α -Klotho was reduced in KTRs, as compared with HCs (median 616 vs. 1,042 pg/mL, $P < 0.001$). Further, α -Klotho was moderately associated with eGFR at baseline ($\rho = 0.30$) and final follow-up ($\rho = 0.29$). In contrast, α -Klotho was inversely correlated with hsIL-6 ($\rho = -0.39$, 95% CI -0.60 – -0.15 , $P = 0.002$). In multivariable linear regression models, $\ln(\alpha\text{-Klotho})$ changes were tied to higher final eGFR (14.8 95% CI 6.2–25.6 mL/min/1.73 m²). During follow-up of 274 person-years, we recorded 153 hospitalizations. In multivariable models, higher $\ln(\alpha\text{-Klotho})$ was independently associated with lower hospitalization rate (IRR 0.31, 95% CI 0.15–0.65, $P = 0.002$). This association persisted after adjustment for baseline eGFR (IRR 0.37, 95% CI 0.20–0.69, $P = 0.002$). Overall, given further validation and standardization of assay technology, serum α -Klotho may be a strong candidate for supplemental, outpatient risk stratification in KTRs.

KEYWORDS

biomarker, hospitalization, inflammation, kidney, Klotho

Introduction

Patients with chronic kidney disease (CKD) and kidney transplant recipients (KTRs) experience enhanced biological aging, variable renal dysfunction and low-grade inflammation, which are drivers of cardiovascular (CV), infectious and neoplastic morbidity [1, 2]. Candidate prognostic markers, such as α -Klotho and sirtuin-1 (SIRT1),

Serum α -Klotho and SIRT1 - Relationship with Graft Function, Inflammation and Hospitalization Rates in Kidney Transplant Recipients

GRAPHICAL ABSTRACT

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are increasingly studied as signature molecules of cytoprotective, anti-inflammatory and renoprotective pathways [2, 3]. The need for improved post kidney transplant (KTx) management, both from a perspective of graft dysfunction and comorbid disorder risk, has prompted research into risk stratification tools [4–7]. The post-KTx setting is unique in that chronic immunosuppression carries the risk of cardiometabolic side-effects that must be balanced against the risk of allograft dysfunction [8].

α -Klotho has been identified both as a transmembrane co-receptor for fibroblast growth factor-23 (FGF-23) and as a soluble circulating form [9, 10]. FGF-23 is known to regulate phosphate and vitamin D metabolism [11, 12], though Klotho also exerts anti-inflammatory and anti-fibrotic effects [13]. The kidney is considered a major source of α -Klotho [14–16], which strengthens its potential utility as a circulating marker. In murine models, lower α -Klotho is tied to premature aging, vascular disease, skin atrophy, and mineral-bone dysregulation [14]. In turn, its overexpression is tied to enhanced longevity, which may be mediated through effects on insulin and insulin-like growth factor-1 signalling pathways [17].

In humans, lower α -Klotho is tied to CV disease and mortality [18]. Population studies report inverse associations with body-mass measures and non-linear relationships with lipid abnormalities [18, 19]. Lower α -Klotho is also linked to metabolic syndrome, with apparent sex-specific patterns [20, 21]. Systemic inflammation is also reported to affect Klotho signalling [22, 23], while in kidney disease α -Klotho may exert vaso- and renoprotective effects [16, 24].

SIRT1 is an NAD⁺-dependent histone deacetylase that acts as an intracellular energy regulator; through deacetylation of key mediators (e.g., FOXO, NF- κ B, p53) it can modulate pathways tied to oxidative stress, inflammation, DNA repair, apoptosis, autophagy and mitochondrial function [25, 26].

Metabolic and inflammatory processes are implicated in development of post-KTx complications, while underlying pathobiological processes are also affected by maintenance immunosuppressive treatment in KTRs [2, 27, 28]. The accelerated aging and chronic inflammation described in the setting of CKD implicate potential roles for both α -Klotho and SIRT1 in disease progression [29, 30]. However, the relationships between α -Klotho, SIRT1, and inflammatory markers in KTRs are not well understood. This study investigated serum concentrations of α -Klotho, SIRT1, and high-sensitivity interleukin-6 (hsIL-6) in KTRs and their associations with hospitalization outcomes and clinical characteristics.

Materials and methods

Study design

This was an observational cohort study that enrolled 127 KTRs under routine ambulatory care at the Nephrology and Transplantology Department of the University Hospital in Kraków, Poland, between 1 September 2016 and 30 June 2019.

Abbreviations: BMI, body mass index; CAD, coronary artery disease; CI, confidence interval; CKD, chronic kidney disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CV, coefficient of variation; eGFR, estimated glomerular filtration rate; ELISA, enzyme-linked immunosorbent assay; FGF-23, fibroblast growth factor 23; GCP, Good Clinical Practice; HbA1c, glycated hemoglobin; HC, healthy control; hslIL-6, high-sensitivity interleukin-6; IQR, interquartile range; IRR, incidence rate ratio; KDIGO, Kidney Disease: Improving Global Outcomes; KTR, kidney transplant recipient; KTx, kidney transplantation; In, natural logarithm; MAP, mean arterial pressure; mTOR, mechanistic target of rapamycin; NAD⁺, nicotinamide adenine dinucleotide; NF- κ B, nuclear factor kappa B; PI3K, phosphoinositide 3-kinase; ROS, reactive oxygen species; SIRT1, sirtuin-1; TGF- β , transforming growth factor beta; TLR4, Toll-like receptor 4; TNF- α , tumor necrosis factor alpha; WHR, waist-to-hip ratio.

The median (IQR) follow-up duration was 29 [25–32] months. All patients provided written informed consent prior to study participation. This study was conducted in accordance with Declaration of Helsinki and ICH/GCP guidelines and represents an extension of our prior work and institutional research into circulating biomarkers in KTRs. Approval from the local Bioethics Committee was granted in extension of the original biomarker assessment study and updated for patient follow-up (Bioethics Committee Decision No. 1072.6120.202.2022).

Data collection

Demographic and clinical data were collected at the time of patient enrolment and over follow-up based on manual chart review. Longitudinal assessments were obtained through screening of electronic and paper-based medical records from consecutive ambulatory clinic visits by physicians, at pre-defined 3-to-6-month intervals. The main outcomes assessed over follow-up included: (i) hospitalization at the University Hospital departments for any cause; (ii) all-cause mortality; and (iii) death-censored graft loss, defined as graft dysfunction requiring permanent return to dialysis or re-transplantation listing. Study outcome data was gathered based on direct patient, family or dialysis centre contact, when available. Individual record censoring occurred at the date of death, dialysis transfer or last ambulatory care visit assessment.

Definitions

Hypertension was defined as outpatient visit blood pressure measurement $\geq 140/90$ mmHg or recorded use of antihypertensive medications. Dyslipidaemia was defined based on existing medical record or use of lipid-lowering agents. Similarly, diabetes was identified based on pre-existing records or use of glucose-lowering therapy and/or biochemical data according to Polish Diabetes Society guidelines. Coronary artery disease (CAD) was defined broadly as a composite outcome of either history of myocardial infarction, stroke/transient ischemic attack, percutaneous coronary intervention or coronary artery bypass grafting, or existing record of chronic coronary syndrome. We did not include heart failure within this definition. Body mass index (BMI) and waist-to-hip ratio (WHR) were calculated based on standard formula. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 2021 race-free equation via *nephro* package [31]. CKD was classified according to KDIGO guidelines [32].

Biochemical assessment

We collected peripheral venous blood samples from patients presenting for first study visit following overnight fast. Serum was obtained and stored in serum separator tubes, allowed to clot at room temperature, and then centrifuged. Aliquots were frozen at -70°C and stored. Assays were conducted later, in batch and under standardized conditions. Procedures were performed by experienced laboratory staff blinded to patient outcomes.

Concentrations of study markers were measured using commercially available enzyme-linked immunosorbent assay

(ELISA) kits, in accordance with manufacturer instructions. For α -Klotho (Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan; Catalog No. 27998) sensitivity was 6.15 pg/mL; measurement range 93.75–6,000 pg/mL; intra-assay coefficient of variation (CV) $< 3.5\%$ and inter-assay CV was $< 11.4\%$. For sirtuin-1 (Wuhan EIAab Science Co., Ltd., Wuhan, China; Catalog No. E3949h) sensitivity < 32 pg/mL; detection range 78–5,000 pg/mL; intra-assay CV was $< 4.3\%$ and inter-assay CV was $< 7.2\%$. For high-sensitivity Interleukin-6 (hsIL-6; Quantikine HS ELISA Human IL-6 Immunoassay; R&D Systems, Inc., Minneapolis, MN, USA; Catalog No. HS600B) the mean minimum detectable dose was 0.039 pg/mL; reference range: 0.447–9.96 pg/mL; intra-assay CV was $< 7.8\%$ and inter-assay CV was $< 9.6\%$.

Routine biochemical tests, including serum creatinine, were measured using standardized methods on automated analysers, as per standard laboratory protocol (Hitachi 917, Hitachi, Tokyo, Japan; Modular P, Roche Diagnostics, Mannheim, Germany).

Statistical analysis

Analyses were performed in R 4.5.3 (R Core Team, 2026; R Foundation for Statistical Computing, Vienna, Austria). Continuous and categorical variables were summarized as median with interquartile range (IQR) and counts with proportion (N, %), respectively. Variable distributions were assessed with visual inspection of density plots and Shapiro-Wilk test. For between-group comparisons we utilized exact Wilcoxon rank-sum tests for continuous variables, whereas Fisher's test was employed for categorical variable comparison. To assess relationships from a robust perspective, we utilized Spearman's rank correlation coefficient (ρ), with 95% confidence intervals (CI) derived using percentile bootstrap over 2000 replicates.

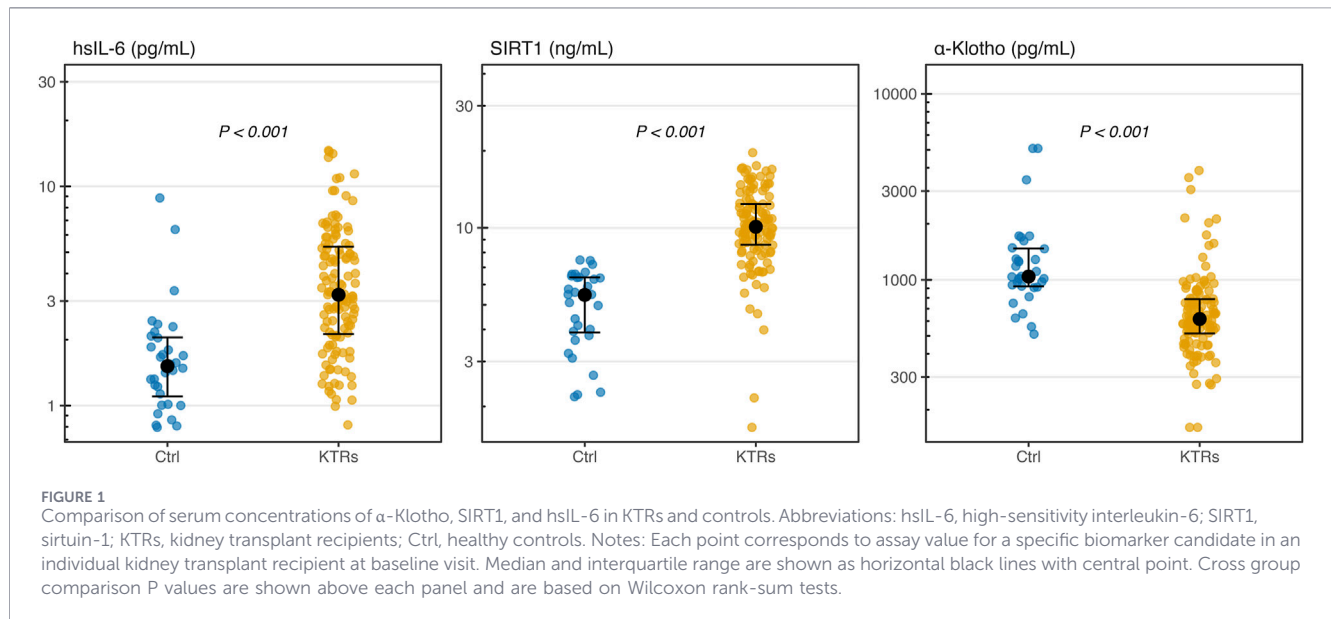
The relationship between $\ln(\alpha\text{-Klotho})$ and final eGFR was modelled using hierarchical linear regression approach. CIs for coefficients were derived using bootstrap methods. We performed influence diagnostics and Cohen's f^2 was utilized to quantify effect size. For additional sensitivity, we confirmed linearity of relationships based on alternative restricted cubic spline (3 knot; $P = 0.07$ for non-linearity).

Due to overdispersion of hospitalization counts, we considered different modelling approaches. Overall, we utilized quasi-Poisson regression, with log-transformed follow-up time as offset variable. To assess whether $\ln(\alpha\text{-Klotho})$ was independently associated with outcome after adjustment for all candidate predictors, we fitted a fully-adjusted bias-reduced Poisson model via *brglm2* package. Additionally, non-parametric bootstrap and a negative binomial sensitivity model were considered. The Firth model was refitted with baseline CKD-EPI eGFR added as a covariate to further test if serum $\alpha\text{-Klotho}$ provides additional information over eGFR. Multicollinearity was assessed with variance inflation factors. Statistical tests were two-tailed and we treated $P < 0.05$ as statistically significant.

Results

Baseline characteristics of the study cohort

This study included 127 KTRs who were followed for a median (IQR) time of 29 [25–32] months. Most patients were of male gender



($N = 86$, 67.7%) and middle-aged, with median (IQR) age of 54 [33–52] years. We compared serum α -Klotho concentrations between KTRs and 32 healthy controls (16 male [50.0%]; mean age 50 years, age range 29–74 years). We observed lower circulating α -Klotho levels in serum of KTRs, with median (IQR) values of 616 (514–788) pg/mL versus 1,042 (922–1,472) pg/mL ($P < 0.001$), which correspond to an estimated difference of 463 (95% CI 346–605) pg/mL (see Figure 1).

For descriptive comparison of clinical characteristics, we stratified patients using a 75th-percentile cut-off (788 pg/mL) into “low” ($n = 95$) and “high” ($n = 32$) α -Klotho groups (see Table 1). However, for inferential analyses, serum α -Klotho was modelled as a continuous variable with natural log transformation due to distributional considerations. Both “low” and “high” α -Klotho groups were comparable in age, sex, body mass indices, metabolic parameters, smoking status, and baseline eGFR. Interestingly, hypertension was more frequently recorded in the low α -Klotho group ($P = 0.016$). The high α -Klotho group also showed lower log-transformed hsIL-6 levels, with median (IQR) values of 0.81 (0.37–1.20) versus 1.31 (0.94–1.73) ($P < 0.001$). Respectively, higher SIRT1 levels, with median (IQR) values of 11.46 (9.78–14.48) versus 9.82 (8.11–11.52) ng/mL were recorded in the “high” α -Klotho group, as compared to “low” α -Klotho group ($P = 0.010$). Of note, this difference in SIRT1 concentrations was not robust to the cut-off, as detailed in Section *Relationships between clinical characteristics and α -Klotho and SIRT1 concentrations in serum of KTRs*.

Relationships between clinical characteristics and α -Klotho and SIRT1 concentrations in serum of KTRs

For monotonic relationship analyses, we followed Cohen’s approach to interpretation of Spearman’s correlation strength ($\rho = 0.10$, weak; $\rho = 0.30$, moderate; $\rho = 0.50$, strong). We observed that serum α -Klotho was consistently associated with allograft function across all timepoints: baseline eGFR ($\rho =$

0.304), follow-up T1 ($\rho = 0.315$), and follow-up T2 ($\rho = 0.29$). Circulating α -Klotho in serum was inversely correlated with hsIL-6 levels ($\rho = -0.39$, 95% CI -0.60–0.15, $P = 0.002$). Importantly, this association was also consistent in alternative analysis with α -Klotho categorization approaches ($P = 0.001$ for the 75th-percentile cut-off, $P = 0.026$ for the median cut-off).

By contrast, the α -Klotho-SIRT1 relationship was weaker and cut-off dependent. In continuous analysis, Spearman ρ was estimated at 0.21 (95% CI -0.04–0.44, $P = 0.098$). Group differences are reported descriptively in Table 1 ($P = 0.010$ for the 75th-percentile cut-off), though were not preserved using a median split ($P = 0.31$). The SIRT1 finding should be interpreted with caution and requires validation. Other monotonic relationships between α -Klotho and clinical features were weak: HbA1c ($\rho = -0.140$), BMI ($\rho = -0.087$), mean arterial pressure ($\rho = 0.099$), waist-to-hip ratio ($\rho = 0.020$), and age ($\rho = 0.006$).

Relationship between changes in kidney allograft function over follow-up

At baseline, median (IQR) eGFR was 57.4 (41.7–72.9) mL/min/1.73 m², with a median change of 0 (–10.4–7.1) mL/min/1.73 m² over follow-up. Most KTRs ($N = 103$, 80%) were characterized with stable graft function, which we defined as absolute change within 15 mL/min/1.73 m². At the last available follow-up visit, KDIGO stage distribution was as follows: G1 (11.8%), G2 (35.4%), G3a (20.5%), G3b (12.6%), G4 (11.8%), and G5 (7.9%).

We observed that when patients were stratified by serum concentrations of α -Klotho categorized into tertiles, they differed by eGFR ($P = 0.010$; Figure 2A). Specifically, patients in the lowest tertile had a median (IQR) final eGFR of 44.2 (25.6–65.9) mL/min/1.73 m², which is markedly lower than the second tertile (63.9 (52.7–76.5); $P = 0.048$) and the third tertile (61.4 (49.6–89.0); $P = 0.024$). Patients with eGFR decline also showed numerically lower median ln (α -Klotho) ($P = 0.14$; Figure 2B). Further, the continuous monotonic relationship between ln (α -Klotho) and final eGFR was formally significant ($\rho = 0.28$, $P = 0.002$; Figure 2C), and the

TABLE 1 Demographic and clinical characteristics of kidney transplant recipients stratified by serum α -Klotho levels.

Characteristic	High α -Klotho (n = 32)	Low α -Klotho (n = 95)	P Value
Age, years	53.0 (40.0–59.3)	56.0 (42.0–61.5)	0.394
Sex, male, N (%)	23 (71.9)	63 (66.3)	0.664
BMI (kg/m ²)	24.27 (23.55–27.22)	26.30 (23.05–29.51)	0.176
Waist-to-hip ratio	0.93 (0.87–0.99)	0.94 (0.86–1.00)	0.780
Mean arterial pressure, mmHg	103.17 (91.83–110.17)	98.67 (93.00–108.00)	0.311
Smoking status, N (%)			0.360
Never	25 (78.1)	71 (76.3)	
Former	3 (9.4)	16 (17.2)	
Current	4 (12.5)	6 (6.5)	
Diabetes mellitus, N (%)	11 (34.4)	23 (24.2)	0.356
Hypertension, N (%)	26 (81.2)	91 (95.8)	0.016
Dyslipidaemia, N (%)	5 (15.6)	13 (13.7)	0.774
Coronary artery disease, N (%)	4 (12.5)	21 (22.1)	0.309
HbA1c, %	5.90 (5.40–6.05)	5.75 (5.47–6.30)	0.778
Fasting glucose, mmol/L	5.60 (5.05–6.00)	5.38 (4.94–5.82)	0.286
Serum creatinine, baseline, μ mol/L	108.0 (89.8–141.0)	130.0 (95.0–169.5)	0.070
Follow-up, months	29.43 (27.31–31.75)	28.50 (24.42–31.83)	0.388
Metabolic syndrome, N (%)	9 (32.1)	21 (26.2)	0.626
SIRT1, ng/mL	11.46 (9.78–14.48)	9.82 (8.11–11.52)	0.010
ln (hsIL-6)	0.81 (0.37–1.20)	1.31 (0.94–1.73)	<0.001
ln (α -Klotho)	6.89 (6.78–7.34)	6.34 (6.10–6.47)	<0.001

Abbreviations: BMI, body mass index; HbA1c, glycated hemoglobin; hsIL-6, high-sensitivity interleukin-6; SIRT1, sirtuin-1.

Data are shown as median (interquartile range) unless otherwise stated. Stratification follows a 75th-percentile cut-off at 788 pg/mL. This cut-off is descriptive only; all inferential analyses utilize serum α -Klotho levels as a continuous variable.

Between-group comparison is based on exact Wilcoxon rank-sum tests and Fisher's exact test for continuous and categorical variables, respectively. For body-mass indices and mean arterial hypertension data were available for n = 123, for HbA1c% n = 71 patients and for fasting glucose n = 110, respectively.

distribution of α -Klotho tertiles across KDIGO stages also supported a significant trend association ($P = 0.003$; Figure 2D).

We then constructed iterative linear regression models with progressive covariate adjustment (see Table 2). In the unadjusted model, each unit increase in ln (α -Klotho) was associated with 15.4 mL/min/1.73 m² higher final eGFR (95% CI 6.7–25.4). The association was preserved after demographic adjustment (Beta 15.3, 95% CI 7.3–25.8) and after additional adjustment for BMI and diabetes status (Beta 14.8, 95% CI 6.2–25.6). However, the effect size was modest ($P^2 = 0.083$). In additional sensitivity analysis after excluding high-influence observations, the relationship was consistently observed (Beta 15.4, 95% CI 3.1–27.7, $P = 0.015$). We also confirmed the plausible linearity of the ln (α -Klotho) effect using comparison with a three-knot restricted cubic spline model ($P = 0.07$ for non-linearity).

Relationship between α -Klotho, SIRT1 and all-cause hospitalization

Over 274 person-years of follow-up, we recorded 153 inpatient admissions that occurred among 54 KTRs (42.5%); 73 (57.5%) were

not hospitalized during follow-up. Of note, we recorded hospitalizations that occurred within the setting of the University Hospital. Recurrent admissions ranged from 1 to 12 per patient, with a median (IQR) of 1 [1, 2] admissions. In age-adjusted quasi-Poisson regression (see Table 3), higher ln (α -Klotho) was significantly associated with lower hospitalization rate (IRR 0.38, 95% CI 0.19–0.74, $P = 0.006$). Coronary artery disease was also associated with higher rate (IRR 2.51, 95% CI 0.98–6.14, $P = 0.049$), while sex, diabetes, hypertension, dyslipidaemia, mean arterial pressure, BMI, ln (hsIL-6), and SIRT1 were not significant predictors.

To evaluate whether ln (α -Klotho) was independently associated with readmission after adjustment for all candidate predictors, we fitted a fully-adjusted Firth-corrected Poisson model containing age, sex, BMI, mean arterial pressure, diabetes mellitus, hypertension, dyslipidaemia, coronary artery disease, ln (hsIL-6), SIRT1, and ln (α -Klotho), with robust standard errors and log (follow-up time) as offset (Supplementary Table S1; Figure 3A). Although exploratory, ln (α -Klotho) was observed with a maintained, strong inverse relationship with hospitalization rate (IRR 0.31, 95% CI 0.15–0.65, $P = 0.002$). Similar observations were noted for

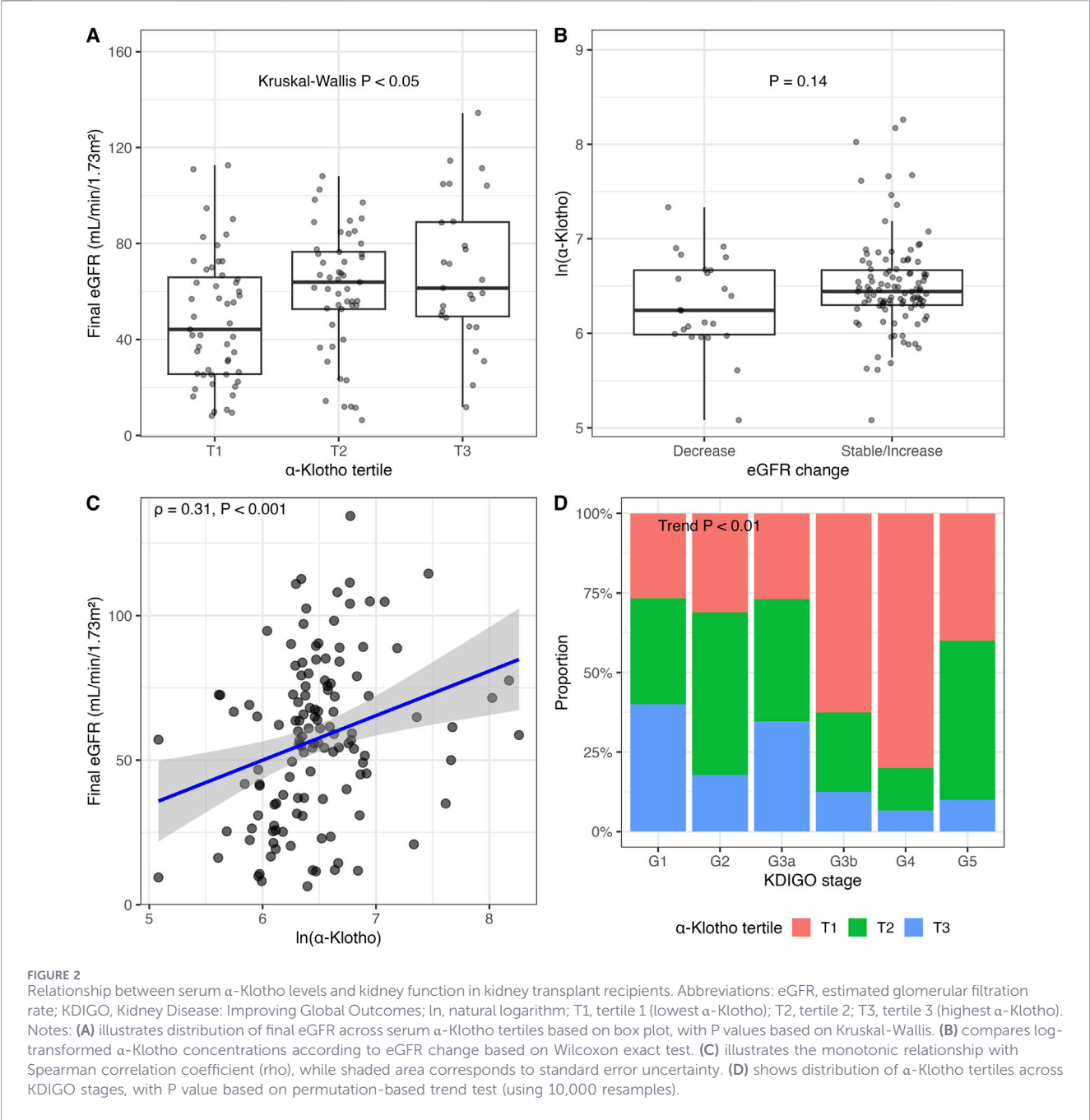


TABLE 2 Relationship between circulating α -Klotho and last available eGFR over follow-up.

Model	Beta (95% CI)	Adj. R ²
Baseline (unadjusted)	15.41 (6.73–25.44)	0.070
Baseline + age + sex	15.31 (7.28–25.81)	0.057
Baseline + BMI + diabetes	14.80 (6.22–25.59)	0.038

Abbreviations: Adj., adjusted; BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate. Beta coefficients should be interpreted as change in final eGFR, per 1 unit change in α -Klotho on natural log scale. 95% CIs are based on bootstrap.

coronary artery disease (IRR 3.02, 95% CI 1.25–7.30, $P = 0.014$). These findings were consistent across different, alternative model constructs, both using a non-parametric bootstrap of the same model ($R = 1,000$) with a 95% CI of 0.10–0.66, as well as a negative binomial regression model (similarly, the estimated IRR was 0.32 95% CI 0.14–0.73, $P = 0.005$). In model diagnostics, all

TABLE 3 Predictors of all-cause hospitalization in kidney transplant recipients.

Predictor	Crude IRR (95% CI)	P	Age-adj. IRR (95% CI)	Age-adj. P
Sex, male	0.71 (0.34–1.52)	0.366	0.71 (0.34–1.52)	0.361
Diabetes mellitus	1.03 (0.42–2.27)	0.937	1.05 (0.42–2.31)	0.916
Hypertension	0.93 (0.30–4.62)	0.912	0.93 (0.30–4.69)	0.919
Dyslipidaemia	1.08 (0.33–2.74)	0.887	1.08 (0.32–2.76)	0.884
Coronary artery disease	1.91 (0.84–3.94)	0.099	2.51 (0.98–6.14)	0.049
BMI, per kg/m ²	1.02 (0.94–1.10)	0.541	1.03 (0.95–1.10)	0.509
MAP, per mmHg	1.00 (0.97–1.03)	0.783	1.00 (0.98–1.03)	0.763
ln (hsIL-6)	1.39 (0.81–2.37)	0.230	1.41 (0.82–2.39)	0.205
SIRT1, per ng/mL	0.91 (0.82–1.02)	0.117	0.91 (0.82–1.02)	0.117
ln (α -Klotho)	0.37 (0.19–0.74)	0.005	0.38 (0.19–0.74)	0.006

Abbreviations: Adj., adjusted; BMI, body mass index; CI, confidence interval; hsIL-6, high-sensitivity interleukin-6; IRR, incidence rate ratio; MAP, mean arterial pressure; SIRT1, sirtuin-1. Predictors are based on a quasi-Poisson regression model with offset based on log (follow-up). Additionally, we performed a fully-adjusted Firth-corrected sensitivity analysis that is available in the [Supplementary Material \(Supplementary Table S1\)](#).

variance inflation factors were <2, which suggesting limited confounding by collinearity.

To further investigate whether α -Klotho may provide additional information over eGFR monitoring, we refitted the fully adjusted Firth model with baseline eGFR added as a covariate. Consistently, ln (α -Klotho) consistently demonstrated an independent inverse association with hospitalization rate (IRR 0.37, 95% CI 0.20–0.69, $P = 0.002$), even after adjustment for baseline eGFR. At the same time, baseline eGFR was a strong predictor of hospitalization (IRR 0.97 per mL/min/1.73 m²; $P < 0.001$).

Discussion

The salient findings of this observational study are that (i) serum α -Klotho concentrations are significantly reduced in KTRs and (ii) are independently associated with future graft function, (iii) inversely correlated with circulating inflammatory burden, and (iv) inversely associated with all-cause hospitalization, even after adjustment for baseline kidney function. Our results extend evidence from prior work and recent meta-analyses that have reported reduced serum α -Klotho levels after kidney donation, while an increase of α -Klotho was observed in individuals post-KTx [53]. Our findings place α -Klotho as a candidate supplemental marker that may add information to standard eGFR-based risk stratification in the post-KTx setting.

In this exploratory study we observed that higher serum α -Klotho concentrations were associated with preserved graft function over follow-up, an observation consistent across multivariable specifications for last available eGFR. This aligns with reports from other KTR cohorts, in which higher circulating α -Klotho was tied to improvement in renal function over time [54–57].

Importantly, the lower hospitalization rate associated with higher α -Klotho remains a novel and clinically relevant finding, strengthened by consistent risk attribution in different models, even when baseline eGFR was added as a covariate to the fully adjusted model. Preliminary reports suggest α -Klotho deficiency may be

detectable at very early stages of CKD [58, 59], though few studies have investigated circulating α -Klotho levels in KTRs, with mixed results [60]. Other research groups have reported associations between α -Klotho levels and glomerular function [56, 57], though variability of α -Klotho concentrations at different post-transplant timepoints has been observed, with decremental trends over time [60, 61]. While baseline eGFR and age remain important confounders and determinants of graft function [33], our analyses suggest serum α -Klotho is an independent predictor of future eGFR and may provide supplemental risk stratification value.

While Klotho functions as a co-receptor for FGF-23 signalling, its full biological roles and protective mechanisms remain unelucidated [13, 34]. High Klotho expression has been demonstrated in the kidney [35], with this organ identified as a major source of circulating α -Klotho [36]. In experimental models of transgenic mice, Klotho overexpression was tied to improved renal function, reduced calcification and higher phosphaturia [14, 17, 24]. In patients with autosomal dominant polycystic kidney disease, serum α -Klotho was associated with cyst size and renal growth [37]. On a cellular level, Klotho is likely to exert anti-apoptotic [38] and anti-fibrotic [39] effects.

We observed higher prevalence of hypertension in patients with lower α -Klotho levels, alongside an inverse correlation with circulating hsIL-6 levels that was robust to both alternative dichotomizations and continuous analysis. On a theoretical level, cytokines such as TNF- α and IL-6 may suppress α -Klotho expression in the kidney [40–42], and evidence from murine studies suggests Klotho acts as a negative regulator of NF- κ B-related inflammation [43]. Oxidative stress, cellular damage and fibrosis due to events such as ischemia-reperfusion are likely to occur post-KTx, with experimental data supportive of a protective role of Klotho [44]. In peritoneal dialysis patients, lower circulating Klotho levels have been associated with higher markers of oxidative stress and inflammation (8-isoprostane and IL-6), although this relationship was not retained in multivariable models [45]. Previous studies in haemodialysis patients have shown that lower Klotho is

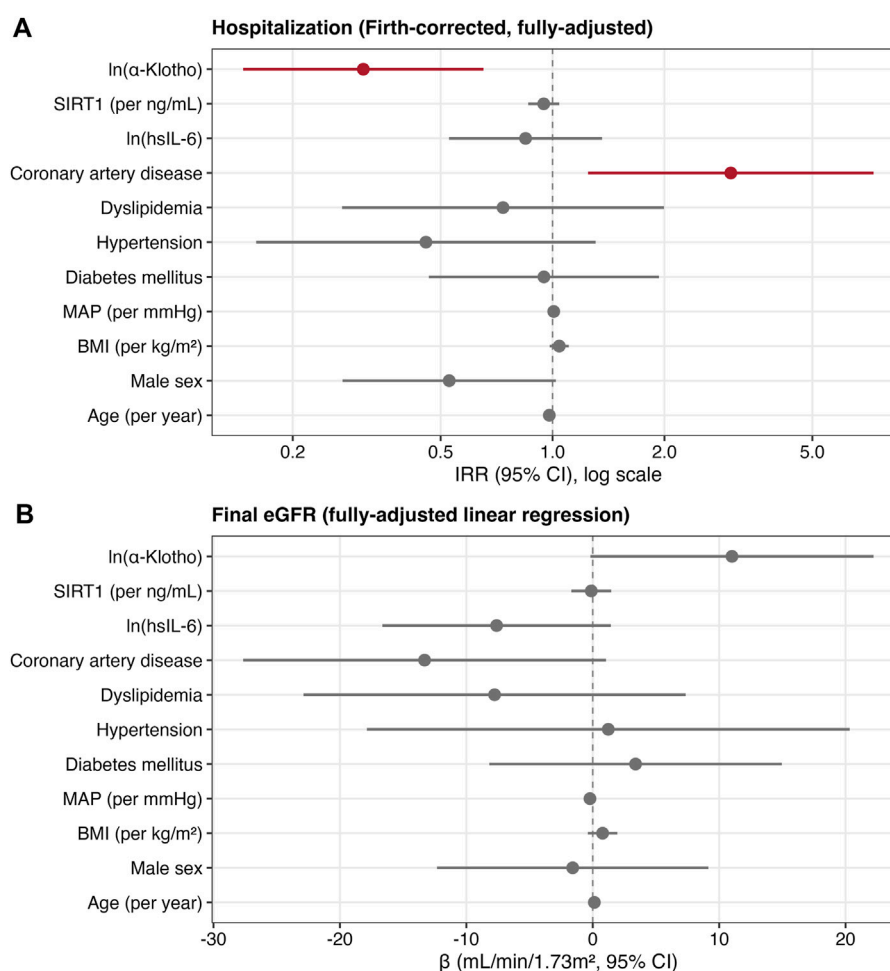


FIGURE 3

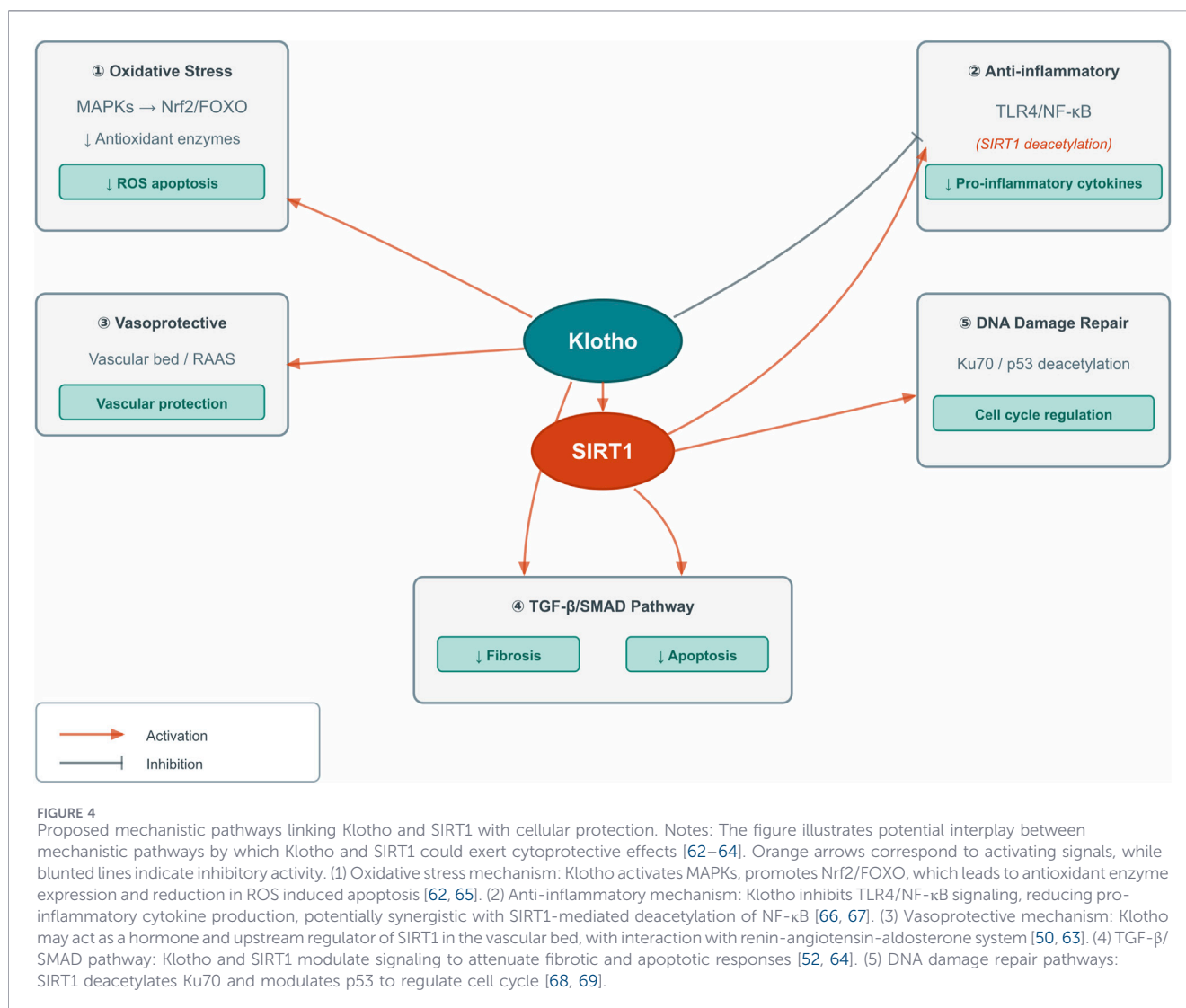
Forest plot of hospitalization risk and final eGFR predictors based on the full multivariable model. Abbreviations: BMI, body mass index; CAD, coronary artery disease; CI, confidence interval; eGFR, estimated glomerular filtration rate; IRR, incidence rate ratio; MAP, mean arterial pressure; SIRT1, sirtuin-1. Notes: (A) illustrates incidence rate ratios for hospitalization from a fully-adjusted Firth-corrected bias-reduced Poisson model ($n = 123$, 151 events). (B) illustrates linear regression coefficients for final eGFR based on a fully-adjusted linear model. Points and horizontal bars correspond to point estimates with 95% confidence intervals. Coloured points are indicative of predictors with $P < 0.05$, while grey points indicate non-significant covariates.

associated with CV events, in a manner independent of common risk factors and other covariates [46]. Since Klotho levels are closely tied to age and renal function, understanding inter-relationships between these factors appears crucial for clinical interpretation. Taken together, α -Klotho deficiency is likely to be an important player in the development of kidney disease and may also promote vascular calcification [34, 47, 48, 58].

Theoretical justification for anti-inflammatory (e.g., via inhibition of NF- κ B signalling and M2 polarization [49]) effects of SIRT1 is increasing. A schematic representation of the potential, cytoprotective pathways involving α -Klotho and SIRT1 is shown in Figure 4 [14, 50]. Acute rejection studies have also reported SIRT1 decline [51]. However, the descriptive group difference in serum SIRT1 between high and low α -Klotho strata observed at present was not retained in models with continuous predictor form, as well as in categorization approaches. Our continuous analysis did not provide sufficient evidence to speculate regarding an α -Klotho-SIRT1 relationship. This null finding may reflect threshold effects, statistical noise, or a combination of both.

The systemic bioactivity of Klotho may be dependent on both membrane-bound and soluble isoform activity. Vasoprotective effects of soluble α -Klotho have been reported, alongside its capacity to inhibit growth factor pathways driving towards pro-fibrotic transition [24, 52]. However, cross-population differences in serum α -Klotho concentrations may affect inter-assay variance and comparability [36, 70]. While our findings align with some adult and paediatric studies [71, 72], research in dialysis and CKD populations shows inconsistent results [73, 74]. These discrepancies may reflect methodological differences, sample heterogeneity, or the complex inflammatory environment in kidney disorders.

The limitations of our study need to be emphasized. First, we measured the level of circulating candidate markers in serum at a single post-KTx timepoint. Caution in interpretation is required due to the declining levels of α -Klotho over time post-KTx, as well as methodological considerations that relate to freeze-thaw cycle and assay standardization [60, 70]). Moreover, the observational design precludes any causal inference at present. At the same time, reverse causation remains a relevant concern, in that impaired graft



function may attenuate α -Klotho levels; therefore, greater mechanistic understanding of potential interactions from experimental models is crucial to build the theoretical framework for Klotho interpretation. Third, it should be stated that serum α -Klotho shows substantial inter-individual variation, while no standardized ELISA is widely accepted. We cannot, therefore, recommend the use of a pragmatic clinical cut-off, although research is encouraged and the use of percentile-based cut-offs seems preferable. Additionally, there are multiple potential confounding factors and exposures, such as diet [75], alcohol consumption [76], exercise [77] or stress [78]. Lastly, the modest sample size, event rate and exploratory nature of this study merit additional caution when interpreting our results on a causal level.

To summarize, in this observational cohort of KTRs, we observed that higher circulating α -Klotho levels were independently associated with reduced all-cause hospitalization rates, even after adjustment for baseline eGFR, and were inversely tied to hsIL-6. Higher KDIGO stage was also associated with lower serum α -Klotho concentrations. Our findings extend the current work in the field that supports circulating α -Klotho as a candidate supplemental marker for the KTx setting. Our findings

also indirectly support the ongoing interest into mechanistic aspects of therapies that are hypothesized to modulate α -Klotho expression, which includes renin-angiotensin-aldosterone system inhibitors [79], active vitamin D analogues such as calcitriol and paricalcitol [80], and mTOR inhibitors such as sirolimus and everolimus [81, 82]. Direct strategies, such as recombinant soluble α -Klotho administration, remain highly experimental. Importantly, mechanistic speculation on clinical utility requires high caution. Future prospective, large studies with standardized assays and serial α -Klotho measurements remain of importance to replicate and validate the associations investigated at present. At the same time, experimental studies that will clarify mechanistic relationship underlying α -Klotho relationship with inflammation, renal disease and metabolic dysfunction are warranted.

Data availability statement

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

Ethics statement

This study was granted approval by the Jagiellonian University Bioethics Committee. 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

AS, KB, KK, and MK conceived the study, performed statistical analyses, and drafted the manuscript; KB, AS, MŻ, and KN collected data; MB, JoM, EK-Ż, KS, JaM, and PM contributed to methodology and validation; AB-P and MK contributed to visualization; MZ and KK acquired funding; MK and KK supervised the project. All authors contributed to the article and approved the submitted version.

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References

1. Betjes MGH, Meijers RWJ, Litjens NHR. Loss of renal function causes premature aging of the immune system. *Blood Purif* (2013) 36:173–8. doi:10.1159/000356084
2. Heldal TF, Ueland T, Jenssen T, Hartmann A, Reisaeter AV, Aukrust P, et al. Inflammatory and related biomarkers are associated with post-transplant diabetes mellitus in kidney recipients: a retrospective study. *Transpl Int* (2018) 31:510–9. doi:10.1111/tri.13116
3. Jallah BP, Kuypers DRJ. Impact of immunosenescence in older kidney transplant recipients: associated clinical outcomes and possible risk stratification for immunosuppression reduction. *Drugs Aging* (2024) 41:219–38. doi:10.1007/s40266-024-01100-5
4. Créon A, Morin L, Garcia V, Aouni L, Rabant M, Terzi F, et al. Early post-transplant urinary EGF as a potential predictor of long-term allograft loss in kidney transplant recipients. *Transpl Int* (2025) 38:15061. doi:10.3389/ti.2025.15061
5. de Rooij ENM, van Duijl TT, Hoogveen EK, Romijn FPHTM, Dekker FW, van Kooten C, et al. Urinary NGAL outperforms 99mTc-MAG3 renography in predicting DCD kidney graft function. *Transpl Int* (2025) 38:13818. doi:10.3389/ti.2025.13818
6. Budhiraja P, Smith BH, Kukla A, Kline TL, Korfiatis P, Stegall MD, et al. Clinical and radiological fusion: a new frontier in predicting post-transplant diabetes mellitus. *Transpl Int* (2025) 38:14377. doi:10.3389/ti.2025.14377
7. Akifova A, Budde K, Oellerich M, Beck J, Bornemann-Kolatzki K, Schütz E, et al. Perspective for donor-derived cell-free DNA in antibody-mediated rejection after kidney transplantation: defining context of use and clinical implications. *Transpl Int* (2024) 37:13239. doi:10.3389/ti.2024.13239
8. Park S, Sellares J, Tinel C, Anglicheau D, Bestard O, Friedewald JJ. European society of organ transplantation consensus statement on testing for non-invasive diagnosis of kidney allograft rejection. *Transpl Int* (2024) 36:12115. doi:10.3389/ti.2023.12115
9. Imura A, Iwano A, Tohyama O, Tsuji Y, Nozaki K, Hashimoto N, et al. Secreted klotho protein in sera and CSF: implication for post-translational cleavage in release of klotho protein from cell membrane. *FEBS Lett* (2004) 565:143–7. doi:10.1016/j.febslet.2004.03.090
10. Matsumura Y, Aizawa H, Shiraki-Iida T, Nagai R, Kuro-o M, Nabeshima Y. Identification of the human klotho gene and its two transcripts encoding membrane and secreted klotho protein. *Biochem Biophys Res Commun* (1998) 242:626–30. doi:10.1006/bbrc.1997.8019

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2026.16186/full#supplementary-material>

11. Kaludjerovic J, Komaba H, Sato T, Erben RG, Baron R, Olauson H, et al. Klotho expression in long bones regulates FGF23 production during renal failure. *FASEB J* (2017) 31:2050–64. doi:10.1096/fj.201601036R
12. Olauson H, Lindberg K, Amin R, Jia T, Wernerson A, Andersson G, et al. Targeted deletion of klotho in kidney distal tubule disrupts mineral metabolism. *J Am Soc Nephrol* (2012) 23:1641–51. doi:10.1681/ASN.2012010048
13. Prud'homme GJ, Kurt M, Wang Q. Pathobiology of the klotho antiaging protein and therapeutic considerations. *Front Aging* (2022) 3:3. doi:10.3389/fragi.2022.931331
14. Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* (1997) 390:45–51. doi:10.1038/36285
15. Lindberg K, Amin R, Moe OW, Hu M-C, Erben RG, Östman Wernerson A, et al. The kidney is the principal organ mediating klotho effects. *J Am Soc Nephrol* (2014) 25:2169–75. doi:10.1681/ASN.2013111209
16. Li S-A, Watanabe M, Yamada H, Nagai A, Kinuta M, Takei K. Immunohistochemical localization of klotho protein in brain, kidney, and reproductive organs of mice. *Cell Struct Funct* (2004) 29:91–9. doi:10.1247/csf.29.91
17. Kurosu H, Yamamoto M, Clark JD, Pastor JV, Nandi A, Gurnani P, et al. Suppression of aging in mice by the hormone klotho. *Science* (2005) 309:1829–33. doi:10.1126/science.1112766
18. Lee J, Kim D, Lee H-J, Choi J-Y, Min J-Y, Min K-B. Association between serum klotho levels and cardiovascular disease risk factors in older adults. *BMC Cardiovasc Disord* (2022) 22:442. doi:10.1186/s12872-022-02885-2
19. Chen Z, Tao T, Huang G, Tong X, Li Q, Su G. Analysis of the association between serum antiaging humoral factor klotho and cardiovascular disease potential risk factor apolipoprotein B in general population. *Medicine (Baltimore)* (2023) 102:e34056. doi:10.1097/MD.00000000000034056
20. Wang Z, Zhang H, Zheng G, Wang Z, Shi L. Gender-specific association between circulating serum klotho and metabolic components in adults. *BMC Endocr Disord* (2024) 24:198. doi:10.1186/s12902-024-01737-8
21. Ch O. The association between metabolic syndrome and the anti-aging humoral factor klotho in middle-aged and older adults. *Diabetes & Metabolic Syndrome* (2022) 16(6), 102522. doi:10.1016/j.dsx.2022.102522

22. Liang Y, Liu Y, Tan Q, Zhou K, Wu Y, Yu L. Systemic immune-inflammation mediates the association between klotho protein and metabolic syndrome: findings from a large-scale population-based study. *Lipids Health Dis* (2024) 23:360. doi:10.1186/s12944-024-02339-y
23. Chen C, Tang P, Zhu W. Systemic immune-inflammation index mediates the association between abdominal obesity and serum klotho levels. *Sci Rep* (2025) 15:4205. doi:10.1038/s41598-025-88015-2
24. Hu MC, Shi M, Zhang J, Quiñones H, Griffith C, Kuro-o M, et al. Klotho deficiency causes vascular calcification in chronic kidney disease. *J Am Soc Nephrol* (2011) 22:124–36. doi:10.1681/ASN.2009121311
25. Yang Y, Liu Y, Wang Y, Chao Y, Zhang J, Jia Y, et al. Regulation of SIRT1 and its roles in inflammation. *Front Immunol* (2022) 13:13. doi:10.3389/fimmu.2022.831168
26. Kong L, Wu H, Zhou W, Luo M, Tan Y, Miao L, et al. Sirtuin 1: a target for kidney diseases. *Mol Med* (2015) 21:87–97. doi:10.2119/molmed.2014.00211
27. Lund KP, von Stemmann JH, Eriksson F, Hansen MB, Pedersen BK, Sørensen SS, et al. IL-10-specific autoantibodies predict major adverse cardiovascular events in kidney transplanted patients - a retrospective cohort study. *Transpl Int* (2019) 32:933–48. doi:10.1111/tri.13425
28. Bolufer M, Soler J, Molina M, Taco O, Vila A, Macía M. Immunotherapy for cancer in kidney transplant patients: a difficult balance between risks and benefits. *Transpl Int* (2024) 37:13204. doi:10.3389/ti.2024.13204
29. Stenvinkel P, Larsson TE. Chronic kidney disease: a clinical model of premature aging. *Am J Kidney Dis* (2013) 62:339–51. doi:10.1053/j.ajkd.2012.11.051
30. Yan Z, Shao T. Chronic inflammation in chronic kidney disease. *Nephron* (2023) 148:143–51. doi:10.1159/000534447
31. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New Creatinine- and cystatin C–Based equations to estimate GFR without race. *N Engl J Med* (2021) 385:1737–49. doi:10.1056/NEJMoa2102953
32. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2024 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int* (2024) 105:S117–S314. doi:10.1016/j.kint.2023.10.018
33. Batko K, Sączek A, Banaszkiewicz M, Małyszko J, Koc-Zórawska E, Zórawski M, et al. Risk prediction of kidney function in long-term kidney transplant recipients. *Front Med* (2025) 12:1469363. doi:10.3389/fmed.2025.1469363
34. Lim K, Lu T-S, Molostvov G, Lee C, Lam FT, Zehnder D, et al. Vascular klotho deficiency potentiates the development of human artery calcification and mediates resistance to fibroblast growth factor 23. *Circulation* (2012) 125:2243–55. doi:10.1161/CIRCULATIONAHA.111.053405
35. Olauson H, Mencke R, Hillebrands J-L, Larsson TE. Tissue expression and source of circulating αKlotho. *Bone* (2017) 100:19–35. doi:10.1016/j.bone.2017.03.043
36. Hu MC, Shi M, Zhang J, Addo T, Cho HJ, Barker SL, et al. Renal production, uptake, and handling of circulating αKlotho. *J Am Soc Nephrol* (2016) 27:79–90. doi:10.1681/ASN.2014101030
37. Pavik I, Jaeger P, Ebner L, Poster D, Krauer F, Kistler AD, et al. Soluble klotho and autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol* (2012) 7:248–57. doi:10.2215/CJN.09020911
38. Panesso MC, Shi M, Cho HJ, Paek J, Ye J, Moe OW, et al. Klotho has dual protective effects on cisplatin-induced acute kidney injury. *Kidney Int* (2014) 85:855–70. doi:10.1038/ki.2013.489
39. Guan X, Nie L, He T, Yang K, Xiao T, Wang S, et al. Klotho suppresses renal tubulointerstitial fibrosis by controlling basic fibroblast growth factor-2 signalling. *J Pathol* (2014) 234:560–72. doi:10.1002/path.4420
40. Moreno JA, Izquierdo MC, Sanchez-Niño MD, Suárez-Alvarez B, Lopez-Larrea C, Jakubowski A, et al. The inflammatory cytokines TWEAK and TNFα reduce renal klotho expression through NFκB. *J Am Soc Nephrol* (2011) 22:1315–25. doi:10.1681/ASN.2010101073
41. Zeng Y, Wang P-H, Zhang M, Du J-R. Aging-related renal injury and inflammation are associated with downregulation of klotho and induction of RIG-I/NF-κB signaling pathway in senescence-accelerated mice. *Aging Clin Exp Res* (2016) 28:69–76. doi:10.1007/s40520-015-0371-y
42. Thurston RD, Larmonier CB, Majewski PM, Ramalingam R, Midura-Kiela M, Laubitz D, et al. Tumor necrosis factor and interferon-gamma down-regulate klotho in mice with colitis. *Gastroenterology* (2010) 138:1384–94. doi:10.1053/j.gastro.2009.12.002
43. Zhao Y, Banerjee S, Dey N, Lejeune WS, Sarkar PS, Brobey R, et al. Klotho depletion contributes to increased inflammation in kidney of the db/db mouse model of diabetes via RelA (serine)536 phosphorylation. *Diabetes* (2011) 60:1907–16. doi:10.2337/db10-1262
44. Hu M-C, Moe OW. Klotho as a potential biomarker and therapy for acute kidney injury. *Nat Rev Nephrol* (2012) 8:423–9. doi:10.1038/nrneph.2012.92
45. Oh HJ, Nam BY, Lee MJ, Kim CH, Koo HM, Doh FM, et al. Decreased circulating klotho levels in patients undergoing dialysis and relationship to oxidative stress and inflammation. *Perit Dial Int* (2015) 35:43–51. doi:10.3747/pdi.2013.00150
46. Memmos E, Sarafidis P, Pateinakos P, Tsiantoulas A, Faitatzidou D, Giamalis P, et al. Soluble klotho is associated with mortality and cardiovascular events in hemodialysis. *BMC Nephrol* (2019) 20:217. doi:10.1186/s12882-019-1391-1
47. Patidar A, Singh DK, Thakur S, Farrington K, Baydoun AR. Uremic serum-induced calcification of human aortic smooth muscle cells is a regulated process involving klotho and RUNX2. *Biosci Rep* (2019) 39:BSR20190599. doi:10.1042/BSR20190599
48. Kim HR, Nam BY, Kim DW, Kang MW, Han J-H, Lee MJ, et al. Circulating α-klotho levels in CKD and relationship to progression. *Am J Kidney Dis* (2013) 61:899–909. doi:10.1053/j.ajkd.2013.01.024
49. Zhao X, Li M, Lu Y, Wang M, Xiao J, Xie Q, et al. Sirt1 inhibits macrophage polarization and inflammation in gouty arthritis by inhibiting the MAPK/NF-κB/AP-1 pathway and activating the Nrf2/HO-1 pathway. *Inflamm Res* (2024) 73:1173–84. doi:10.1007/s00011-024-01890-9
50. Gao D, Zuo Z, Tian J, Ali Q, Lin Y, Lei H, et al. Activation of SIRT1 attenuates klotho deficiency-induced arterial stiffness and hypertension by enhancing AMP-activated protein kinase activity. *Hypertension* (2016) 68:1191–9. doi:10.1161/HYPERTENSIONAHA.116.07709
51. Aksungur N, Kizilkaya M, Altundaş N, Balkan E, Kara S, Demirci E, et al. Molecular crosstalk between SIRT1, Wnt/β-Catenin signaling, and inflammatory pathways in renal transplant rejection: role of miRNAs, lncRNAs, IL-1, IL-6, and tubulointerstitial inflammation. *Medicina (Kaunas)* (2025) 61:1073. doi:10.3390/medicina61061073
52. Doi S, Zou Y, Togao O, Pastor JV, John GB, Wang L, et al. Klotho inhibits transforming growth factor-β1 (TGF-β1) signaling and suppresses renal fibrosis and cancer metastasis in mice. *J Biol Chem* (2011) 286:8655–65. doi:10.1074/jbc.M110.174037
53. Thongprayoon C, Neyra JA, Hansrivijit P, Medaura J, Leeaphorn N, Davis PW, et al. Serum klotho in living kidney donors and kidney transplant recipients: a meta-analysis. *J Clin Med* (2020) 9:1834. doi:10.3390/jcm9061834
54. Kim SM, Kim SJ, Ahn S, Min S-I, Min S-K, Ha J. The potential role of klotho as a prognostic biomarker in deceased donor kidney transplantation. *Transplantation* (2018) 102:S539. doi:10.1097/01.tp.0000543389.60945.78
55. Deng G, Yang A, Wu J, Zhou J, Meng S, Zhu C, et al. The value of older Donors' Klotho Level in Predicting Recipients' short-term renal function. *Med Sci Monit* (2018) 24:7936–43. doi:10.12659/MSM.913274
56. Tsujita M, Kosugi T, Masuda T, Okada M, Futamura K, Hiramitsu T, et al. Serum αKlotho as a predictor of graft dysfunction after kidney transplantation. *Transpl Proc* (2018) 50:3440–4. doi:10.1016/j.transproceed.2018.09.008
57. Shikida Y, Mizobuchi M, Yoshitake O, Kato T, Ogata H, Koiwa F, et al. Lower soluble klotho levels in the pretransplant period are associated with an increased risk of renal function decline in renal transplant patients. *Ther Apher Dial* (2021) 25:331–40. doi:10.1111/1744-9987.13578
58. Barker SL, Pastor J, Carranza D, Quiñones H, Griffith C, Goetz R, et al. The demonstration of αKlotho deficiency in human chronic kidney disease with a novel synthetic antibody. *Nephrol Dial Transpl* (2015) 30:223–33. doi:10.1093/ndt/gfu291
59. Shimamura Y, Hamada K, Inoue K, Ogata K, Ishihara M, Kagawa T, et al. Serum levels of soluble secreted α-Klotho are decreased in the early stages of chronic kidney disease, making it a probable novel biomarker for early diagnosis. *Clin Exp Nephrol* (2012) 16:722–9. doi:10.1007/s10157-012-0621-7
60. Donate-Correa J, Matos-Perdomo E, González-Luis A, Martín-Olivera A, Ortiz A, Mora-Fernández C, et al. The value of klotho in kidney transplantation. *Transplantation* (2023) 107:616–27. doi:10.1097/TP.0000000000004331
61. Bleskestad IH, Thorsen IS, Jonsson G, Skadberg Ø, Bergrem H, Gøransson LG. Soluble klotho and intact fibroblast growth factor 23 in long-term kidney transplant patients. *Eur J Endocrinol* (2015) 172:343–50. doi:10.1530/EJE-14-0457
62. Lim SW, Jin L, Luo K, Jin J, Shin YJ, Hong SY, et al. Klotho enhances FoxO3-mediated manganese superoxide dismutase expression by negatively regulating PI3K/AKT pathway during tacrolimus-induced oxidative stress. *Cell Death Dis* (2017) 8:e2972. doi:10.1038/cddis.2017.365
63. de Borst MH, Vervloet MG, ter Wee PM, Navis G. Cross talk between the renin-angiotensin-aldosterone system and vitamin D-FGF-23-klotho in chronic kidney disease. *J Am Soc Nephrol* (2011) 22:1603–9. doi:10.1681/ASN.2010121251
64. García-Vizcaino EM, Liarte S, Alonso-Romero JL, Nicolás FJ. Sirt1 interaction with active Smad2 modulates transforming growth factor-β regulated transcription. *Cell Commun Signal* (2017) 15:50. doi:10.1186/s12964-017-0205-y
65. Zhu H, Gao Y, Zhu S, Cui Q, Du J. Klotho improves cardiac function by suppressing reactive oxygen species (ROS) mediated apoptosis by modulating Mapks/Nrf2 signaling in doxorubicin-induced cardiotoxicity. *Med Sci Monit* (2017) 23:5283–93. doi:10.12659/MSM.907449
66. Bi F, Liu W, Wu Z, Ji C, Chang C. Antiaging factor klotho retards the progress of intervertebral disc degeneration through the toll-like receptor 4-NF-κB pathway. *Int J Cell Biol* (2020) 2020:8319516. doi:10.1155/2020/8319516
67. Wang Y, Wang K, Bao Y, Zhang T, Ainiwaer D, Xiong X, et al. The serum soluble klotho alleviates cardiac aging and regulates M2a/M2c macrophage polarization via inhibiting TLR4/Myd88/NF-κB pathway. *Tissue Cell* (2022) 76:101812. doi:10.1016/j.tice.2022.101812

68. Yi J, Luo J. SIRT1 and p53, effect on cancer, senescence and beyond. *Biochim Biophys Acta* (2010) 1804:1684–9. doi:10.1016/j.bbapap.2010.05.002
69. Jeong SM, Haigis MC. Sirtuins in cancer: a balancing act between genome stability and metabolism. *Mol Cells* (2015) 38:750–8. doi:10.14348/molcells.2015.0167
70. Adema AY, Vervloet MG, Blankenstein MA, Heijboer AC. α -Klotho is unstable in human urine. *Kidney Int* (2015) 88:1442–4. doi:10.1038/ki.2015.238
71. Gamrot Z, Adamczyk P, Świętochowska E, Roszkowska-Bjanid D, Gamrot J, Szczepanska M. Tumour necrosis factor alpha (TNF α) and alpha-Klotho (α KL) in children and adolescents with chronic kidney disease (CKD). *Endokrynol Pol* (2021) 72: 625–33. doi:10.5603/EP.a2021.0082
72. Wang Q, Su W, Shen Z, Wang R. Correlation between soluble α -Klotho and renal function in patients with chronic kidney disease: a review and meta-analysis. *Biomed Res Int* (2018) 2018:9481475. doi:10.1155/2018/9481475
73. Lisowska KA, Storonik H, Soroczyńska-Cybula M, Maziewski M, Dębska-Słizień A. Serum levels of α -Klotho, inflammation-related cytokines, and mortality in hemodialysis patients. *J Clin Med* (2022) 11:6518. doi:10.3390/jcm11216518
74. Seiler S, Wen M, Roth HJ, Fehrenz M, Flügge F, Herath E, et al. Plasma klotho is not related to kidney function and does not predict adverse outcome in patients with chronic kidney disease. *Kidney Int* (2013) 83:121–8. doi:10.1038/ki.2012.288
75. Jurado-Fasoli L, Amaro-Gahete FJ, Martínez-Tellez B, Ruiz JR, Gutiérrez Á, Castillo MJ, et al. Adherence to the mediterranean diet, dietary factors, and S-Klotho plasma levels in sedentary middle-aged adults. *Exp Gerontol* (2019) 119:25–32. doi:10.1016/j.exger.2019.01.019
76. Jurado-Fasoli L, Amaro-Gahete FJ, Gutiérrez Á, Castillo MJ. Alcohol consumption and S-Klotho plasma levels in sedentary healthy middle-aged adults: a cross sectional study. *Drug Alcohol Depend* (2019) 194:107–11. doi:10.1016/j.drugalcdep.2018.09.024
77. Amaro-Gahete FJ, Gutiérrez Á, Jurado-Fasoli L, Ruiz JR, Castillo MJ, Gutiérrez Á. Role of exercise on S-Klotho protein regulation: a systematic review. *Curr Aging Sci* (2018) 11:100–7. doi:10.2174/1874609811666180702101338
78. Prather AA, Epel ES, Arenander J, Broestl L, Garay BI, Wang D, et al. Longevity factor klotho and chronic psychological stress. *Transl Psychiatry* (2015) 5:e585. doi:10.1038/tp.2015.81
79. Lim SC, Liu J-J, Subramaniam T, Sum CF. Elevated circulating alpha-klotho by angiotensin II receptor blocker losartan is associated with reduction of albuminuria in type 2 diabetic patients. *J Renin Angiotensin Aldosterone Syst* (2014) 15:487–90. doi:10.1177/1470320313475905
80. Lau WL, Leaf EM, Hu MC, Takeno MM, Kuro-o M, Moe OW, et al. Vitamin D receptor agonists increase klotho and osteopontin while decreasing aortic calcification in mice with chronic kidney disease fed a high phosphate diet. *Kidney Int* (2012) 82: 1261–70. doi:10.1038/ki.2012.322
81. Mizusaki K, Hasuike Y, Kimura T, Nagasawa Y, Kuragano T, Yamada Y, et al. Inhibition of the mammalian target of rapamycin may augment the increase in soluble klotho levels in renal transplantation recipients. *Blood Purif* (2019) 47(Suppl. 2):12–8. doi:10.1159/000496630
82. Wu Q-J, Zhang T-N, Chen H-H, Yu X-F, Lv J-L, Liu Y-Y, et al. The sirtuin family in health and disease. *Sig Transduct Target Ther* (2022) 7:402. doi:10.1038/s41392-022-01257-8



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Delayed graft function and long-term outcomes after deceased donor kidney transplantation: insights from mate-kidney analysis

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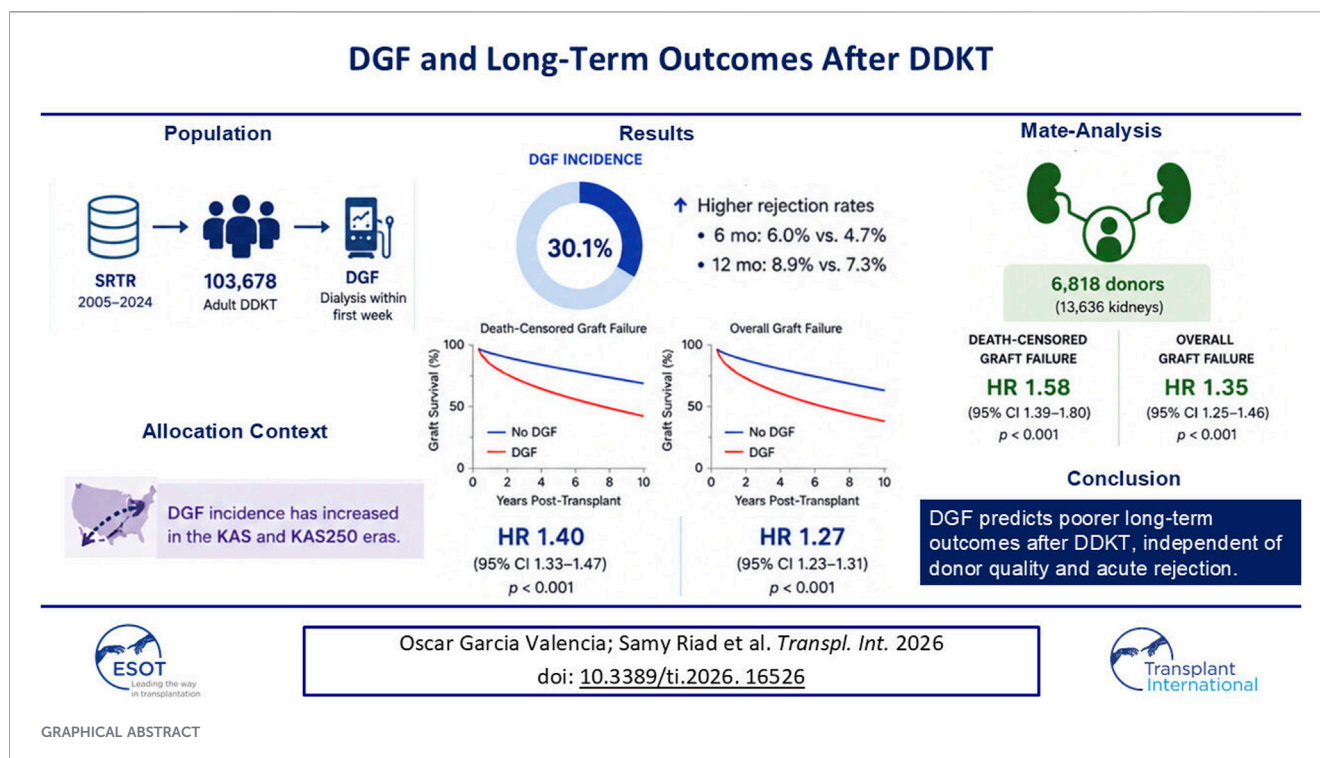
Delayed graft function (DGF) is a frequent complication after deceased donor kidney transplantation, yet its long-term impact remains understudied. Heterogeneous populations, short follow-up, and inadequate control of donor-related factors have limited prior studies. Using the Scientific Registry of Transplant Recipients (2005–2024), we evaluated the association between DGF and long-term graft outcomes, including a mate-kidney (same donor, discordant DGF status) analysis to isolate its independent effect. We identified 103,678 dialysis-dependent deceased donor kidney recipients maintained on tacrolimus and mycophenolate. Mixed-effects Cox models, adjusted for donor, recipient, immunologic, and procurement factors. DGF occurred in 30.1% of recipients and was associated with increased death-censored graft failure (HR 1.40; 95% CI 1.33–1.47) and overall graft failure (HR 1.27; 95% CI 1.23–1.31). DGF recipients had higher rejection rates at 6 (6.0% vs. 4.7%) and 12 months (8.9% vs. 7.3%), longer hospital stay, and higher 1-year rehospitalization. In the mate-kidney analysis (6,818 donors), DGF remained strongly associated with death-censored graft failure (HR 1.58; 95% CI 1.39–1.80) and overall graft failure (HR 1.35; 95% CI 1.25–1.46), confirming the independent effect of DGF. DGF is a strong predictor of adverse long-term outcomes irrespective of rejection and warrants targeted prevention strategies and intensified follow-up.

KEYWORDS

allocation policy change, delayed graft function, long-term outcomes, mate kidney, rejection

Highlights

- Delayed graft function is associated with significantly increased long-term risks of both death-censored and overall kidney graft failure.



- This association persists independent of acute rejection and after controlling for donor characteristics using mate-kidney analysis, confirming that delayed graft function itself, rather than donor quality, is a key determinant of long-term outcomes.
- The rising incidence of DGF in the KAS and KAS250 eras underscores important clinical and policy implications in the current allocation landscape.

Introduction

Delayed graft function (DGF), commonly defined as the need for dialysis within the first week after kidney transplantation, is a frequent complication after deceased donor kidney transplantation [1], affecting approximately 25%–35% of recipients [2]. Despite substantial advances in organ preservation, perioperative care, and immunosuppression, DGF remains a major clinical and operational challenge.

Short- and intermediate-term consequences of DGF, including longer hospital stays, higher healthcare costs, increased rejection,

and reduced 1–5-year graft survival are well documented. However, the long-term impact of DGF beyond 5 years remains poorly understood, with prior studies limited by small sample sizes, heterogeneous immunosuppression, and inadequate control for donor factors that strongly influence graft survival [3–6].

Delayed graft function arises from a complex interplay of donor-, recipient-, and procurement-level factors, including ischemia-reperfusion injury, donor quality, cold ischemia time, and recipient comorbidity. Because donor characteristics substantially affect both early graft function and long-term outcomes, distinguishing the independent effect of DGF from donor quality is challenging [7, 8]. Mate-kidney analysis, which compares outcomes between kidneys from the same donor with discordant for DGF status, is a powerful approach to overcoming this confounding but has rarely been applied in studies with long follow-up.

In parallel, kidney allocation in the United States has undergone substantial changes over the past decade. Prior to 2014, allocation was largely based on donation service areas and waiting time, contributing to geographic disparities. The Kidney Allocation System (KAS) [9], implemented in 2014 and revised in 2021 (KAS250) [10], introduced prioritization based on recipient and donor characteristics and expanded geographic sharing through a 250-nautical-mile radius. These changes have influenced donor utilization and transplant logistics, with potential downstream effects on delayed graft function and long-term transplant outcomes.

To address these gaps, we conducted a national analysis of all primary dialysis-dependent deceased donor kidney transplants in the United States between 2005 and 2024. Our objectives were to:

1. Evaluate the association between DGF and long-term death-censored and overall graft failure, and

Abbreviations: BMI, Body Mass Index; cPRA, Calculated Panel Reactive Antibody; DCGF, Death censored graft failure; DCD, Donation After Cardiac Death; DGF, Delayed graft function; DM, Diabetes Mellitus; DSA, Donor specific alloantibodies; ESKD, End Stage Kidney Disease; GFR, glomerular filtration rate; HLA, human leukocyte antigen; HRSA, The Health Resources and Services Administration; IL-2RA, Interleukin 2 receptor antagonists; KAS, Kidney allocation system; KAS 250, 250-nautical mile fixed-radius allocation; KDIGO, Kidney Disease Improving Global Outcomes; OPTN, Organ Procurement and Transplantation Network; PCKD, Polycystic Kidney Disease; PVD, Peripheral Vascular Disease; rATG, rabbit Anti-thymocyte globulin; SRTR, Scientific Registry of Transplant Recipients; US, United States; UNOS, United network for organ sharing.

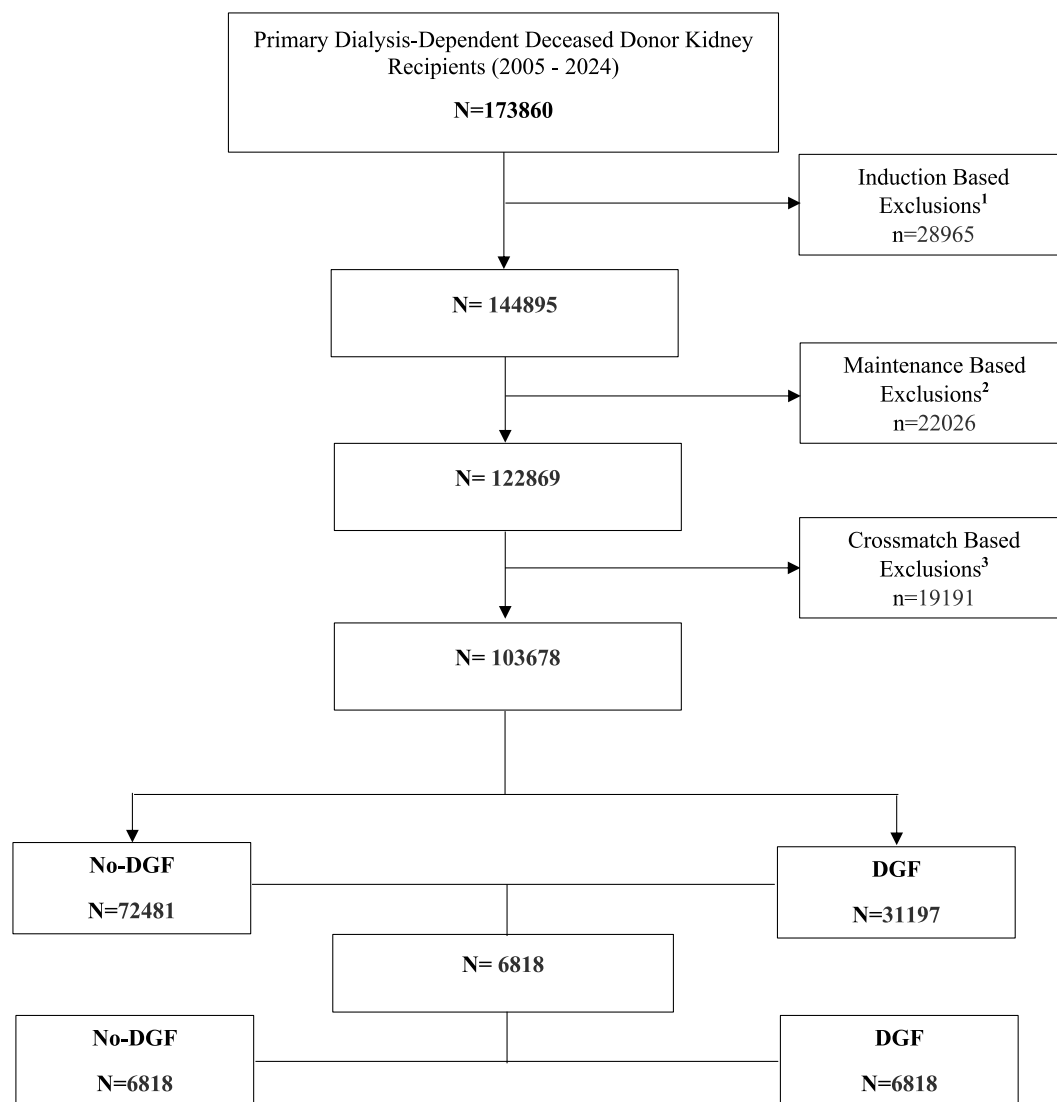


FIGURE 1
Study Population. 1. Induction-based exclusions: use of unusual agents, combination agents, missing or no-induction. 2. Maintenance-based exclusions: missing immunosuppression or regimens other than Tacrolimus/MMF with or without steroids. 3. Missing, positive, or weakly positive crossmatch.

2. Perform a mate-kidney analysis to isolate the independent effect of DGF from donor-related factors.

Human Services provide oversight to the activities of the OPTN and SRTR contractors.

Study population

Materials and methods

Data source

This study used data from the Scientific Registry of Transplant Recipients (SRTR). The SRTR data system includes data on all donors, wait-listed candidates, and transplant recipients in the US, submitted by the members of the Organ Procurement and Transplantation Network (OPTN). The Health Resources and Services Administration (HRSA), U.S. Department of Health and

Using the Scientific Registry of Transplant Recipients standard analysis file, we identified all dialysis-dependent primary deceased donor kidney recipients ($N = 173,860$) between 2005 and 2024. We excluded recipients discharged on maintenance immunosuppression other than tacrolimus and mycophenolate with or without steroids ($n = 22,026$). Moreover, we excluded recipients of no induction or mixed induction ($n = 28,965$) and those with a positive or unknown crossmatch ($n = 19,191$). Thus, our cohort consisted of 103,678 recipients and was divided into two groups of recipients based on the DGF status: the DGF group ($n =$

31,197) and the No-DGF group ($n = 72,481$). Delayed graft function (DGF) was defined according to the SRTR/OPTN registry criteria as the need for at least one dialysis treatment within the first week after kidney transplantation.

An additional mate-kidney analysis was performed among recipients with kidneys from the same donor (i.e., mate kidneys) ($n = 13,636$ kidneys from 6,818 donors). Pairs of kidneys from the same donor who met the above inclusion criteria and were identified where one recipient experienced DGF while the other did not (Figure 1).

Outcomes of interest

The primary outcome of the study was death-censored kidney graft failure and overall graft failure by recipient's DGF status, with overall graft failure defined as graft loss or death. Secondary outcomes included 6-month and 1-year rejection rate, length of stay, rehospitalization within the first year, BK polyoma virus infection rate, and 1 year creatinine according to recipient DGF status.

Statistical analyses

Continuous variables were summarized with the mean and standard deviation. Differences between groups were tested using analysis of variance or pooled t-tests. For categorical variables, data were summarized as counts and within-group percentages. Proportions were compared by the chi-square test. Time to event data were summarized with Kaplan-Meier estimates of incidence and curves for death-censored and overall graft survival through 10 years post-transplant. We left-censored the cohort at 90 days to limit the contribution of early complications, including primary non-function and death. Log-rank tests were used to identify statistically significant differences between groups.

Mixed effect Cox proportional hazard models examined the association between DGF and outcomes of interest. Models were adjusted for variables selected *a priori* based on medical relevance after review by the study team including donor (age, ethnicity, sex, DBD vs. DCD, KDRI), recipient (age, ethnicity, sex, ESKD etiology, dialysis vintage, presence of vascular disease, induction type and maintenance), immunologic (HLA mismatch, cPRA) and procurement factors (preservation). Transplant center was used as a random effect in these models. For the mate analysis, a random effect was also used for the donor. Linearity in all tests was evaluated using splines for continuous variables. Plots of Schoenfeld residuals were used to test the assumption of proportionality. We also performed sensitivity analyses on the full cohort, without left censoring, to assess the robustness of our findings.

To further evaluate the relationship between delayed graft function (DGF) and acute rejection, we performed additional analyses stratified by rejection status within the first-year post-transplant. Separate Cox proportional hazards models were constructed among recipients with and without rejection. In addition, an interaction term between DGF and rejection was included in the full cohort model to assess for effect modification. All models were adjusted using the same covariates as the primary analysis. All analyses were performed using R v4.2.2

(R Foundation for Statistical Computing, Vienna, Austria). P-values less than 0.05 were considered statistically significant.

Results

Baseline characteristics

Our cohort consisted of 103,678 deceased donor kidney transplant recipients of whom 72,481 (69.90%) recipients did not experience DGF and 31,197 (30.09%) did. Recipients with DGF were older (54.8 vs. 52.7 years, $p < 0.001$), less likely to be women (31.8% vs. 41.5%, $p < 0.001$) and more likely to be Black (41.5% vs. 35.4%, $p < 0.001$). Diabetes mellitus as an etiology of end-stage kidney disease was more frequently observed in recipients with DGF (40.1% vs. 29.8%, $p < 0.001$), while glomerulonephritis (15.4% vs. 20.4%, $p < 0.001$) was less frequently observed compared to recipients without DGF. Recipients with DGF had longer waiting time on dialysis and had a higher KDRI (Table 1).

Recipients who experienced DGF were more likely to have received kidneys from older donors (42.0 vs. 38.1 years $p < 0.001$), donation after cardiac death (DCD) donors (DCD, 32.5% vs. 16.8%, $p < 0.001$), and imported kidneys (37.3% vs. 28.6%, $p < 0.001$). These kidneys had longer cold ischemia times (19.9 vs. 17.4 h, $p < 0.001$), and their donors were less frequently supported with inotropic agents before donation (34.3% vs. 41.4%, $p < 0.001$). A higher number of HLA-mismatches was noted among the recipients with DGF. The induction type was similar between the two groups (Table 1).

Unadjusted outcomes

The DGF rate increased over the study period. Before the KAS was implemented in 2014, the DGF rate averaged around 26.3%, while after KAS the DGF rate averaged around 30.6%. In March of 2021, KAS250 was implemented, and the rate of DGF has since averaged 35.0% (Figure 2). The increase in DGF rates over time was most pronounced following implementation of the KAS and KAS250 allocation policies, with a relative increase of approximately 16% from the pre-KAS to post-KAS250 era. This trend likely reflects evolving donor utilization practices, including increased use of donation after circulatory death (DCD) donors and longer ischemia times.

On univariable analysis, DGF was associated with worse short-term outcomes (Table 2). The length of stay was longer in the DGF group (8.2 days, vs. 5.7 days) compared to the recipients without DGF ($p < 0.001$). The rejection rate was also higher in DGF recipients at 6 months (6.0% vs. 4.7% $p < 0.001$). This difference remained through the 1-year mark (8.9% vs. 7.3% $p < 0.001$). BK infection rate within the first year was not different between groups (8.8% vs. 9.4%, $p = 0.14$) in recipients with and without DGF, respectively, although BK data was only available on 28,147 recipients (27.1%). The 1-year creatinine level was higher in the DGF group, at 1.58 mg/dL (± 0.7) compared to 1.37 mg/dL (± 0.6) ($p < 0.001$).

In the Kaplan-Meier analysis, DGF was associated with worse overall and death-censored graft survival (both log-rank $p < 0.001$) (Figure 3). Overall graft survival at 5 and 10 years in patients without

TABLE 1 Baseline characteristics of recipients by delayed graft function status.

Variable	No DGF N = 72,481	DGF N = 31,197	p value
Recipient variables			
Recipient age	52.7 (13.3)	54.8 (12.2)	<0.001
Recipient sex (female)	30,092 (41.5%)	9,922 (31.8%)	<0.001
Ethnicity			<0.001
Black	25,648 (35.4%)	12,950 (41.5%)	
Hispanic	13,183 (18.2%)	6,076 (19.5%)	
Other	6,993 (9.6%)	3,142 (10.1%)	
White	26,657 (36.8%)	9,029 (28.9%)	
ESKD etiology			<0.001
DM	21,593 (29.8%)	12,515 (40.1%)	
GN	14,757 (20.4%)	4,809 (15.4%)	
Other	30,236 (41.7%)	11,981 (38.4%)	
PKD	5,881 (8.1%)	1,882 (6.0%)	
Recipient BMI	28.3 (5.41)	29.6 (5.49)	<0.001
Time on dialysis (years)	4.26 (3.1)	5.04 (3.2)	<0.001
Recipient vascular disease	7,743 (18.7%)	3,862 (23.2%)	<0.001
Donor variables			
Donor age	38.1 (16.1)	42.0 (14.7)	<0.001
Donor Race			<0.001
Black	10,675 (14.7%)	4,306 (13.8%)	
Hispanic	10,273 (14.2%)	4,114 (13.2%)	
Other	2,564 (3.5%)	1,248 (4.0%)	
White	48,969 (67.6%)	21,529 (69.0%)	
Donor sex (female)	28,995 (40.0%)	11,248 (36.1%)	<0.001
Donor cause of death			<0.001
Anoxia	25,652 (35.4%)	12,811 (41.1%)	
Cerebrovascular	19,957 (27.5%)	9,316 (29.9%)	
Head trauma	24,434 (33.7%)	7,892 (25.3%)	
Other	2,438 (3.4%)	1,178 (3.8%)	
Donor diabetes	24,274 (34.3%)	13,761 (45.4%)	<0.001
KDRI	1.130 (0.284)	1.198 (0.274)	<0.001
Procurement variables			
DCD	12,147 (16.8%)	10,124 (32.5%)	<0.001
Pumped	33,453 (46.2%)	16,087 (51.6%)	<0.001
Cold ischemia time (hrs.)	17.4 (8.0)	19.9 (8.4)	<0.001
Laterality			<0.001
Left	33,349 (46.0%)	14,516 (46.5%)	
Right	36,929 (50.9%)	15,945 (51.1%)	
<i>En-bloc</i>	1,500 (2.1%)	357 (1.1%)	
Sequential	703 (1.0%)	379 (1.2%)	

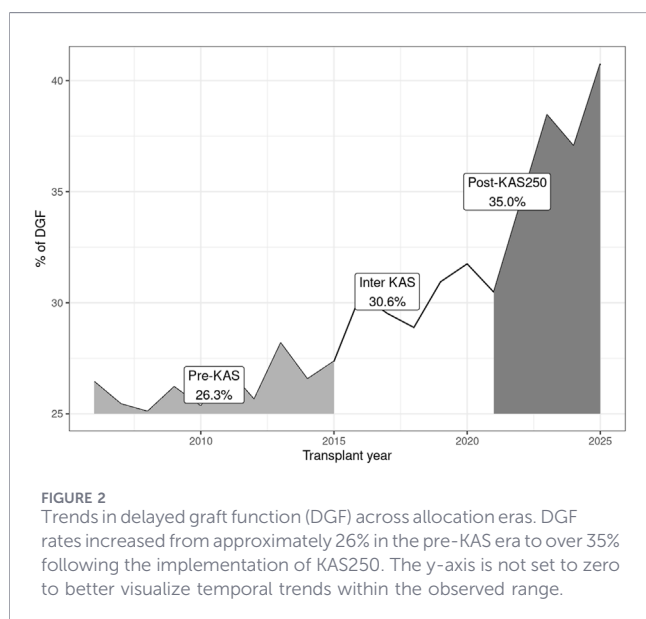
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TABLE 1 Continued

Variable	No DGF N = 72,481	DGF N = 31,197	p value
Inotropic support (yes)	29,974 (41.4%)	10,694 (34.3%)	<0.001
Local organ	51,734 (71.4%)	19,565 (62.7%)	<0.001
Immunologic variables			
cPRA	20.3% (32.8)	18.2% (30.6)	<0.001
HLA-mismatch			<0.001
0	4,442 (6.1%)	1,283 (4.1%)	
1–3	13,395 (18.5%)	5,208 (16.7%)	
4–6	54,642 (75.4%)	24,706 (79.2%)	
Induction			<0.001
Alemtuzumab	11,171 (15.4%)	4,933 (15.8%)	
IL-2RA	14,344 (19.8%)	5,576 (17.9%)	
rATG	46,966 (64.8%)	20,688 (66.3%)	
Steroid maintenance	49,825 (68.7%)	23,138 (74.2%)	<0.001

Values are shown as mean (SD) for continuous variables and n (%) for categorical variables. P-values were calculated using t-tests or ANOVA, for continuous variables and chi-square tests for categorical variables.

Abbreviations: BMI, body mass index; ESKD, end-stage kidney disease; PCKD, polycystic kidney disease; PVD, peripheral vascular disease; HLA, human leukocyte antigen; cPRA, calculated panel reactive antibody; IL-2RA, interleukin-2 receptor antagonist.



DGF was 77.7% (CI 77.3, 78.0) and 48.7% (CI 48.1, 49.2) vs. 68.9% (CI 68.3, 69.6) and 37.4% (CI 36.5, 38.3) in those with DGF, respectively.

Adjusted outcomes

To further examine the impact of DGF, an adjusted analysis was performed. In the Cox proportional hazards model, DGF was associated with a 40% increased risk of death-censored graft failure [HR 1.40, CI (1.33, 1.47), $p < 0.001$] and a 27% increased risk of overall graft loss by 10 years post-transplant [HR 1.27, CI

(1.23, 1.31), $p < 0.001$]. Older recipient age was associated with lower death-censored graft failure [HR 0.97, CI (0.97, 0.97), $p < 0.001$] but higher overall graft failure [HR 1.02, CI (1.02, 1.02), $p < 0.001$]. Death-censored and overall graft failure were slightly lower in recipients of Hispanic or other ethnicities compared to white recipients. Black recipients had an increased risk of death-censored graft failure [HR 1.42, CI (1.34, 1.51), $p < 0.001$], but not overall graft failure [HR 0.99, CI (0.95, 1.02), $p = 0.42$] compared to white recipients. Compared to diabetes as a cause of ESRD, other ESRD etiologies were associated with lower death-censored and overall graft failure (Table 3).

Compared to the pre-KAS era, the KAS era was associated with 15% decreased death-censored graft failure [HR 0.85, CI (0.80, 0.90), $p < 0.001$] and 4% increased risk of overall graft failure [HR 1.04, CI (1.00, 1.08), $p = 0.04$]. The post-KAS 250 was not associated with a higher risk of death-censored graft failure [HR 0.95, CI (0.72, 1.25), $p = 0.72$] or overall graft failure [HR 1.04, CI (0.89–1.21), $p = 0.64$].

KDRI, DBD and donor age were significantly associated with a higher risk of death censored and overall graft failure, whereas vascular disease and dialysis time were only significantly associated with the latter. Compared to rabbit Anti-thymocyte globulin (r-ATG), neither alemtuzumab nor Interleukin 2 Receptor Antagonists (IL-2RA) was associated with increased death-censored graft failure. However, IL-2RA was associated with a 5% increased risk of overall graft failure [HR 1.05, CI (1.01, 1.10), $p = 0.021$]. Steroid use was associated with an increased risk of overall graft failure but not death-censored kidney graft failure. Compared to conventional single kidney transplants, en-bloc transplants were associated with lower death-censored and overall graft failure; dual kidney transplants were associated with lower overall graft failure but not death-censored graft failure (Table 3).

TABLE 2 Short-term outcomes by DGF status.

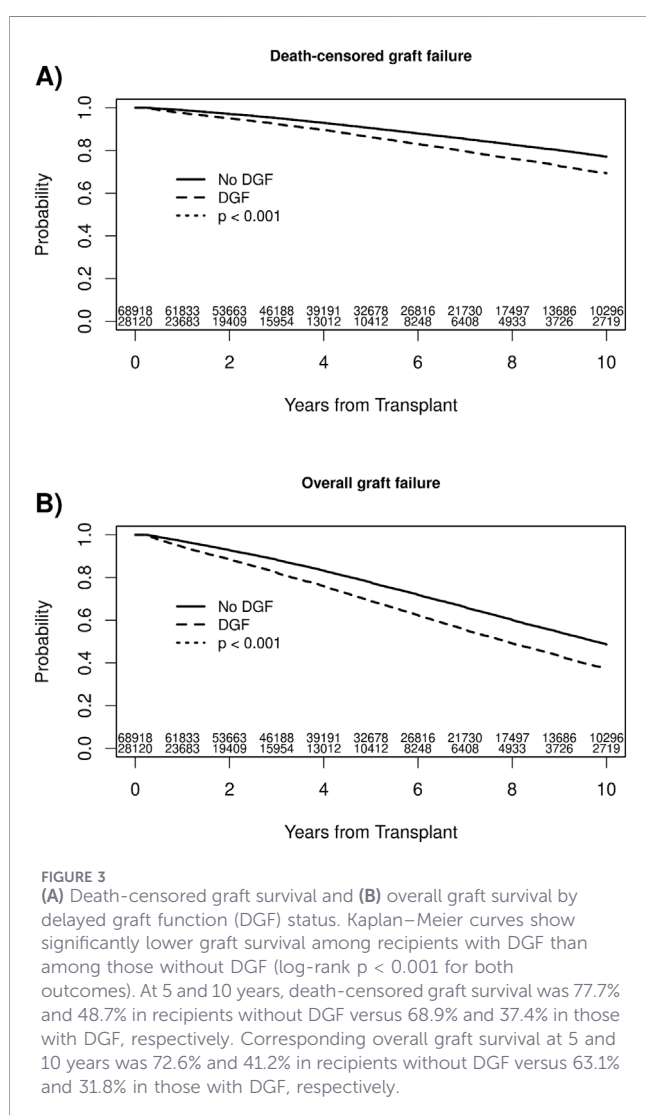
Outcome	No DGF (N = 72,481)	DGF (N = 31,197)	p value
Six-month rejection ¹	3,140 (4.7%)	1,615 (6.0%)	<0.001
One-year rejection	4,601 (7.3%)	2,180 (8.9%)	<0.001
Length of stay (Days)	5.7 (10.15)	8.2 (12.0)	<0.001
One-year re-hospitalization	28,812 (45%)	1,308 (54.1%)	<0.001
BK infection ²	1970 (9.4%)	639 (8.8%)	0.141
One-year creatinine (mg/dL)	1.37 (0.59)	1.58 (0.71)	<0.001

Values are presented as mean (SD) for continuous variables and as n (%) for categorical variables. P-values were calculated using t-tests for continuous variables and chi-square tests for categorical variables.

Abbreviations: DGF, delayed graft function; BK, BK, polyomavirus.

¹Rejection is defined as biopsy-proven or treated rejection.

²BK, virus data available through 2014 only.



To further explore the relationship between delayed graft function (DGF) and acute rejection, we performed stratified analyses based on rejection status within the first-year post-transplant. DGF remained significantly associated with both

death-censored and overall graft failure in recipients with and without rejection. No significant interaction between DGF and rejection status was observed, suggesting that DGF's adverse effect on long-term outcomes is independent of rejection status (Table 4).

Outcomes among mate-kidneys cohort

To control for unaccounted donor factors, we performed a mate-kidney analysis, and we found, in the unadjusted analysis, that those who experienced DGF, had lower death-censored kidney survival (log-rank $p < 0.001$) and overall kidney graft survival (log-rank $p < 0.001$), compared to those without DGF (Figure 4). In the mate-kidneys cohort with fully adjusted models (Table 5), delayed graft function (DGF) was associated with an 58% higher risk of death-censored graft failure (HR 1.58; 95% CI 1.39–1.80; $p < 0.01$) and a 35% higher risk of overall graft failure (HR 1.35; 95% CI 1.25–1.46; $p < 0.01$).

Discussion

In this national analysis of more than 100,000 deceased donor kidney recipients, we demonstrate that DGF is associated with substantially increased risks of long-term death-censored and overall graft failure, with adverse effects persisting for up to a decade after transplantation. Notably, even after restricting the analysis to mate kidneys (thereby fully controlling for donor quality) DGF remained a powerful independent predictor of inferior allograft survival. These findings highlight the lasting clinical significance of DGF well beyond the perioperative period.

Our results extend and significantly strengthen prior work. Existing literature has documented the short-term consequences of DGF, including higher rejection risk, prolonged recovery, and increased hospital utilization [11–19]. However, long-term outcomes have been inconsistently reported, limited by small sample sizes or short follow-up. The present study confirms that the detrimental effects of DGF persist well into late post-transplant years and are not explained solely by baseline donor characteristics or center-level practice variation.

TABLE 3 Cox proportional hazard models for death-censored and overall kidney graft failure by DGF status.

Variable	Death-censored graft failure [HR, (95% C.I), p-value]	Overall graft failure [HR, (95% C.I), p-value]
DGF	[HR 1.40 (1.33, 1.47), p < 0.01]	[HR 1.27, (1.23, 1.31), p < 0.01]
Recipient age	[HR 0.97 (0.97, 0.97), p < 0.01]	[HR 1.02, (1.02, 1.02), p < 0.01]
Recipient sex (male)	[HR 1.05, (0.99, 1.10), p = 0.08]	[HR 1.09, (1.06, 1.13), p < 0.01]
Ethnicity		
White	Ref	Ref
Black	[HR 1.42 (1.34, 1.51), p < 0.01]	[HR 0.99, (0.95, 1.02), p = 0.42]
Hispanic	[HR 0.86, (0.80, 0.93), p < 0.01]	[HR 0.77, (0.74, 0.81), p < 0.01]
Other	[HR 0.87, (0.79, 0.96), p < 0.01]	[HR 0.73, (0.69, 0.78), p < 0.01]
ESKD etiology		
DM	Ref	Ref
PCKD	[HR 0.61, (0.54, 0.68), p < 0.01]	[HR 0.49, (0.46, 0.52), p < 0.01]
GN	[HR 0.87, (0.79, 0.94), p < 0.01]	[HR 0.68, (0.65, 0.71), p < 0.01]
Other	[HR 0.95, (0.9, 1.01), p = 0.08]	[HR 0.75, (0.72, 0.77), p < 0.01]
Dialysis vintage	[HR 1.00, (1, 1.01), p = 0.39]	[HR 1.03, (1.02, 1.03), p < 0.01]
Vascular disease	[HR 1.06, (0.99, 1.15), p = 0.09]	[HR 1.26, (1.21, 1.31), p < 0.01]
Single kidney	Ref	Ref
<i>En-bloc</i>	[HR 0.54, (0.43, 0.68), p < 0.01]	[HR 0.63, (0.55, 0.73), p < 0.01]
Dual kidney	[HR 0.82, (0.67, 1.01), p = 0.08]	[HR 0.82, (0.73, 0.93), p < 0.01]
Local organ	[HR 0.94, (0.88, 1.01), p = 0.09]	[HR 0.99, (0.95, 1.04), p = 0.79]
KDRI	[HR 1.89, (1.68, 2.13), p < 0.01]	[HR 1.48, (1.38, 1.60), p < 0.01]
DBD	[HR 1.13, (1.05, 1.21), p < 0.01]	[HR 1.11, (1.06, 1.16), p < 0.01]
Donor age	[HR 1.01, (1.01, 1.01), p < 0.01]	[HR 1.01, (1.0, 1.01), p < 0.01]
Donor ethnicity		
White	Ref	Ref
Black	[HR 1.12, (1.04, 1.20), p < 0.01]	[HR 1.05, (1.00, 1.10), p = 0.03]
Hispanic	[HR 0.94, (0.88, 1.02), p = 0.12]	[HR 0.95, (0.91, 1.00), p = 0.04]
Other	[HR 1.15, (1.02, 1.30), p = 0.02]	[HR 1.09, (1.01, 1.18), p = 0.02]
Donor sex (male)	[HR 0.98, (0.94, 1.03), p = 0.49]	[HR 0.98, (0.95, 1.01), p = 0.12]
Pumped	[HR 1.01, (0.95, 1.07), p = 0.80]	[HR 1.00, (0.96, 1.04), p = 0.97]
Cold ischemia time	[HR 1.00, (1, 1.01), p = 0.13]	[HR 1.00, (1, 1.01), p = <0.01]
Pre-KAS	Ref	
KAS	[HR 0.85, (0.80, 0.90), p < 0.01]	[HR 1.04, (1.00, 1.08), p = 0.04]
KAS-250	[HR 0.95, (0.72, 1.25), p = 0.72]	[HR 1.04, (0.89, 1.21), p = 0.64]
HLA-mismatch		
0	Ref	Ref
1–3	[HR 1.25, (1.10, 1.41), p < 0.01]	[HR 1.09, (1.02, 1.17), p < 0.01]
4–6	[HR 1.37, (1.22, 1.53), p < 0.01]	[HR 1.13, (1.06, 1.21), p < 0.01]
cPRA	[HR 1.09, (1.01, 1.19), p = 0.04]	[HR 1.11, (1.05, 1.17), p < 0.01]

(Continued)

TABLE 3 Continued

Variable	Death-censored graft failure [HR, (95% C.I), p-value]	Overall graft failure [HR, (95% C.I), p-value]
Induction type		
r-ATG	Ref	Ref
IL-2RA	[HR 1.05, (0.98, 1.13) p = 0.15]	[HR 1.05, (1.01, 1.10) p = 0.02]
Alemtuzumab	[HR 1.05, (0.97, 1.14) p = 0.23]	[HR 0.99, (0.94, 1.05) p = 0.79]
Steroid maintenance	[HR 1.03, (0.96, 1.1) p = 0.47]	[HR 1.07, (1.03, 1.12) p < 0.01]

Hazard ratios (HR) with 95% confidence intervals (CI) are reported. Models were adjusted for recipient demographics, donor characteristics, immunologic factors, and procurement variables as specified in the Methods section.

Continuous variables are modeled per-unit increases: recipient age and dialysis vintage per year, KDRI, per 0.1-unit increase, and cold ischemia time per hour.

Abbreviations: HR, hazard ratio; CI, confidence interval; DGF, delayed graft function; ESKD, end-stage kidney disease; KDRI, kidney donor risk index; DBD, donation after brain death; KAS, kidney allocation system; cPRA, calculated panel reactive antibody.

TABLE 4 Cox proportional hazard models for delayed graft function and graft failure stratified by acute rejection status.

Model	Death-censored graft failure [HR, (95% C.I), p-value]	Overall graft failure [HR, (95% C.I), p-value]
Model 1 (recipients with rejection)		
DGF	[HR 1.31, (1.15, 1.50); <0.01]	[HR 1.24, (1.12, 1.37); <0.01]
Model 2 (recipients without rejection)		
DGF	[HR 1.28, (1.21, 1.37); <0.01]	[HR 1.21, (1.17, 1.26); <0.01]
Model 3 (all recipients)		
DGF	[HR 1.3, (1.23, 1.39); <0.01]	[HR 1.22 (1.18, 1.27); <0.01]
Acute rejection	[HR 2.50, (2.30, 2.71); <0.01]	[HR 1.78, (1.67, 1.89); <0.01]
Interaction between DGF and rejection	[HR 0.92, (0.81, 1.06); 0.27]	[HR 0.98, (0.89, 1.08); 0.71]

Hazard ratios (HR) with 95% confidence intervals (CI) are reported. Models were adjusted for recipient demographics, donor characteristics, immunologic factors, and transplant-related variables consistent with the primary analysis.

Model 1: recipients with acute rejection in year 1; Model 2: recipients without rejection; Model 3: full cohort, with both delayed graft function and acute rejection as covariates, plus their interaction term.

All models were adjusted for the covariates as in the primary analysis.

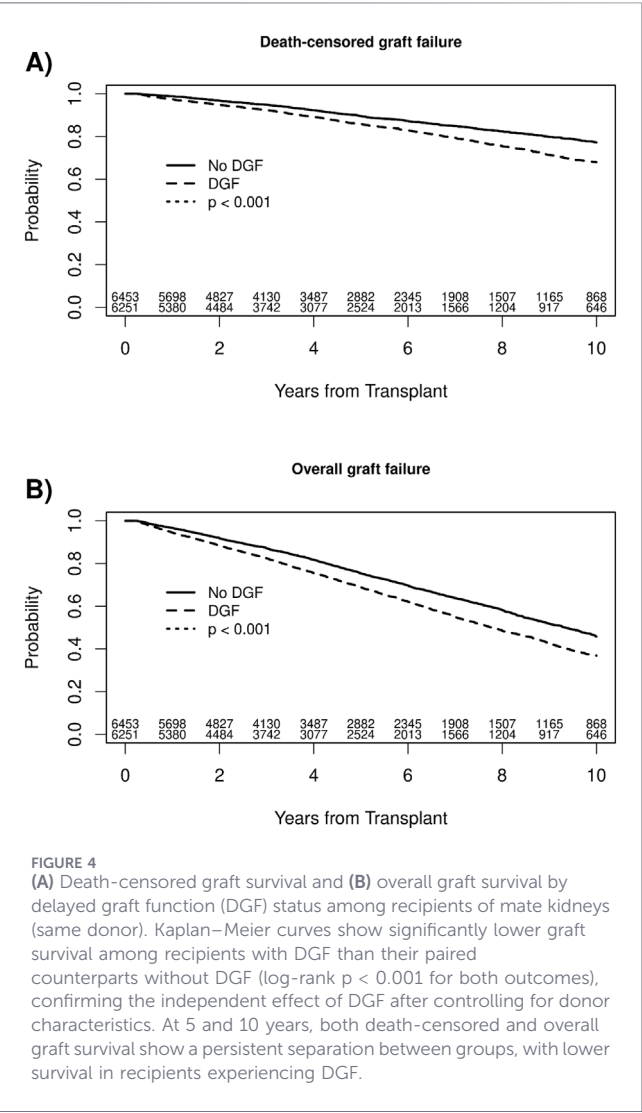
Abbreviations: HR, hazard ratio; CI, confidence interval; DGF, delayed graft function.

Mechanistically, these findings are biologically plausible. DGF reflects significant ischemia-reperfusion injury, which induces tubular epithelial cell damage, inflammatory activation, and heightened immunogenicity [13, 20, 21]. These early injuries may predispose the graft to chronic interstitial fibrosis, subclinical alloimmunity, and progressive decline in graft function. The higher rates of early rejection, worse early creatinine, and greater rehospitalization among DGF recipients in our cohort support this mechanistic pathway. However, in our additional analyses, the association between DGF and long-term graft failure persisted irrespective of rejection status, and no significant interaction was observed. These findings suggest that while rejection contributes to worse outcomes, DGF is independently associated with sustained effect on graft survival.

The mate-kidney analysis (using kidneys from the same donor) confirmed the independent association between DGF and adverse transplant outcomes. Recipients that experienced DGF had an 80% and a 45% increased risk of long-term death-censored and overall

graft failure, respectively. Again, these findings emphasize the relevance of DGF beyond the immediate post-transplant period. Moreover, the disparity observed between mate kidneys demonstrates the lasting impact of experiencing DGF on allograft survival, while also highlighting the pivotal contribution of recipient-specific factors in determining long-term transplant outcomes, independent of donor quality.

Machine perfusion (pump preservation) has been studied as a strategy to reduce the ischemia-reperfusion injury and the incidence of DGF. Some reports have demonstrated that its use is associated with a reduction in DGF [22–25], however its benefits might not be universally observed, as other studies have reported that machine perfusion was not associated with a clear benefit [26]. In our cohort, after adjusting for donor, recipient, immunologic and procurement factors, machine perfusion itself was not independently associated with improved long-term graft survival. These findings suggest that while the use of machine perfusion might provide some advantages in the short term, the long-term effect is not observed.



Our findings extend on the work by Lim et al [6] where they analyzed 74 pairs of DCD kidneys, with a median follow up of 1.9 years, and reported a greater proportion of recipients with DGF had experienced overall graft loss and death censored graft loss at 3 years compared to those without DGF. Compared to their study, our analysis leveraged a markedly larger and more diverse cohort (over 100,000 recipients including both DBD and DCD donors) thereby enhancing statistical power and generalizability. Our longer follow-up also allowed us to find late graft failures and show the sustained effect of DGF. Furthermore, by restricting our cohort to

recipients with uniform maintenance immunosuppression and applying a mixed-effects Cox model with center-level random effects we were able to minimize confounding from treatment heterogeneity and practice variation.

The Kidney Allocation System (KAS) was designed to improve allocation equity [6]; however, our findings highlight the increasing prevalence of DGF over our study period, particularly after the implementation of the KAS in 2014 and KAS250 in 2021, consistent with previous reports [27–29]. The reasons behind this trend are likely multifactorial and probably reflect continuous changes in donor and recipient selection criteria, the increased utilization of donation after circulatory death (DCD) organs (which are more prone to ischemia-reperfusion injury), as well as other logistical factors prolonging ischemia time [29, 30].

Strengths and limitations

This study has several limitations inherent to its retrospective, registry-based design. Despite adjustment for a comprehensive set of donor, recipient, immunologic, and procurement variables, residual confounding from unmeasured or incompletely captured factors may persist, and causal inferences cannot be definitively established. Although the mate-kidney analysis strengthens causal inference by controlling for donor-related factors, it does not account for center-level practices or recipient-specific clinical decisions that may influence outcomes.

Delayed graft function (DGF) was defined using the standard SRTR/OPTN definition (dialysis within the first week post-transplant); however, this definition does not capture the severity or duration of DGF, as the registry records it as a dichotomous variable. As such, we were unable to evaluate potential dose-response relationships between DGF severity and long-term outcomes. In addition, variability in clinical practice, particularly differences in dialysis initiation thresholds across centers, may lead to misclassification of DGF and introduce bias.

The SRTR also lacks granular data on immunosuppression management, including the timing of calcineurin inhibitor initiation, dosing, and drug levels. Therefore, we could not assess whether delayed or subtherapeutic tacrolimus exposure contributed to the higher rejection rates or worse outcomes observed in recipients with DGF.

Despite these limitations, our study has several and notable strengths. It includes a large cohort of transplant recipients, long-term follow-up and comprehensive reporting of outcomes and risk factors. Most importantly, the subgroup analysis of mate kidneys

TABLE 5 Cox proportional hazard models for death-censored and overall kidney graft failure by DGF Status among recipients of kidney pairs from the same donor (mate kidneys).

Variable	Death-censored graft failure	Overall graft failure
	[HR, (95% C.I.), p-value]	[HR, (95% C.I.), p-value]
DGF	[HR 1.58, (1.39, 1.80), $p < 0.01$]	[HR 1.35, (1.25, 1.46), $p < 0.01$]

Hazard ratios (HR) with 95% confidence intervals (CI) are reported. Models were adjusted for the same covariates as the primary analysis, including recipient demographics, immunologic factors, transplant-related variables, and additional adjustment for donor pairing. Abbreviations: HR, hazard ratio; CI, confidence interval; DGF, delayed graft function.

allowed us to control for donor-related confounders. Finally, the large sample size provided enough statistical power to detect statistically significant differences.

Conclusion

DGF is a strong, independent, and durable predictor of inferior long-term kidney allograft outcomes. Importantly, the association persists even after controlling for all donor-related factors using mate-kidney analysis. As DGF continues to rise in the current allocation era, strategies focused on prevention, early identification, and enhanced post-transplant management are critical to improving long-term outcomes for deceased donor kidney recipients.

Data availability statement

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

Ethics statement

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

Author contributions

OG: Concept/design, Drafting article, Data interpretation, Critical revision of article, Approval of article. AA: Concept/design, Drafting article, Data interpretation, Critical revision of article, Approval of article. MM: Data interpretation, Drafting article, Critical revision of article, Approval of article. GM: Concept/design, Data interpretation, Critical revision of article, Approval of article. BS: Data analysis/interpretation, Critical revision of article, Approval of article. AB: Concept/design, Data interpretation, Critical revision of article, Approval of article. NI: Concept/design, Data interpretation, Critical revision of article, Approval of article. CJ: Concept/design, Data interpretation, Critical revision of article, Approval of article. AC: Concept/design, Data interpretation, Critical revision of article, Approval of article. HA: Concept/design, Data interpretation, Critical revision of article, Approval of article. SR: Concept/design, Data analysis/interpretation, Drafting article, Critical revision of article, Approval of

article. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

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References

- Mallon DH, Summers DM, Bradley JA, Pettigrew GJ. Defining delayed graft function after renal transplantation: simplest is best. *Transplantation* (2013) 96(10):885–9. doi:10.1097/TP.0b013e3182a19348
- Mannon RB. Delayed graft function: the AKI of kidney transplantation. *Nephron* (2018) 140(2):94–8. doi:10.1159/000491558
- Yarlagadda SG, Coca SG, Formica RN, Jr, Parikh CR. Association between delayed graft function and allograft and patient survival: a systematic review and meta-analysis. *Nephrol Dial Transplant* (2008) 24(3):1039–47. doi:10.1093/ndt/gfn667
- Shoskes DA, Cecka JM. Effect of delayed graft function on short- and long-term kidney graft survival. *Clin Transpl* (1997) 297–303.
- Helfer MS, Pompeo JC, Costa ORS, Vicari AR, Ribeiro AR, Manfro RC. Long-term effects of delayed graft function duration on function and survival of deceased donor kidney transplants. *Braz J Nephrol* (2018) 41(2):231–41. doi:10.1590/2175-8239-jbn-2018-0065
- Lim WH, McDonald SP, Russ GR, Chapman JR, Ma MK, Pleass H, et al. Association between delayed graft function and graft loss in donation after cardiac death kidney Transplants—a paired kidney registry analysis. *Transplantation* (2017) 101(6):1139–43. doi:10.1097/TP.0000000000001323
- Swanson KJ, Bhattarai M, Parajuli S. Delayed graft function: current status and future directions. *Curr Opin Organ Transpl* (2023) 28(1):1–7. doi:10.1097/MOT.0000000000001034
- Cooper M, Wiseman AC, Doshi MD, Hall IE, Parsons RF, Pastan S, et al. Understanding delayed graft function to improve organ utilization and patient outcomes: report of a scientific workshop sponsored by the national kidney foundation. *Am J Kidney Dis* (2023) 83:360–9. doi:10.1053/j.ajkd.2023.08.018
- HraS A. Kidney allocation system (KAS). *Health Resour Serv Adm* (2025). Available online at: <https://www.hrsa.gov/optn/professionals/resources/kidney-pancreas/kidney-allocation-system> (Accessed January 5, 2026).
- Sharing UNfO. Community update: policy development based on continuous distribution (2025). Available online at: <https://unos.org/news/community-update-continuous-distribution-policy-development/> (Accessed January 5, 2026).
- Wu WK, Famure O, Li Y, Kim SJ. Delayed graft function and the risk of acute rejection in the modern era of kidney transplantation. *Kidney Int* (2015) 88(4):851–8. doi:10.1038/ki.2015.190
- Jadlowiec CC, Hippen B, Gill J, Heilman R, Stewart D, Reddy KS, et al. Current opinions on DGF management practices: a survey of the United States and Canada. *Clin Transplant* (2023) 37(5):e14949. doi:10.1111/ctr.14949
- Melih KV, Boynuegri B, Mustafa C, Nilgun A. Incidence, risk factors, and outcomes of delayed graft function in deceased donor kidney transplantation. *Transpl Proc* (2019) 51(4):1096–100. doi:10.1016/j.transproceed.2019.02.013
- Brier ME, Ray PC, Klein JB. Prediction of delayed renal allograft function using an artificial neural network. *Nephrol Dial Transplant* (2003) 18(12):2655–9. doi:10.1093/ndt/gfg439
- Gentil M, Alcaide M, Algarra G, Pereira P, Toro J, González-Roncero F, et al. Impact of delayed graft function on cadaveric kidney transplant outcome. *Transplant Proc* (2003) 35(2):689–91. doi:10.1016/s0041-1345(03)00048-4
- Jadlowiec CC, Heilman RL, Smith ML, Khamash HA, Huskey JL, Harbell J, et al. Transplanting kidneys from donation after cardiac death donors with acute kidney injury. *Am J Transplant* (2020) 20(3):864–9. doi:10.1111/ajt.15653
- Helfer MS, Vicari AR, Spuldar F, Gonçalves LFS, Manfro RC. Incidence, risk factors, and outcomes of delayed graft function in deceased donor kidney transplantation in a Brazilian center. *Transplant Proc* (2014/07/01/2014) 46(6):1727–9. doi:10.1016/j.transproceed.2014.05.026
- de Sandes-Freitas TV, Mazzali M, Manfro RC, Andrade LGM, Vicari AR, Sousa MV, et al. Exploring the causes of the high incidence of delayed graft function after kidney transplantation in Brazil: a multicenter study. *Transpl Int* (2021) 34(6):1093–104. doi:10.1111/tri.13865
- Hu X, Zheng J, Li Y, Tian XH, Tian PX, Xiang HL, et al. Prediction of kidney transplant outcome based on different DGF definitions in Chinese deceased donation. *BMC Nephrol* (2019/11/13 2019) 20(1):409. doi:10.1186/s12882-019-1557-x
- Siedlecki A, Irish W, Brennan DC. Delayed graft function in the kidney transplant. *Am J Transpl* (2011) 11(11):2279–96. doi:10.1111/j.1600-6143.2011.03754.x
- Ponticelli C, Reggiani F, Moroni G. Delayed graft function in kidney transplant: risk factors, consequences and prevention strategies. *J Pers Med* (2022) 12(10):1557. doi:10.3390/jpm12101557
- O'callaghan J, Morgan R, Knight S, Morris P. Systematic review and meta-analysis of hypothermic machine perfusion versus static cold storage of kidney allografts on transplant outcomes. *J Br Surg* (2013) 100(8):991–1001.
- Gill J, Dong J, Eng M, Landsberg D, Gill JS. Pulsatile perfusion reduces the risk of delayed graft function in deceased donor kidney transplants, irrespective of donor type and cold ischemic time. *Transplantation* (2014) 97(6):668–74. doi:10.1097/01.TP.0000438637.29214.10
- Moers C, Pirenne J, Paul A, Ploeg RJ, Machine Preservation Trial Study Group. Machine perfusion or cold storage in deceased-donor kidney transplantation. *New Engl J Med*. (2012) 366(8):770–1. doi:10.1056/NEJMc1111038
- Li Z, Mahgoub MY, Alrifai AZA, Smith B, Ryan RJ, Mour GK, et al. A comprehensive analysis on the impact of hypothermic machine perfusion and its influence on delayed graft function. *Transplant Direct* (2025) 11(12):e1875. doi:10.1097/TXD.0000000000001875
- Watson C, Wells A, Roberts R, Akoh JA, Friend PJ, Akyol M, et al. Cold machine perfusion versus static cold storage of kidneys donated after cardiac death: a UK multicenter randomized controlled trial. *Am J Transplant* (2010) 10(9):1991–9. doi:10.1111/j.1600-6143.2010.03165.x
- Taber DJ, DuBay D, McGillicuddy JW, Nadig S, Bratton CF, Chavin KD, et al. Impact of the new kidney allocation system on perioperative outcomes and costs in kidney transplantation. *J Am Coll Surg* (2017) 224(4):585–92. doi:10.1016/j.jamcollsurg.2016.12.009
- Parker WF, Ross LF, Richard Thistlethwaite J, Jr., Gallo AE. Impact of the kidney allocation system on young pediatric recipients. *Clin Transpl* (2018) 32(4):e13223. doi:10.1111/ctr.13223
- Zhang X, Potluri VS, Molinari M, Giuntella O, Hariharan S, Puttarajappa CM. Impact of kidney allocation system 250 policy on 1-year graft loss. *Am J Transplant* (2025) 25(6):1253–63. doi:10.1016/j.ajt.2024.12.011
- Cron DC, Husain SA, Adler JT. The new distance-based kidney allocation system: implications for patients, transplant centers, and organ procurement organizations. *Curr Transpl Rep* (2022) 9(4):302–7. doi:10.1007/s40472-022-00384-z



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Comparable outcomes with anti-thymocyte globulins versus basiliximab in kidney transplantation from controlled circulatory death donors

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Due to organ shortage, donation after circulatory death (DCD) has increased. As DCD kidney grafts have higher rates of delayed graft function (DGF), the French national protocol mandates the use of hypothermic machine perfusion and routine anti-thymocyte globulin (ATG) induction therapy. However, evidence favoring ATG over interleukin-2 receptor antagonists, such as basiliximab, remains scarce. We retrospectively analysed a single-center cohort of 158 low immunological risk patients who underwent DCD kidney transplantation between 2015 and 2023. Patients transplanted before March 2020 received ATG (n = 64), while those transplanted thereafter (during and after the COVID-19 pandemic) received basiliximab (n = 94). Baseline characteristics were comparable, except for recipient age (lower in the basiliximab group), and cold ischemia times were similar. There were no

differences in primary non-function, DGF rates (17% after ATG versus 12% after basiliximab), biopsy-proven rejection, one-year renal function or graft survival. ATG was associated with prolonged EBV and BK viremia without increased complications. Hospital stays were similar, but ATG induction was 1.7 times more expensive. These findings were confirmed in an independent French multicenter cohort (n = 506). In conclusion, basiliximab appears to be a cost-effective alternative to ATG for low-immunological-risk patients receiving DCD kidney grafts, for whom ATG is not clearly indicated.

KEYWORDS

antithymocyte globulin, basiliximab, DCD (donation after circulatory death), delayed graft function, induction

Introduction

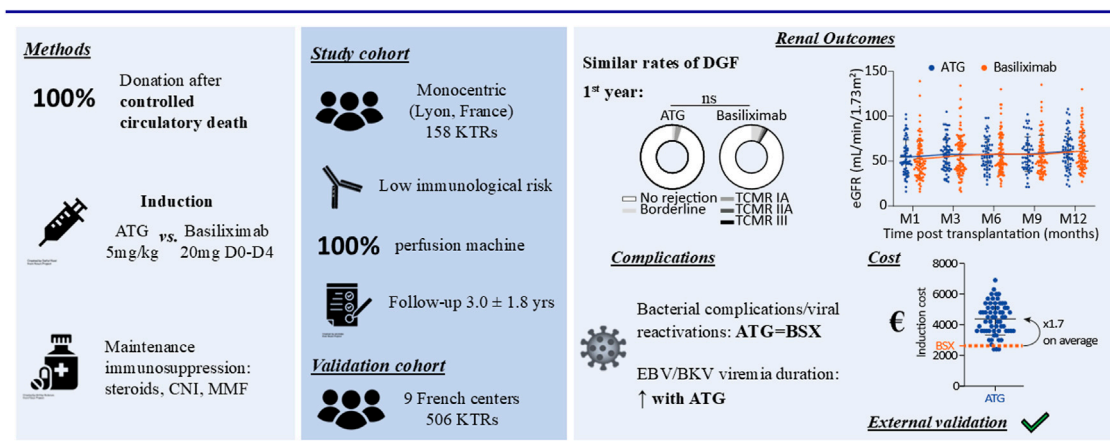
Kidney transplantation is the preferred treatment for patients with end-stage renal disease (ESRD), offering both improved quality of life [1] and significantly better survival [2, 3] for only a fraction of the costs of hemodialysis. However, due to the shortage of organ available there is a growing imbalance between the number of patients waiting for a graft and the number of transplantations performed each year [4]. As a result, many patients die each year while waiting for a compatible graft [4, 5].

To address the problem of organ shortage and optimize access to transplants, various strategies have been implemented to expand the donor pool. These include regenerative medicine approaches and xenotransplantation - currently still in the research phase [6, 7] - as well as the already established use of living donors and the extension of criteria for transplantation from deceased donors. In France, the “Maastricht 3 program”, launched in 2015, enabled the procurement of organs from donors after controlled circulatory death (DCD) [8]. This program has significantly contributed to increase the availability of kidney transplants. Between 2014 and 2024, the total number of

kidney transplants from deceased donors in France rose by 16.2% (2718 in 2014 vs. 3159 in 2024), with DCD donors accounting for nearly all this increase, while the number of donations after brain dead remained stable [4]. A similar trend has been observed in the United States [5].

However, due to the unavoidable warm ischemia time before organ retrieval, DCD transplants are inherently associated with an increased risk of delayed graft function (DGF) [9, 10]. In France, several measures have been proposed to mitigate the risk of DGF, and, more critically, primary non-function (PNF). First, all grafts from DCD donors must undergo hypothermic machine perfusion, which has been shown to reduce the risk of DGF [11, 12] particularly in donors aged over 60 [13]. Second, cold ischemia time (CIT), which is a known variable associated with DGF [12–14], is limited to 18 h (with a strong recommendation to keep it below 12 h). Third, at the time the program was set up, the French regulatory agency recommended the systematic use of depleting induction therapy with polyclonal antibodies (either anti-thymocyte globulins (ATG) or anti-lymphocyte globulins) to mitigate ischemia-reperfusion injury [15] and to promote early graft function [16] by allowing delayed introduction of calcineurin inhibitors. Interestingly, the

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GRAPHICAL ABSTRACT

combination of hypothermic machine perfusion and polyclonal antibodies may have synergistic effects in reducing graft immunogenicity and lowering rejection rates following transplantation with a DCD graft. Hypothermic perfusion facilitates the release of tissue-resident leukocytes into the perfusion fluid [17], while polyclonal antibodies deplete circulating passenger leukocytes. Given that several allorecognition pathways rely directly on donor passenger leukocytes [18–20], this approach could contribute to a longer-term reduction in rejection.

Notably, the expected short and long-term efficacy of polyclonal antibodies after DCD transplantation has never been prospectively compared with interleukin-2 receptor antagonists (IL-2RA) such as basiliximab or daclizumab. Therefore, we retrospectively analyzed prospectively collected data from a single center to compare the short-, medium-, and long-term outcomes of ATG versus basiliximab induction in kidney transplant recipients from DCD donors and assess their cost-effectiveness. These findings were subsequently evaluated in an independent validation cohort.

Patients and methods

Study design

The study cohort consisted in a monocentric cohort of kidney transplant patients from Lyon University Hospital, France. It was used to retrospectively compare the impact of induction treatment in patients receiving kidneys from controlled circulatory death donors. All patients receiving a kidney transplantation from a DCD donor between January 1, 2015 and December 31, 2023 were enrolled. Data were prospectively collected. The validation cohort (DIVAT, Données Informatisées et Validées en Transplantation) included 506 adult recipients of DCD kidney transplants from 9 French centers [Necker (Paris), Saint Louis (Paris), Foch (Suresnes), le Kremlin-Bicêtre, Saint-Etienne, Nantes, Nancy, Montpellier, Nice] between January 1, 2015, and December 31, 2023.

Inclusion and exclusion criteria

In France, the Maastricht III program, which defines the rules for transplants using controlled DCD donors, imposes strict conditions. Donors must be strictly under the age of 70 and must not have acute organ failure. The agonic phase must last less than 3 h and the functional warm ischemia phase (time interval during which the organs are hypoperfused and then no longer perfused at all due to circulatory failure) less than 120 min for the kidneys. *Ex vivo* hypothermic perfusion is mandatory and must last at least 2 hours, while CIT must be < 18h. Recipients can only receive a first, HLA-compatible transplant.

Patients who received both available induction treatments were excluded from the analysis.

Definition of delayed graft function

Delayed graft function was defined as the need for at least one hemodialysis session after the transplantation.

Histological evaluation

Light microscopy was performed on systematic or “for cause” kidney biopsy samples fixed in formalin-acetic alcohol after staining with haematoxylin and eosin. Histological lesions were scored according to the Banff 2022 classification [21].

Statistical analysis

Qualitative variables were expressed as percentages and compared with the chi-square test or Fisher’s exact test when the conditions of application of chi-square were not met. Quantitative variables were expressed as mean $\pm$ SD and compared using Student’s t-test. All tests were two-sided. When repeated measurements of the same parameter were performed over time, two-way ANOVA or mixed effects model were used with Sidak’s multiple comparisons test. No imputation was performed. Analyses were performed using variable-specific complete-case analyses, excluding only observations with missing data for the variable under consideration. Incidence and survival data were analysed by Kaplan-Meier curve and compared using a log-rank test. A multivariable Cox proportional hazards model was used to assess patient and graft survival in the validation cohort. All data were analysed using Prism (GraphPad) or the R software. All analyses were performed using R Statistical Software (v4.5.1); R Core Team [22].

Study approval

The study was carried out in accordance with French legislation on biomedical research and the Declaration of Helsinki. All patients gave informed consent for the utilization of clinical data [Données Informatiques Validées en Transplantation (DIVAT cohort)] for research purpose (www.divat.fr, CNIL (Commission Nationale de l’Informatique et des Libertés) no. 914184, ClinicalTrials.gov: NCT02900040).

Results

Impact of the COVID-19 pandemic on induction therapy selection for DCD kidney transplants

Between January 2015 and December 2023, 163 patients received a transplant from a DCD donor. All received induction treatment with ATG (Thymoglobulin, Sanofi®, target dose 5 mg/kg) or an IL-2RA, basiliximab (Simulect, Novartis®, 20 mg day 0 and day 4). As they did not tolerate ATG, 5 patients were switched to basiliximab and received both molecules; they were therefore excluded from the study. Of the remaining 158 patients, the vast majority of those transplanted before the onset of COVID-19 pandemic in France (March 2020) have received ATG as an induction, as specified in the National protocol [23]. To limit the risk of COVID-19 induced mortality in the post-transplantation period [24, 25], almost all patients transplanted after March 2020 were induced with basiliximab (Figure 1A). However, the change in induction treatment was not accompanied by changes in

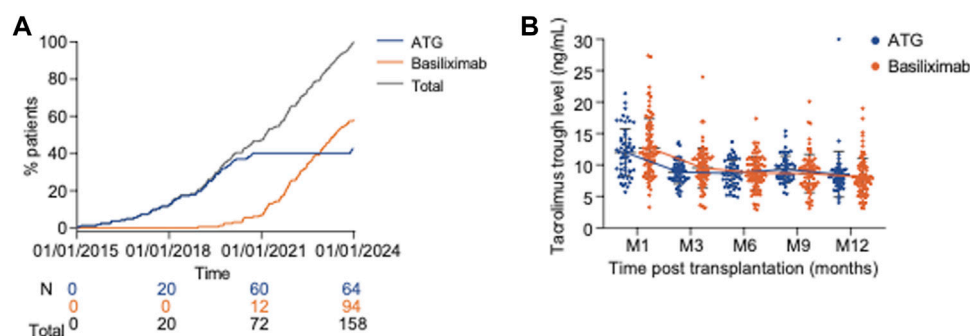


FIGURE 1

Evolution of Immunosuppressive Strategies Over Time. (A) Curve depicting the incidence of transplanted patients receiving a graft from a DCD donor, overall (black curve), induced with ATG (blue curve), or induced with basiliximab (orange curve). (B) Evolution of tacrolimus levels over time based on the induction therapy received. Mixed effect model, ns. M, month.

the maintenance immunosuppressive regimen. In particular, the target residual doses of calcineurin inhibitors were the same for all patients (Figure 1B; of note, the few patients ($n = 5$; 3%) on ciclosporin were excluded from this analysis), regardless of the induction treatment.

Taking advantage of this situation, we underwent the present study aiming at comparing the effect of induction on the prognosis of transplants with DCD donors. Therefore, transplant outcomes (in terms of renal parameters and complications) were compared between the 64 patients induced with ATG and the 94 patients induced with basiliximab, whose characteristics are presented in Table 1. Briefly, the mean recipient age was 56.2 ± 11.7 and 52.0 ± 12.6 years and the mean donor age was 50.6 ± 12.6 and 50.8 ± 13.6 years, in ATG and basiliximab groups, respectively. The type of initial kidney disease leading to end-stage renal disease was similar in both groups. The mean follow-up was 3.0 ± 1.8 years; nine patients died during follow-up.

The type of induction therapy has no impact on renal graft function or immunological outcomes

The cold-ischemia time was comparable between the groups (787 ± 214 min in ATG group vs. 769 ± 264 min in basiliximab group; unpaired t-test, $p = 0.66$; Table 1), and all kidneys were preserved on a perfusion machine. The rate of PNF was low (5; 3.16%) and did not differ according to the induction received (1 [1.56%] patient in ATG group, vs. 4 [4.25%] patients in basiliximab group; Fisher's exact test, $p = 0.65$; Figure 2A). Among the remaining 153 patients, 15% experienced DGF, 11 in ATG group and 12 in basiliximab group (chi-square test, $p = 0.48$; Figure 2B). Thus, induction does not affect the early outcomes after transplantation.

Since some studies have indicated a lower risk of biopsy-proven rejection with ATG induction compared to IL-2RA [26], we sought to determine whether this also holds true for DCD transplants, considered more immunogenic [27]. In our cohort, patients had very low levels of immunization (19.6% with preformed anti-HLA antibodies, none with preformed donor-specific antibodies; chi-square test for comparison between groups, $p = 0.86$; Table 1),

and the number of HLA-A/B/DR mismatches was also similar between the groups (3.7 ± 1.1 and 3.7 ± 1.2 in ATG and basiliximab groups, respectively; chi-square test, $p = 0.86$; Table 1). Overall, 5 patients experienced full blown T-cell mediated rejection (2 and 1 grade IA, 0 and 1 grade IIA, 0 and 1 grade III, in ATG and basiliximab groups, respectively) during the first year of follow-up, whereas one patient with concomitant BK nephropathy was excluded from this analysis. Over the same period of 1 year of follow-up, 6 other patients had borderline lesions (1 in ATG group and 5 in basiliximab group). The intensity of inflammatory lesions (borderline lesions or TCMR) did not differ between the groups (chi-square test, $p = 0.45$; Figure 2C). Of these 11 patients, all but two (with borderline lesions) were treated with corticosteroids and the control biopsy performed at 192 ± 83 days showed resolution of inflammation in all cases. Two of these 9 treated patients developed post-transplant diabetes mellitus. In addition, three patients in the basiliximab group developed microvascular inflammation (MVI) without DSA.

The evolution of renal function and proteinuria during the first year was comparable regardless of induction (Figures 2D,E). Patients had a mean one-year estimated glomerular filtration rate of 61.0 ± 19.6 mL/min/1.73 m² and 61.3 ± 20.3 mL/min/1.73 m² in ATG and basiliximab groups, respectively (unpaired t-test, $p = 0.93$; Figure 2D). At 1 year, among patients with an available proteinuria measurement, 10 of 59 and 12 of 80 had significant proteinuria ≥ 50 mg/mmol creatininuria in ATG and basiliximab groups, respectively (chi-square test, $p = 0.76$; Figure 2E).

At the systematic one-year evaluation, 102 patients had a biopsy (41 in the ATG group and 61 in the basiliximab group, mean delay 383 ± 23 days post transplantation [range 275–455 days]). Among all biopsies for which the following Banff scores were available, there was no difference in terms of tubulointerstitial ($i + t$; evaluated on 91 biopsies) or microvascular ($g + ptc$; 89 biopsies) acute inflammatory lesions, nor in chronic glomerular lesions (cg ; 90 biopsies) or interstitial fibrosis/tubular atrophy (IFTA; 88 biopsies; Figure 2F). Very few patients developed *de novo* donor-specific antibodies (2 in ATG group, 1 in basiliximab group, log-rank test, $p = 0.36$).

Finally, to integrate all the risks (PNF, DGF, rejection...) that could potentially affect graft function on the long term, we

TABLE 1 Patients characteristics—Original cohort.

Mean $\pm$ SD or n (%)	Whole cohort N = 158	ATG N = 64	BSX N = 94	p-value
Recipient characteristics at the time of transplantation				
Female	47 (30)	23 (36)	24 (26)	0.160
Age (years)	54 $\pm$ 12.4	56 $\pm$ 11.7	52 $\pm$ 12.6	0.046
BMI (kg/m ²)	26.2 $\pm$ 4.6	26.3 $\pm$ 5.1	26.1 $\pm$ 4.3	0.791
Blood group				0.044
A	64 (41)	20 (31)	44 (47)	
B	20 (13)	13 (20)	7 (8)	
O	65 (41)	26 (41)	39 (41)	
AB	9 (5)	5 (8)	4 (4)	
Renal disease				0.539
Diabete mellitus	24 (15)	10 (16)	14 (15)	
Glomerulonephritis	43 (27)	17 (27)	26 (28)	
Tubular-interstitial, genetic	12 (8)	20 (31)	19 (20)	
Vascular	22 (14)	11 (17)	11 (12)	
Uropathy	1 (1)	0	1 (1)	
Undetermined	34 (21)	11 (17)	23 (24)	
Anti-HLA antibody	31 (20)	13 (20)	18 (19)	0.857
DSA	0	0	0	NA
Donor characteristics				
Female	43 (27)	15 (23)	28 (30)	0.379
Age (years)	51 $\pm$ 13.2	51 $\pm$ 12.6	51 $\pm$ 13.6	>0.999
BMI (kg/m ²)	25.3 $\pm$ 5.3	25.5 $\pm$ 5.3	25.1 $\pm$ 5.3	0.642
Transplantation characteristics				
Cold ischemia time (min)	776 $\pm$ 244	787 $\pm$ 214	769 $\pm$ 264	0.651
Perfusion machine	158 (100)	64 (100)	94 (100)	NA
No. of HLA A/B/DR mismatches	3.7 $\pm$ 1.1	3.7 $\pm$ 1.1	3.7 $\pm$ 1.2	>0.999
Induction therapy dose (mg/kg for ATG, mg for BSX)	ND	4.9 $\pm$ 0.6	40 $\pm$ 2	NA
Maintenance immunosuppression at hospital discharge				
Tacrolimus	152 (96)	58 (91)	94 (100)	0.009
Cyclosporine	5 (3)	5 (8)	0	
MMF	154 (97)	63 (98)*	91 (97)	0.647
Steroids mTORi	157 (99)*	63 (98)*	94 (100)	0.405
Belatacept	2 (1)	0	2 (2)	0.515
	1 (1)	0	1 (1)	>0.999
CMV status				0.200
D ⁻ /R ⁻	29 (18)	14 (22)	15 (16)	
D ⁺ /R ⁻	21 (13)	5 (8)	16 (16)	
R ⁺	108 (69)	45 (70)	63 (68)	
EBV status				0.247
D ⁻ /R ⁻	1 (1)	0	1 (1)	
D ⁺ /R ⁻	3 (2)	0	3 (3)	
R ⁺	154 (97)	64 (100)	90 (96)	

Abbreviations: ATG, anti-thymocyte globulins; BSX, basiliximab; BMI, body mass index; DSA, donor-specific antibodies; ND, not determined; MMF, mycophenolate mofetil; mTORi, mechanistic target of rapamycin inhibitor; CMV, cytomegalovirus; EBV, Epstein-Barr virus; D/R, donor/recipient; *, death of a patient at day 17 of transplantation.

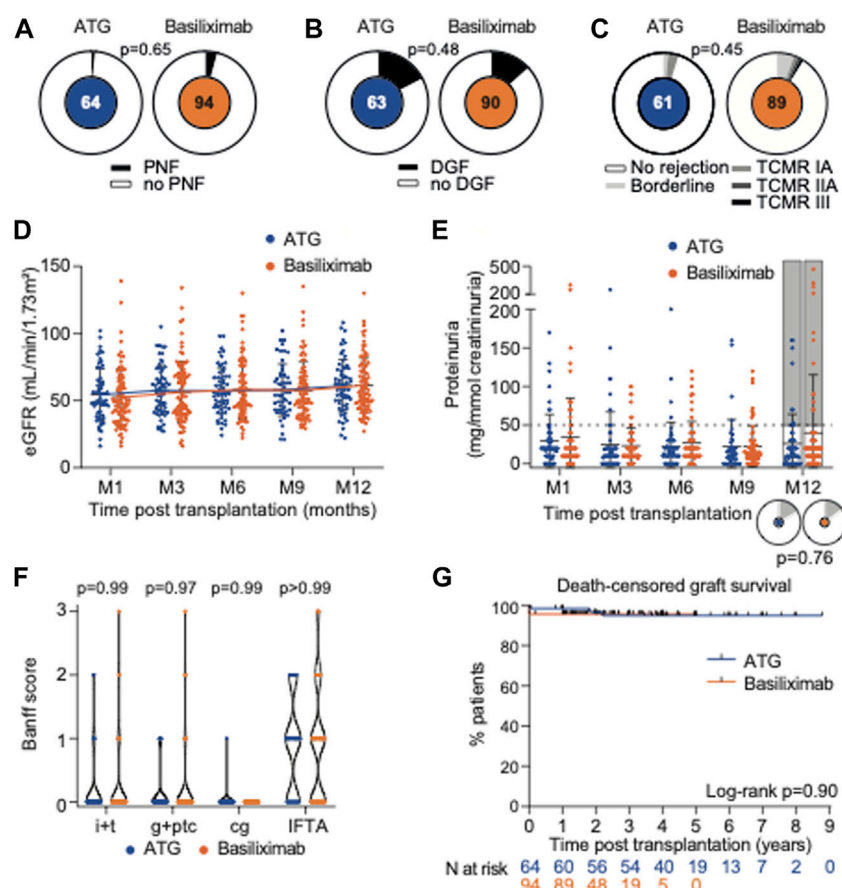


FIGURE 2

Comparison of renal functional and immunological outcomes in patients receiving ATG or basiliximab. (A–C) The prevalence of (A) primary non-function (PNF), (B) delayed graft function (DGF) and (C) rejection was compared between groups (A) in the entire cohort, (B) in patients without PNF and (C) in patients without PNF who were alive at the one-year follow-up. Fisher's test (A,B) and chi-square test (C), ns. (D,E) Evolution of (D) estimated glomerular filtration rate (eGFR) and (E) proteinuria levels over time based on the induction therapy received. Mixed effect models, ns. (F) Comparison of the Banff i + t, g + ptc, cg and IFTA scores between groups in the one-year biopsy. Two-way ANOVA, ns. (G) Kaplan-Meier survival curve for death-censored graft survival according to induction therapy. M; month. TCMR; T-cell mediated rejection.

compared the death-censored graft survival of the two groups and found no difference (log-rank test, $p = 0.90$; Figure 2G).

The type of induction therapy has no impact on infectious and cancer complications

Having demonstrated that graft survival was similar in the two groups of patients, we wanted to compare the safety of the two induction therapies.

Patient and graft survival were similar between the 2 groups (log-rank test, $p = 0.58$; Figure 3A). The number of bacterial complications (including bacteremia, pyelonephritis and bacterial pneumonia) during the first year was equivalent (chi-square test, $p = 0.46$; Figure 3B). Regarding viral complications, we observed no difference in the incidence of cytomegalovirus (CMV) viremia between the two groups (log-rank test, $p = 0.94$; Figure 3C). However, this result should be interpreted with caution because patients received protocolized prophylaxis against CMV with valganciclovir, the duration of which varied according to the serological status of the donor/recipient pair (6 months for the

high-risk seropositive donor/seronegative recipient group [D+/R-] and 3 months for intermediate-risk group [R+]). Although the difference was not statistically significant, there were more D+/R-high-risk pairs in the basiliximab than in the ATG group (16% vs. 8% respectively; Table 1). Regarding EBV, there were only 4 high-risk pairs (D+/R-) and all received basiliximab as induction. In the rest of patients (D+/R+ or D-/R+), the incidence of EBV reactivation was comparable between groups (log-rank test, $p = 0.75$; Figure 3D) and the maximum viral load was similar (mean maximum viral load 3.1 ± 0.7 log vs. 3.1 ± 0.6 log in the ATG and basiliximab groups, respectively; unpaired t-test, $p = 0.92$; Figure 3E), but the duration of viremia was significantly longer in patients who had received ATG (log-rank test, $p = 0.0028$; Figure 3F). Despite this difference, no patient of the cohort developed EBV-related post-transplant lymphoproliferative disease during the follow-up period. For the analysis focusing on BK virus, we excluded from the analysis the 5 patients who experienced PNF and remained on dialysis. The results were reminiscent to those obtained for EBV. The incidence of viremia (log-rank test, $p = 0.91$; Figure 3G), and maximum

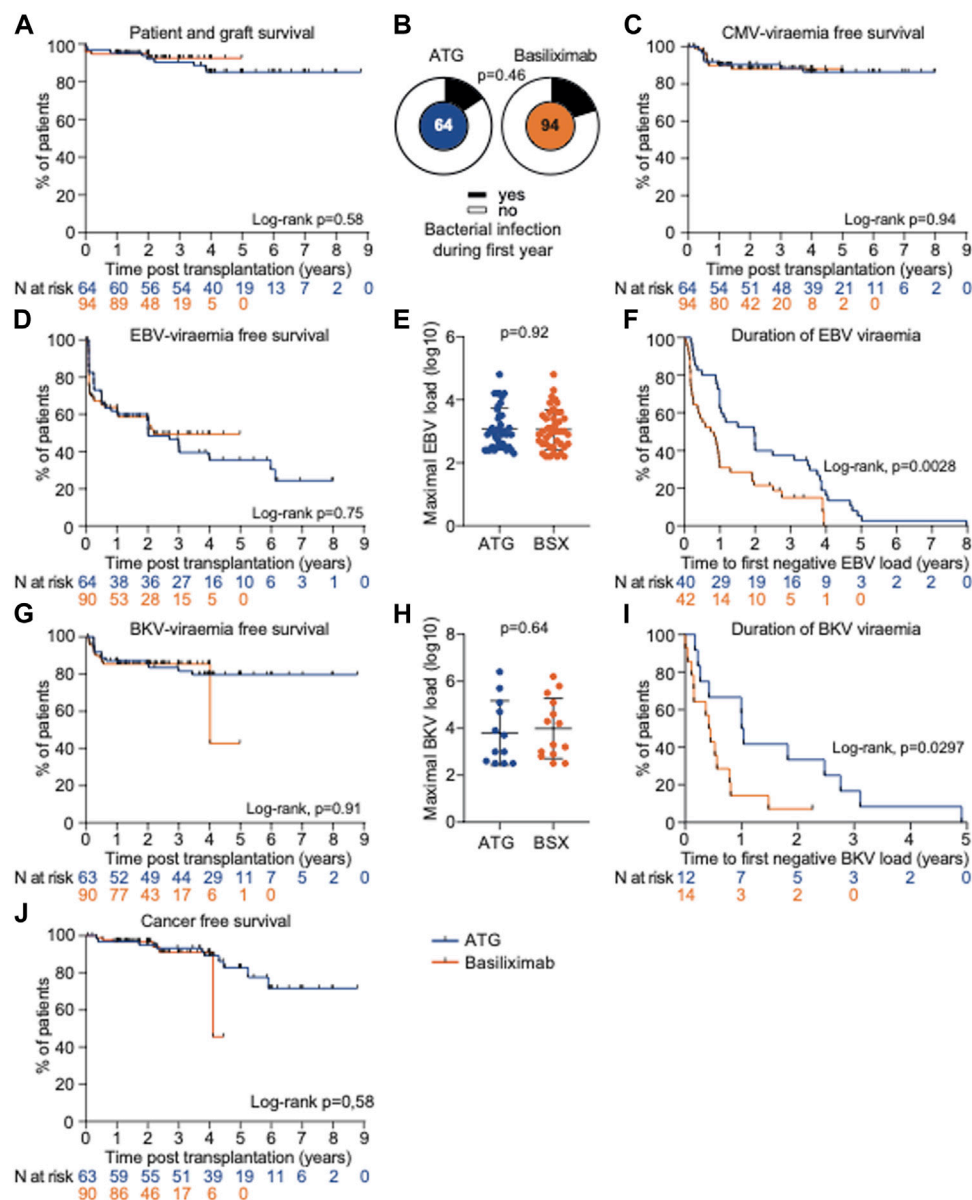


FIGURE 3

Comparison of infectious and cancer complications in patients receiving ATG or basiliximab. (A) Kaplan-Meier survival curve for patient and graft survival according to induction therapy. (B) The prevalence of bacterial infections during the first year was compared between each group in the entire cohort. Chi-square test, ns. (C,D) Kaplan-Meier survival curve for (C) CMV-viraemia and (D) EBV-viraemia free survival according to induction therapy in the entire cohort. (E) Comparison of the maximal EBV viral load between groups. Student's t-test, ns. (F) Kaplan-Meier curve of the delay between EBV reactivation and viral clearance according to induction therapy. (G) Kaplan-Meier survival curve for BKV-viraemia free survival according to induction therapy in patients without primary non function. (H) Comparison of the maximal BKV viral load between groups. Student's t-test, ns. (I) Kaplan-Meier curve of the delay between the first BKV viraemia and viral clearance according to induction therapy. (J) Kaplan-Meier survival curve for cancer free survival according to induction therapy.

viral loads (mean maximum viral load 3.8 ± 1.4 log vs. 4.0 ± 1.3 log in ATG and basiliximab groups, respectively; unpaired t-test, $p = 0.71$; Figure 3H), were similar in the two groups but patients from the ATG group exhibited longer duration of viremia (log-rank test, $p = 0.0297$; Figure 3I). However, this difference did not translate into a higher risk of BK virus-associated nephropathy (BKVN), as only 4 patients developed BKVN, 1 in the group ATG and 3 in the group basiliximab (log-rank test, $p = 0.41$).

Finally, the risk of cancer was equivalent in each group (log-rank test, $p = 0.58$; Figure 3J).

Validation of these results in an independent cohort

To assess the external validity of our findings, we analysed an independent validation cohort comprising 521 kidney transplant recipients from nine additional French centers who received a DCD graft between January 2015 and December 2023. Fifteen patients were excluded due to missing induction data. Among the 506 patients analysed, 408 received ATG and 98 received basiliximab. The characteristics of these patients are presented in

TABLE 2 Patients characteristics—Validation cohort.

Mean + SD or n (%)	Whole cohort N = 506	ATG N = 408	BSX N = 98	p-value
Recipient characteristics at the time of transplantation				
Female	176 (35)	150 (37)	26 (27)	0.073
Age (years)	54 ± 12.6	54 ± 12.6	52 ± 12.6	0.198
BMI (kg/m ²)	25.3 ± 4.4 ¹	25.4 ± 4.4 ¹	24.5 ± 4.4	0.049
Blood group				0.282
A				
B	218 (43)	171 (42)	47 (48)	
O	46 (9)	39 (10)	7 (7)	
AB	216 (43)	181 (44)	35 (36)	
ND	21 (4)	15 (3)	6 (6)	
	5 (1)	2 (1)	3 (3)	
Renal disease				0.888
Diabete mellitus	52 (10)	41 (10)	11 (11)	
Glomerulonephritis	120 (24)	97 (24)	23 (24)	
Tubular-interstitial, genetic	196 (39)	155 (38)	41 (42)	
Vascular	64 (13)	54 (13)	10 (10)	
Undetermined	74 (14)	61 (15)	13 (13)	
Anti-HLA antibody	266 (61 ²)	200 (60 ³)	66 (68 ⁴)	0.203
DSA	63 (15 ⁵)	47 (14 ⁶)	16 (16 ⁷)	1
Donor characteristics				
Female	144 (28)	121 (30)	23 (23)	0.274
Age (years)	52 ± 12.9 ⁸	52 ± 12.9	52 ± 13.4 ⁸	0.959
BMI (kg/m ²)	26.0 ± 5.5 ⁹	26.0 ± 5.6 ⁹	25.9 ± 5.5	0.846
Transplantation characteristics				
Cold ischemia time (min)	614 ± 247 ¹⁰	607 ± 250 ¹¹	643 ± 234 ¹²	0.181
Perfusion machine	450 (93 ¹³)	373 (93 ¹⁴)	77 (92 ¹⁵)	0.580
No. of HLA A/B/DR mismatches	3.5 ± 1.1 ¹⁶	3.5 ± 1.1	3.5 ± 1.1 ¹⁶	0.879
Maintenance immunosuppression at hospital discharge				
Tacrolimus	441 (87)	343 (84)	98 (100)	<0.001
Cyclosporine	63 (12)	63 (15)	0	
MMF	485 (96)	401 (98)	84 (86)	<0.001
Steroids mTORi	497 (98)	405 (99)	92 (94)	0.002
Belatacept	17 (3)	2 (1)	15 (15)	<0.001
	0	0	0	
CMV status				0.016
D ⁻ /R ⁻	124 (24)	94 (23)	30 (31)	
D ⁺ /R ⁻	86 (17)	63 (15)	23 (23)	
R ⁺	292 (58)	248 (61)	44 (45)	
ND	4 (1)	3 (1)	1 (1)	

(Continued)

TABLE 2 Continued

Mean + SD or n (%)		Whole cohort N = 506	ATG N = 408	BSX N = 98	p-value
EBV status					<0.001
	D ⁻ /R ⁻	1 (0)	1 (0)	0	
	D ⁺ /R ⁻	30 (6)	16 (4)	14 (14)	
	R ⁺	472 (93)	389 (95)	83 (85)	
	ND	3 (1)	2 (1)	1 (1)	

Abbreviations: ATG, anti-thymocyte globulins; BSX, basiliximab; BMI, body mass index; DSA, donor-specific antibodies; ND, not determined; MMF, mycophenolate mofetil; mTORi, mechanistic target of rapamycin inhibitor; CMV, cytomegalovirus; EBV, Epstein-Barr virus; D/R, donor/recipient.

¹ND for 1 recipient.

²ND for 73 recipients.

³ND for 72 recipients.

⁴ND for 1 recipient.

⁵ND for 83 recipients.

⁶ND for 82 recipients.

⁷ND for 1 recipient.

⁸ND for 1 donor.

⁹ND for 2 donors.

¹⁰ND for 6 grafts.

¹¹ND for 4 grafts.

¹²ND for 2 grafts.

¹³ND for 23 grafts.

¹⁴ND for 9 grafts.

¹⁵ND for 14 grafts.

¹⁶ND for 1 D/R pair.

Table 2. Of note, a higher proportion of patients in this cohort were immunised, with 61% having preformed anti-HLA antibodies and 15% having day-0 DSA. Furthermore, 7% of grafts in both groups were not preserved using a perfusion machine.

The distribution of induction regimens was more balanced over time than in the study cohort (Figure 4A). The rates of PNF, DGF (after exclusion of PNF), and rejections occurring during the first-year post-transplantation were similar between groups (Figures 4B,C). Kidney function and proteinuria at 1 year were also comparable between patients receiving ATG or basiliximab (Figure 4D).

Protocol biopsies were less systematically performed in this cohort, with fewer than one third of patients undergoing a one-year biopsy; therefore, no biopsy analysis at 1 year is reported.

Importantly, death-censored graft survival did not differ between the groups (log-rank test, $p = 0.44$; Figure 4E). Multivariable Cox regression adjusted for recipient age, HLA-A/B/DR mismatches and cold ischemia time confirmed that survival was comparable between basiliximab and ATG groups (HR 1.26, 95% CI 0.65–2.45; $p = 0.49$; Figure 4F).

From a safety perspective, the two groups were compared in terms of viral and malignancy-related complications. The incidences of CMV, EBV and BKV viremia were comparable (log-rank tests, $p = 0.26$, $p = 0.42$ and $p = 0.2$, respectively; Figures 5A–C). A non-significant trend towards an increased risk of cancer (all types combined) was observed in the ATG group (log-rank test, $p = 0.12$; Figure 5D).

ATG vs. basiliximab induction: comparable efficacy and risks, divergent costs

Basiliximab-treated patients exhibited slightly more early inflammatory kidney lesions without long-term graft impact,

while ATG-treated patients experienced prolonged EBV and BKV viremia without apparent severity. These non-significant differences balance the risks, offering no clear advantage for either agent. A pragmatic approach is to consider tolerance - measured here by the length of the initial hospital stay - and cost. Hospital stays were similar in both groups (mean 12 ± 6 days for ATG vs. 12 ± 7 days for basiliximab, $p = 0.80$; and 12 ± 8 days for ATG vs. 12 ± 7 days for basiliximab, $p = 0.48$; in the study and validation cohorts, respectively; Figure 6A). However, at our institution, ATG induction is, on average, 1.7 times (range 0.92–2.65) more expensive than basiliximab (€300 per ATG vial, median 15 vials per patient versus €1,315 per basiliximab vial, 2 vials per patient; Figure 6B). Therefore, we conclude that in the absence of a compelling clinical reason to prefer ATG, basiliximab appears to be a reasonable and cost-effective choice for induction in DCD kidney transplant recipients.

Discussion

In this study, we demonstrate, in two independent cohorts including a total of 664 patients, that induction therapy with ATG or basiliximab is associated with similar outcomes following kidney transplantation from DCD donors in recipients with a low immunological risk. ATG did not provide any advantage in terms of DGF or rejection incidence. However, it was also not associated with a higher risk of infectious or cancer-related complications.

In fact, at the time the program was set up in France, the rationale for preferring ATG to basiliximab appeared to be weak, as highlighted by the British and European recommendations on this topic [28, 29]. Since then, two studies have specifically compared ATG and IL-2RA in DCD kidney transplantation. In the first study [30], IL2-RA induction was not associated with a higher risk of DGF.

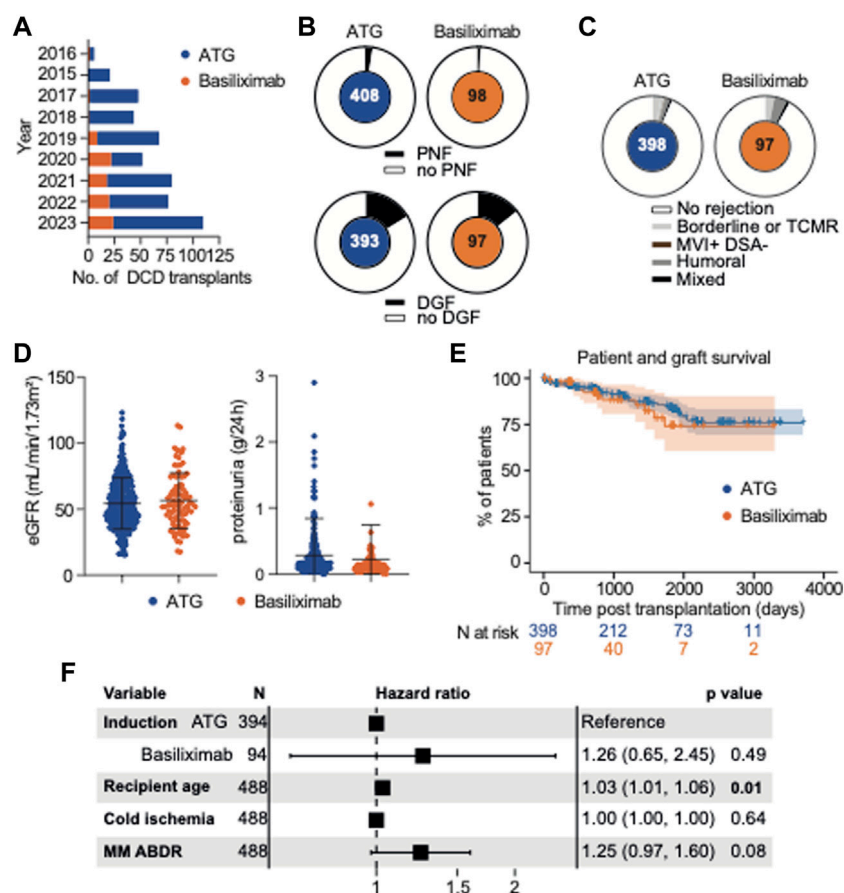


FIGURE 4

Renal functional and immunological outcomes in the validation cohort. (A) Histogram showing the annual number of DCD transplants by induction therapy in the validation cohort. (B) Prevalence of primary non-function (PNF) in the entire validation cohort and delayed graft function (DGF) in patients without PNF. Chi-square and Fisher's exact test, ns. (C) Prevalence of rejection in patients without PNF during first year of follow-up. Chi-square test, ns. (D) Comparison of estimated glomerular filtration rate (eGFR) and proteinuria level at 1 year's evaluation. Student's t-test, ns. (E) Kaplan-Meier survival curve for death-censored graft survival according to induction therapy. (F) Forest plot representing the results of the multivariable Cox regression analysis. Abbreviations: MVI, microvascular inflammation; DSA, donor-specific antibodies.

In the second study [31], ATG was associated with a lower incidence of acute rejection but did not reduce the risk of DGF. Notably, the rates of DGF in both studies were very high, compared to our study which found around 15% DGF, with comparable rates between the groups. Limiting ischemia as much as possible seems to be crucial for these particular grafts, but our CIT were comparable to those previously published. Therefore, this difference is more likely due to the systematic use of normothermic regional perfusion [32] and hypothermic perfusion machine [11, 12], which was not the case in the two published studies. Consequently, our results on induction must be interpreted as part of a strict protocol, which imposes the two technical constraints mentioned above. They cannot therefore be extrapolated outside this specific framework.

One of the strengths of our study is that many patients of the cohort study (65.8% of those with a functioning graft at 1 year) had a screening renal biopsy 1 year post-transplantation, allowing for the detection of subclinical graft inflammation that would not have been seen based only on for cause biopsy and functional parameters [33]. Additionally, the relatively long follow-up period reinforces the robustness of our conclusions.

The theoretical benefit of ATG in mitigating ischemia-reperfusion injury is based on experimental data suggesting that these polyclonal cocktails also contain antibodies specific of adhesion molecules [34, 35] potentially limiting ischemia-reperfusion damage [15] by modulating the inflammatory infiltrate of the graft. As ischemia-reperfusion injury can theoretically still contribute to long-term graft remodeling, ATG should limit chronic graft dysfunction (which can be evaluated by cg and IF/TA lesions). However, our findings do not support any benefit of ATG, or this mechanism may not be relevant in DCD transplant recipients.

Interestingly, we also observed lower rates of T cell-mediated rejection in the basiliximab group compared to previous reports, despite no apparent differences in maintenance immunosuppression. One possible explanation is that perfusion may help clear ischemia-induced pro-inflammatory mediators and donor-derived immune cells, thereby reducing graft immunogenicity and potentially allowing for less intensive immunosuppression. These results confirm that ATG and IL-2RA give similar results in terms of rejection in patients at low immunological risk, as shown in

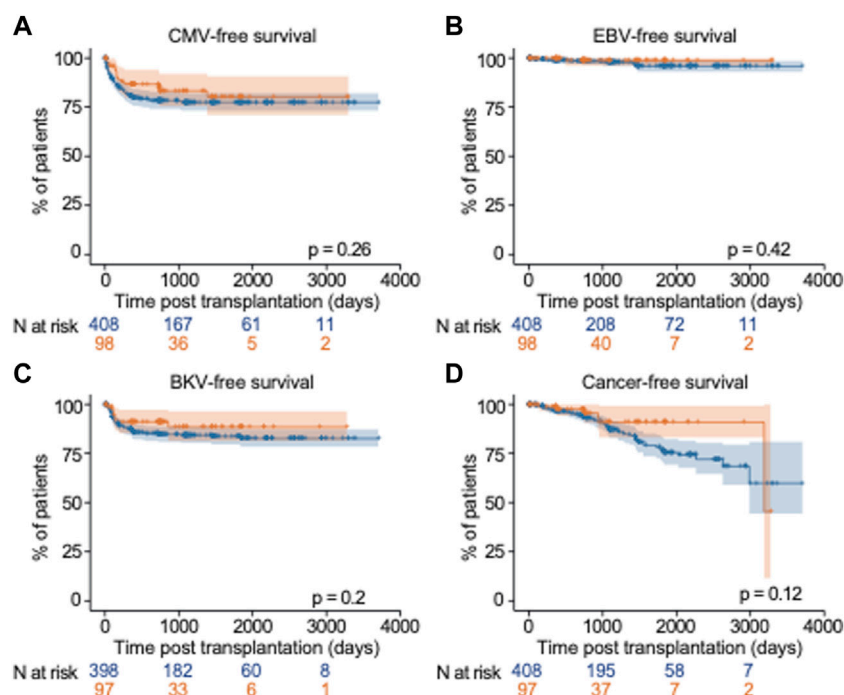


FIGURE 5
Viral and cancer complications in the validation cohort. (A,B) Kaplan-Meier survival curve for (A) CMV-viraemia and (B) EBV-viraemia survival according to induction therapy in the entire cohort. (C) Kaplan-Meier survival curve for BKV-viraemia free survival according to induction therapy in patients without primary non function. (D) Kaplan-Meier survival curve for cancer free survival according to induction therapy.

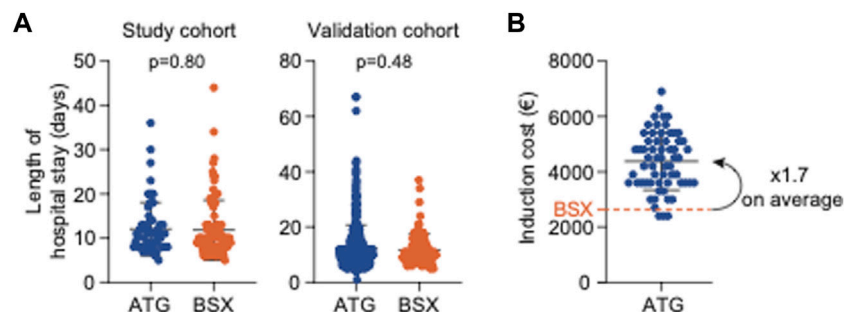


FIGURE 6
Comparison of hospital stay duration in patients receiving ATG or basiliximab and treatment costs. (A) Comparison of the length of hospital stay between groups in the study (left) and the validation (right) cohorts. Student's t-test, ns. (B) Comparison of the costs of each treatment. The dashed line represents the fixed cost of induction with basiliximab.

previously published studies in kidney transplantation with brain-dead donors [36–38].

While we expected more infectious complications, particularly viral, in the ATG group [39–44], we did not observe any excess risk associated with the use of this induction therapy in our study. This result has probably various explanations, such as the lower dose of ATG used in our study (5 mg/kg versus 5–10 mg/kg in other studies), and the systematic use of anti-CMV prophylaxis in patients at risk.

Everything else being equal, it makes sense to prioritize a less expensive drug that does not require the use of animal for its production. While this study was not designed to assess

environmental impact, it is reasonable to assume that monoclonal antibodies have a lower ecological footprint. Since one of the key advantages of transplantation over hemodialysis is its lower societal cost—both financially and in terms of resource consumption (such as plastic disposables and other materials)—optimizing certain parameters, such as induction, could further strengthen this economic and environmental benefit.

Our study has some limitations. First, while the cohort sizes are sufficiently large to support statistical conclusions, the population is highly homogeneous, which limits the generalizability of our findings to more diverse groups, such as

older recipients or those at higher immunological risk. Second, the study is retrospective. However, as the choice of induction therapy in the study cohort was primarily determined by the transplantation period, clinician-driven selection bias was minimized. Notably, induction strategy was the only variable that differed between the two study periods. Furthermore, in the independent validation cohort, the use of induction therapy was more evenly distributed over time, with comparable results, suggesting that the potential bias mentioned above is likely negligible. Third, the follow-up duration may not have been sufficient to fully assess the long-term impact of induction therapy on late complications, such as cancer. Finally, although our study suggests a longer duration of BKV and EBV replication in the ATG group, these findings should be interpreted with caution due to the lack of standardized viral monitoring, which may have introduced bias into the results.

In conclusion, our study does not support selecting induction therapy based solely on DCD donor criteria, as it does not influence transplant outcomes. From a cost-effectiveness perspective, basiliximab should be the preferred choice for patients who do not require ATG to prevent the recurrence of their initial nephropathy. Further studies are needed to determine the optimal approach for high immunological risk recipients.

Data availability statement

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

Ethics statement

The requirement of ethical approval was waived by Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé for the studies involving humans because All patients gave informed consent for the utilization of clinical data [Données Informatiques Validées en Transplantation (DIVAT)] for research purpose at transplantation. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because It was required at transplantation.

References

- Maglakelidze N, Pantsulaia T, Tchokhonelidze I, Managadze L, Chkhotua A. Assessment of health-related quality of life in renal transplant recipients and dialysis patients. *Transplant Proc* (2011) 43(1):376–9. doi:10.1016/j.transproceed.2010.12.015
- Tonelli M, Wiebe N, Knoll G, Bello A, Browne S, Jadhav D, et al. Systematic review: Kidney transplantation compared with dialysis in clinically relevant outcomes. *Am J Transplant* (2011) 11(10):2093–109. doi:10.1111/j.1600-6143.2011.03686.x
- Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LYC, et al. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med* (1999) 341(23):1725–30. doi:10.1056/NEJM199912023412303
- Agence de la biomédecine. *Agence de la biomédecine*. Available online at: <https://rams.agence-biomedecine.fr/greffe-renale-0> (Accessed April 9, 2025).
- Kidney - Scientific Registry of Transplant Recipients. Kidney - Scientific registry of transplant recipients. Available online at: <https://srtr.transplant.hrsa.gov/ADR/Chapter?name=Kidney&year=2023> (Accessed April 9, 2025).
- Montgomery RA, Stern JM, Lonze BE, Tatapudi VS, Mangiola M, Wu M, et al. Results of two cases of pig-to-human kidney xenotransplantation. *N Engl J Med* (2022) 386(20):1889–98. doi:10.1056/NEJMoa2120238
- Kawai T, Williams WW, Elias N, Fishman JA, Crisalli K, Longchamp A, et al. Xenotransplantation of a porcine kidney for end-stage kidney disease. *N Engl J Med* (2025) 392(19):1933–40. doi:10.1056/NEJMoa2412747
- Lamy FX, Atinault A, Thuong M. Prélèvement d'organes en France: état des lieux et perspectives. *La Presse Médicale* (2013) 42(3):295–308. doi:10.1016/j.lpm.2012.05.018

Author contributions

MD, AK, GO, FB, EM, OT, and XC designed the study. All authors collected the clinical data. XC and MD analysed the data and formatted the results. XC, MD, and OT wrote the first draft of the manuscript. All authors reviewed the article and approved the submitted version.

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Conflict of interest

EM has received speaker fees from Sanofi.

The remaining author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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9. Hoogland ERP, Snoeijis MGJ, Winkens B, Christaans MHL, Van Heurn LWE. Kidney transplantation from donors after cardiac death: uncontrolled *versus* controlled donation. *Am J Transplant* (2011) 11(7):1427–34. doi:10.1111/j.1600-6143.2011.03562.x
10. Summers DM, Watson CJE, Pettigrew GJ, Johnson RJ, Collett D, Neuberger JM, et al. Kidney donation after circulatory death (DCD): state of the art. *Kidney Int* (2015) 88(2): 241–9. doi:10.1038/ki.2015.88
11. Moers C, Smits JM, Maathuis MHJ, Treckmann J, Van Gelder F, Napieralski BP, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med* (2009) 360(1):7–19. doi:10.1056/NEJMoa0802289
12. Phillips BL, Ibrahim M, Greenhall GHB, Mumford L, Dorling A, Callaghan CJ. Effect of delayed graft function on longer-term outcomes after kidney transplantation from donation after circulatory death donors in the United Kingdom: a national cohort study. *Am J Transplant* (2021) 21(10):3346–55. doi:10.1111/ajt.16574
13. Cantafio AW, Dick AAS, Halldorson JB, Bakthavatsalam R, Reyes JD, Perkins JD. Risk stratification of kidneys from donation after cardiac death donors and the utility of machine perfusion. *Clin Transplant* (2011) 25(5):E530–E540. doi:10.1111/j.1399-0012.2011.01477.x
14. Locke JE, Segev DL, Warren DS, Dominici F, Simpkins CE, Montgomery RA. Outcomes of kidneys from donors after cardiac death: implications for allocation and preservation. *Am J Transplant* (2007) 7(7):1797–807. doi:10.1111/j.1600-6143.2007.01852.x
15. Aiello S, Cassis P, Mister M, Solini S, Rocchetta F, Abbate M, et al. Rabbit anti-rat thymocyte immunoglobulin preserves renal function during ischemia/reperfusion injury in rat kidney transplantation: thymoglobuline in post-transplant I/R injury. *Transpl Int* (2011) 24(8):829–38. doi:10.1111/j.1432-2277.2011.01263.x
16. Sabah TK, Khalid U, Ilham MA, Ablorsu E, Szabo L, Griffin S, et al. Induction with ATG in DCD kidney transplantation; efficacy and relation of dose and cell markers on delayed graft function and renal function. *Transpl Immunol* (2021) 66:101388. doi:10.1016/j.trim.2021.101388
17. Hullege-Peelen DM, Hesselink DA, Dieterich M, Minnee RC, Peeters A, Hoogduijn MJ, et al. Tissue-resident lymphocytes are released during hypothermic and normothermic machine perfusion of human donor kidneys. *Transplantation* (2024). 108(7). doi:10.1097/TP.0000000000004936
18. Larsen CP, Morris PJ, Austyn JM. Migration of dendritic leukocytes from cardiac allografts into host spleens. A novel pathway for initiation of rejection. *The J Experimental Medicine* (1990) 171(1):307–14. doi:10.1084/jem.171.1.307
19. Charmetant X, Chen CC, Hamada S, Goncalves D, Saison C, Rabeyrin M, et al. Inverted direct allorecognition triggers early donor-specific antibody responses after transplantation. *Sci Transl Med* (2022) 14(663):eabg1046. doi:10.1126/scitranslmed.abg1046
20. Charmetant X, Pettigrew GJ, Thauant O. Allorecognition unveiled: integrating recent breakthroughs into the current paradigm. *Transpl Int* (2024) 37:13523. doi:10.3389/ti.2024.13523
21. Naesens M, Roufosse C, Haas M, Leflaucheur C, Mannon RB, Adam BA, et al. The banff 2022 kidney meeting report: reappraisal of microvascular inflammation and the role of biopsy-based transcript diagnostics. *Am J Transplant* (2024) 24(3):338–49. doi:10.1016/j.ajt.2023.10.016
22. R Core Team. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing (2023). Available online at: <https://www.R-project.org/> (Accessed February 2, 2026).
23. Protocole des conditions à respecter pour réaliser des. Agence de la biomédecine (2003). Available online at: <https://www.agence-biomedecine.fr/Protocole-des-conditions-a-respecter-pour-realiser-des-prelevements-d-organes> (Accessed April 14, 2025).
24. Thauant O, Legeai C, Anglicheau D, Couzi L, Blanche G, Hazzan M, et al. IMPact of the COVID-19 epidemic on the moRTality of kidney transplant recipients and candidates in a French nationwide registry sTudy (IMPORTANT). *Kidney Int* (2020) 98(6):1568–77. doi:10.1016/j.kint.2020.10.008
25. Caillard S, Anglicheau D, Matignon M, Durrbach A, Greze C, Frimat L, et al. An initial report from the French SOT COVID registry suggests high mortality due to COVID-19 in recipients of kidney transplants. *Kidney Int* (2020) 98(6):1549–58. doi:10.1016/j.kint.2020.08.005
26. Brennan DC, Daller JA, Lake KD, Cibrik D, Del Castillo D, Thymoglobulin Induction Study Group. Rabbit antithymocyte globulin *versus* basiliximab in renal transplantation. *N Engl J Med* (2006) 355(19):1967–77. doi:10.1056/NEJMoa060068
27. Fuquay R, Renner B, Kulik L, McCullough JW, Amura C, Strassheim D, et al. Renal ischemia-reperfusion injury amplifies the humoral immune response. *J Am Soc Nephrol* (2013) 24(7):1063–72. doi:10.1681/ASN.2012060560
28. Andrews PA, Burnapp L, Manas DBritish Transplantation Society. Summary of the British transplantation society guidelines for transplantation from donors after deceased circulatory death. *Transplantation* (2014) 97(3):265–70. doi:10.1097/01.TP.0000438630.13967.c0
29. Van Heurn LWE, Talbot D, Nicholson ML, Akhtar MZ, Sanchez-Fructuoso AI, Weekers L, et al. Recommendations for donation after circulatory death kidney transplantation in Europe. *Transpl Int* (2016) 29(7):780–9. doi:10.1111/tri.12682
30. Favi E, Puliatti C, Iesari S, Monaco A, Ferraresso M, Cacciola R. Impact of donor age on clinical outcomes of primary single kidney transplantation from maastricht Category-III donors after circulatory death. *Transplant Direct* (2018) 4(10):e396. doi:10.1097/TXD.0000000000000835
31. Asderakis A, Sabah TK, Watkins WJ, Khalid U, Szabo L, Stephens MR, et al. Thymoglobulin *versus* alemtuzumab *versus* basiliximab kidney transplantation from donors after circulatory death. *Kidney Int Rep* (2022) 7(4):732–40. doi:10.1016/j.ekir.2022.01.1042
32. Oniscu GC, Randle LV, Muesan P, Butler AJ, Currie IS, Perera MTPR, et al. *In situ* normothermic regional perfusion for controlled donation after circulatory death—the United Kingdom experience. *Am J Transplant* (2014) 14(12):2846–54. doi:10.1111/ajt.12927
33. Thauant O, Legendre C, Morelon E, Kreis H, Mamzer-Bruneel MF. To biopsy or not to biopsy? Should we screen the histology of stable renal grafts? *Transplantation* (2007) 84(6):671–6. doi:10.1097/01.tp.0000282870.71282.ed
34. Michallet MC, Previle X, Flacher M, Fournel S, Genestier L, Revillard JP. Functional antibodies to leukocyte adhesion molecules in antithymocyte globulins1. *Transplantation* (2003) 75(5):657–62. doi:10.1097/01.TP.0000053198.99206.E6
35. Bonnefoy-Bérard N, Vincent C, Revillard JP. Antibodies against functional leukocyte surface molecules in polyclonal antilymphocyte and antithymocyte globulins. *Transplantation* (1991) 51(3):669–73. doi:10.1097/00007890-199103000-00024
36. Lebranchu Y, Bridoux F, Büchler M, Meur YL, Etienne I, Toupance O, et al. Immunoprophylaxis with basiliximab compared with antithymocyte globulin in renal transplant patients receiving MMF-Containing triple therapy. *Am J Transplant* (2002) 2(1):48–56. doi:10.1034/j.1600-6143.2002.020109.x
37. Mourad G, Rostaing L, Legendre C, Garrigue V, Thervet E, Durand D. Sequential protocols using basiliximab *versus* anti-thymocyte globulins in renal-transplant patients receiving mycophenolate mofetil and steroids. *Transplantation* (2004) 78(4):584–90. doi:10.1097/01.tp.0000129812.68794.cc
38. Ciancio G, Burke GW, Gaynor JJ, Carreno MR, Cirocco RE, Mathew JM, et al. A randomized trial of three renal transplant induction antibodies: early comparison of tacrolimus, mycophenolate mofetil, and steroid dosing, and newer Immune-Monitoring1. *Transplantation* (2005) 80(4):457–65. doi:10.1097/01.tp.0000165847.05787.08
39. Mourad G, Garrigue V, Squifflet JP, Besse T, Berthouix F, Alamartine E, et al. Induction *versus* noninduction in renal transplant recipients with tacrolimus-based immunosuppression1. *Transplantation* (2001) 72(6):1050–5. doi:10.1097/00007890-200109270-00012
40. Charpentier B, Rostaing L, Berthouix F, Lang P, Civati G, Touraine JL, et al. A three-arm study comparing immediate tacrolimus therapy with antithymocyte globulin induction therapy followed by tacrolimus or cyclosporine A in adult renal transplant recipients1. *Transplantation* (2003) 75(6):844–51. doi:10.1097/01.TP.0000056635.59888.EF
41. Bamouli J, Staack O, Crépin T, Halleck F, Saas P, Brakemeier S, et al. Anti-thymocyte globulins in kidney transplantation: focus on current indications and long-term immunological side effects. *Nephrol Dial Transpl* (2016) 32(10):1601–8. doi:10.1093/ndt/gfw368
42. Thangaraju S, Gill J, Wright A, Dong J, Rose C, Gill J. Risk factors for BK polyoma virus treatment and association of treatment with kidney transplant failure: insights from a paired kidney analysis. *Transplantation* (2016) 100(4):854–61. doi:10.1097/TP.0000000000000890
43. Schold JD, Rehman S, Kayler LK, Magliocca J, Srinivas TR, Meier-Kriesche HU, et al. Treatment for BK virus: incidence, risk factors and outcomes for kidney transplant recipients in the United States. *Transpl Int* (2009) 22(6):626–34. doi:10.1111/j.1432-2277.2009.00842.x
44. Prince O, Savic S, Dickenmann M, Steiger J, Bubendorf L, Mihatsch MJ. Risk factors for polyoma virus nephropathy. *Nephrol Dial Transplant* (2009) 24(3):1024–33. doi:10.1093/ndt/gfm671



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Not all antibodies are created equal: total IgG glycosylation and severity of antibody-mediated rejection in kidney transplantation

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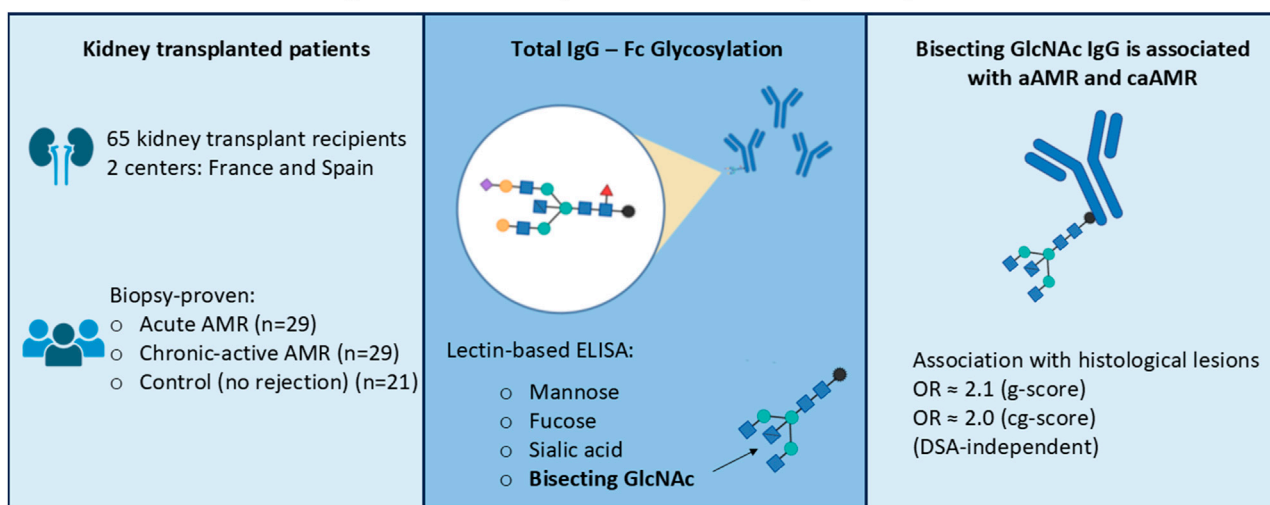
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Antibody-mediated rejection (AMR) is a leading cause of kidney transplant failure and is primarily driven by donor-specific anti-HLA antibodies (DSA), although DSA presence alone does not fully explain the heterogeneity of AMR severity. We prospectively studied 79 kidney transplant recipients from 2 European centers, including 29 with active AMR (aAMR), 29 with chronic-active AMR (caAMR), and 21 controls without rejection, to investigate the association between total Immunoglobulin-G (IgG) glycosylation profiles and AMR. IgG glycosylation was quantified using lectin-based ELISA, assessing relative levels of mannose, core fucose, α 2,6-linked sialic acid, and bisecting N-acetylglucosamine (GlcNAc). Bisecting GlcNAc levels were higher in caAMR compared with controls and aAMR (both $p < 0.001$), while core fucosylation and mannose levels were increased in aAMR and caAMR relative to controls. Higher levels of core fucose, mannose, and α 2,6-sialylation were associated with increasing glomerulitis severity. We found that bisecting GlcNAc (PHA-E/Fc) was significantly associated with both higher g- and cg-score (in ordinal models, OR = 2.1 [95% CI: 1.2–4.1; $p = 0.017$], and OR = 2.0 [95% CI: 1.1–4.3; $p = 0.046$], respectively). Mannose level (ConA/Fc) was significantly associated with higher g-score (2.1 [1.1–4.2], $p = 0.017$). These findings indicate that distinct total IgG glycosylation features are independently associated with specific histological patterns of AMR severity.

KEYWORDS

antibody-mediated rejection, glycosylation, immunoglobulin G, N-acetylglucosamine, renal transplant

Not all alloantibodies are created equal: IgG glycosylation and severity of antibody-mediated rejection in kidney transplantation



GRAPHICAL ABSTRACT

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Introduction

Antibody-mediated rejection (AMR) is a major cause of kidney transplant loss [1]. Although the pathophysiology of AMR is not fully understood, donor-specific anti-HLA antibodies (DSA) are thought to play an important pathogenic role. When present, these antibodies target endothelial graft cells, leading to their direct activation [2], the initiation of the complement cascade, and the recruitment of immune cells [3, 4]. Antibodies can also promote endothelial injury through antibody-dependent cellular cytotoxicity (ADCC) and the release of pro-inflammatory cytokines, both events that are primarily mediated by natural killer (NK) cells [5–8].

While DSA are central to AMR pathogenesis - triggering complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and proinflammatory injury - not all patients with DSA experience graft loss [9]. This disconnect suggests that titer of DSA alone fails to explain clinical outcomes.

Evidence has shown that post-translational modification of antibodies affects their function [10, 11]. The type and level of antibody glycosylation can influence its ability to activate complement and induce ADCC, as well as their stability and circulating half-life [12]. Glycosylation of Immunoglobulin G (IgG) involves the addition of carbohydrate at a conserved site in the Fc

region (Asn297), and, less frequently, in the Fab region [13]. Unlike the conserved site in the Fc region, glycan sites within the Fab region are primarily introduced during somatic hypermutation. N-linked glycosylation starts with the transfer of a high mannose glycan from a dolichol-glycan donor in the endoplasmic reticulum (ER) and is critical for protein folding quality control (Supplementary Figure 1A). Upon exit from the ER and entry into the Golgi apparatus, the high mannose structures are matured by sequential removal of all but three mannoses, leaving a pentasaccharide core (Supplementary Figure 1B), and then rebuilt through the addition of N-acetylglucosamine (GlcNAc) residues (Supplementary Figure 1C) and varying degrees of additional capping glycans (Supplementary Figure 1D). This results in complex N-glycans carrying varied amounts of GlcNAc branching, core fucosylation, galactose and sialic acid residues. Glycan structures found on IgG are typically bi-antennary, and may contain bisecting GlcNAc (Supplementary Figure 1D), a branching modification that influences IgG function and enhances Fc receptor binding. Functionally, sialylation (addition of terminal N-acetylneuraminic acid with an α 2,6 linkage to the underlying galactose) and core fucosylation (fucose attached with an α 1,6 linkage to the asparagine-proximal GlcNAc) are associated with reduced ADCC properties by reducing Fc gamma receptors (FcγR)III affinity [14].

IgG glycosylation profile has been associated with changes in the severity of various autoimmune diseases [15]. A study in kidney transplant recipients showed that low sialylation and high bisecting GlcNAc of anti-donor specific HLA antibodies (DSA) were associated with higher risk of AMR [16]. However, this study assessed only post-translational modifications in IgG3. Herein, we hypothesized that total IgG glycosylation profile may be associated with the risk and the severity of both AMR and caAMR.

Abbreviations: AMR, Antibody-mediated rejection; ADCC, Antibody-dependent cellular cytotoxicity; cg, glomerular chronicity score; ConA, Concanavalin A; DSA, Donor-specific Antibody; g, glomerulitis; GlcNAc, N-acetylglucosamine; IgG, Immunoglobulin G; LCA, Lens culinaris agglutinin; ptc, peritubular capillaritis; MVI, microvascular inflammation; Neu5Ac, sialic acid; SNA, Sambucus nigra agglutinin.

Materials and methods

Study population

We used serum samples from prospectively followed-up kidney transplant recipients from two centers (Grenoble-Alpes, Grenoble, France and Hospital Del Mar of Barcelona, Barcelona, Spain) between January 2016 and May 2024 with a biopsy-proven AMR and control kidney transplant patients with no signs of microvascular inflammation (MVI). All samples were collected at the time of biopsy. All control biopsies were per protocol, taken at 3 months, 1, and 3 years post-transplant. All biopsies were scored according to Banff 2022 and the indications were protocol biopsies or for cause [17]. In brief, AMR was defined by the presence of MVI, i.e., glomerulitis (g) and peritubular capillaritis (ptc). If the MVI score was 2 or above, AMR was confirmed by the presence of C4d deposition in peritubular capillaries and vasa recta and/or by the presence of HLA DSA. If other features of AMR, such as acute thrombotic microangiopathy were present, but the MVI score was below 2, AMR was confirmed by the presence of C4d deposition, regardless of DSA status. C4d deposition was quantified using anti-C4d antibody on formalin-fixed, frozen paraffin-embedded sections. Chronic active AMR (caAMR) was defined by the presence of active lesions (including C4d) and chronic lesions (transplant glomerulopathy (cg) and/or peritubular capillary multilayering).

All patients signed an informed consent form. For Grenoble Hospital, all medical data were collected from the Grain-DB database [CNIL (French National committee for data protection) approval number 1987785v0 for the Nephrology and Kidney transplantation department of Grenoble-France]. The study was also approved by the Institutional Review Board of the Consorci Mar Parc de Salut de Barcelona (Research Project No. 2020/9117/I) and was conducted in accordance with the principles of the Declaration of Helsinki and the Declaration of Istanbul.

Anti-HLA antibody measurement

Anti-HLA antibodies were measured at the time of biopsy in all patients using One Lambda-ThermoFisher LabScreen, Class I, and Class II or Lifecodes LSA Class I and/or Class II test (Immucor GTI Diagnostics, Inc®). Antibody specificity was identified using a Luminex® 200 Instrument (Luminex Corporation, Austin, TX). The MFI threshold of positivity for a DSA was 500 for patients followed-up in Grenoble and 750 for those Barcelona. The sera used for the post-translational analysis were leftovers from the anti-HLA analysis at the Etablissement Français du Sang (EFS) of Grenoble, HLA department and study-dedicated sera for patients enrolled in Barcelona.

Immunoglobulin G purification

We purified total serum IgG using Protein A IgG Purification Kit (Pierce™, Thermo Scientific™, CAT: 44667) and following manufacturer's instructions. Briefly, the procedure consisted of passing the sera sample into the column and then eluting the IgG bound to the resin using an elution buffer (pH 2.8). For each sample, IgG concentration was measured by absorbance at 280 nm and purity was confirmed using SDS-PAGE.

Quantification of glycosylation by lectin ELISA

Purified IgG were diluted to 1 ng/μL in PBS, plated into a 96-well high-binding ELISA plate (Microton High Binding; Greiner BioOne) and incubated overnight at 4 °C. The plate was blocked with carbohydrate free blocking solution (Vector Labs) for 1 h at room temperature. Biotinylated lectins (Vector Labs) were diluted to 1 μg/mL in carbohydrate-free blocking solution and incubated for 1 h at room temperature. Different biotinylated lectins were used: Sambucus nigra agglutinin (SNA): binds to α2,6-linked Neu5Ac and is used to quantify the sialylation of IgG. Concanavalin A (ConA): binds to high mannose and hybrid N-glycans and correlates with more immature IgG. Lens culinaris agglutinin (LCA): binds to core fucosylated N-glycans. Phaseolus vulgaris Erythroagglutinin (PHA-E) binds to bisecting GlcNAc to quantify bisecting GlcNAc on IgG. Anti-Fc measures the amount of immobilized IgG to normalize the results. The amount of IgG coated was 0, 100ng and 200 ng to assess saturation of the signal, and we thereafter used the results of 100 ng for consistency. Signal was detected using europium-conjugated streptavidin (Perkin Elmer) and time-resolved fluorescence as measured in a Victor Nivo plate reader.

Statistical analysis

Normally distributed quantitative variables were expressed as mean ± standard deviation (SD). Non-normally distributed quantitative variables were expressed as median [interquartile ranges (IQR)]. All glycosylation values were non-normally distributed (p-value from Shapiro-Wilk Test <0.005). Qualitative variables were expressed as numbers and percentages. For statistical comparison between multiple groups, we used Kruskal-Wallis test. For statistical comparisons between two groups, we used unpaired Mann-Whitney U Tests. The chi-squared test was used to compare categorical data. Statistical significance was defined as a two-sided p-value <0.05.

To identify factors associated with each ordinal outcome variable (i.e., scores of Banff components), we employed ordinal logistic regressions using the proportional odds model using the *clm* function from the ordinal R package (R version 4.4.2, <https://www.r-project.org>). Ordinal logistic regression models for g- cg- and pc-scores were fitted for each glycan, and adjusted for DSA, recipient age, sex, delay of biopsy since transplantation, and eGFR at biopsy whenever they were statistically significant to avoid overfitting given the limited sample size. These covariates were included, as they may affect both glycosylation and histological outcomes independently of any direct glycosylation-mediated effect [18, 19]. To visualize the ordinal regression model for cg score, we plotted the fitted predicted probability for each cg score level as a function of standard unit change of bisecting GlcNAc (PHA-E/Fc).

Results

Baseline characteristics of the study population

The study included 79 kidney transplant recipients, 29 (36.7%) with biopsy-proven aAMR, 29 (36.7%) with caAMR, and 21 (26.6%)

TABLE 1 Baseline characteristics of study participants.

Characteristics	CTL (N = 21)	aAMR (N = 29)	caAMR (N = 29)	<i>p</i> -value
Age - years	51.7 ± 12	43.7 ± 17	43.4 ± 17	0.179
Gender M/F ratio	3.2	0.9	1.7	0.125
History of previous transplantation - N (%)	0	8 (27.6)	3 (10.3)	0.065
Nephropathy - N (%)				0.522
Diabetes	1 (4.7)	0	2 (6.9)	
Drug-related	1 (4.7)	0	0	
Nephrotic disease	1 (4.7)	3 (10.3)	2 (6.9)	
Genetic	1 (4.7)	1 (3.4)	0	
Uropathy	1 (4.7)	2 (6.9)	3 (10.3)	
Unknown	7 (33.2)	7 (24.1)	5 (17.24)	
Glomerulonephritis	1 (4.7)	7 (24.1)	6 (20.7)	
TMA	1 (4.7)	1 (3.4)	1 (3.4)	
Hypertensive nephropathy	2 (9.5)	3 (10.3)	3 (10.3)	
Interstitial nephropathy	2 (9.5)	1 (3.4)	2 (6.9)	
Acute tubular necrosis	1 (4.7)	0	0	
Polycystic disease	4 (19)	2 (6.9)	4 (13.8)	
Time of biopsy - months after transplant	38.5 [3–43]	10.9 [2–58]	47.3 [35–149]	0.004
PRA (%)	11 ± 28	49 ± 44	30 ± 45	0.021
Living donor - N (%)	4 (19)	7 (24.1)	6 (6.9)	0.902
Donor age - years	55.2 ± 14	49.5 ± 16	45.8 ± 16	0.192
HLA mismatch A/B/DQ - number	5.0 [3–6]	6.0 [5–7]	5.5 [5–7]	0.218
History of diabetes - N (%)	1 (4.7)	0	2 (6.9)	0.329
Anti-thymoglobulin induction - N (%)	9 (42.7)	21 (72.4)	20 (68.9)	0.725
MVI ≥2 - N (%)	0	24 (82.7)	28 (96.5)	<0.001
C4d positivity- N (%)	0	22 (75.8)	10 (34.5)	<0.001
Donor-specific antibodies - N (%)	1 (4.7)	23 (79.3)	21 (72.4)	<0.001
cg-score positivity- N (%)	0	0	29 (100)	<0.001
eGFR at the time of biopsy - mg/dL	43 [31–63]	45 [32–66]	36 [23–49]	0.145
Proteinuria at rejection - g/g	0.1 [0.1–0.4]	0.3 [0.1–0.7]	0.8 [0.3–2.0]	<0.001

FSGS, Focal segmental glomerulosclerosis; HLA, Human Leukocyte Antigen; IgAN, IgA nephropathy; MVI, microvascular inflammation; PRA, panel reactivity antibody; eGFR, estimated glomerular filtration rate; TMA, thrombotic microangiopathy.

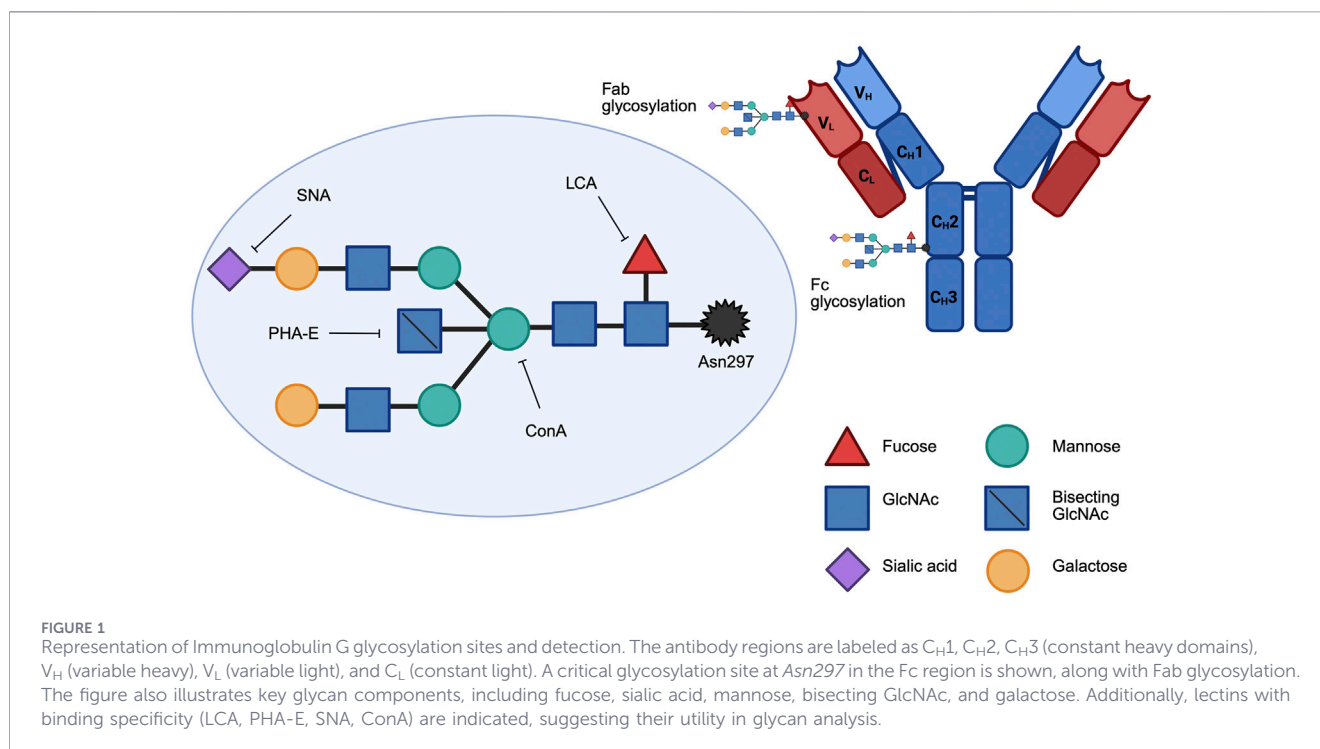
controls with no histological signs of rejection at per-protocol biopsy (Table 1). There was no difference among patients with aAMR, caAMR and controls in the main characteristics such as the age at the time of biopsy, gender, previous transplantation number, type of donor, HLA mismatches, history of diabetes, and induction therapy. The median time between the transplantation and the biopsy was 0.9 years [0.1–4.8] in the aAMR group, 3.9 [2.9–12.3] for the caAMR group, and 3.2 [0.3–3.6] for the control group, $p = 0.004$. The immunosuppressive regimen consisted of tacrolimus, mycophenolate mofetil, and steroids for all patients. Patients with aAMR or caAMR had higher proteinuria than controls (Table 1).

Twenty-three aAMR patients (79.3%) had donor-specific antibody (DSA) at the time of the biopsy, compared to 21 (72.4%) in the caAMR group ($p = 0.539$). One patient in the control group had DSA. The number of DSA was also similar between the two rejection groups (1.5 ± 0.9 vs. 1.4 ± 0.7 , $p =$

0.539) and most of DSA were class II (81% versus 85%, $p = 0.731$) for aAMR and caAMR, respectively. The mean fluorescence intensity (MFI) of the immunodominant DSA was also similar (8226 ± 6549 vs. 4915 ± 5968 , $p = 0.192$).

Associations between IgG glycosylation and the risk of AMR

The quantification of IgG glycan composition (relative amounts of mannose, fucose, sialic acid, and bisecting GlcNAc) was assessed using the ConA, LCA, SNA, and PHA-E lectins, respectively, and normalized with the Fc levels (Figure 1). We found a positive correlation between mannose, core fucose, $\alpha 2,6$ -linked sialic acids. In contrast, bisecting GlcNAc was not significantly associated with the other glycoforms (Supplementary Figure 2). There was no



correlation between the glycan level and the total amount of IgG (data not shown).

We first compared the different IgG glycoforms among the 3 groups. The amounts of bisecting GlcNAc (PHA-E/Fc), core fucose (LCA/Fc), and mannose (ConA/Fc) were significantly lower in the control group than in the caAMR group. Bisecting GlcNAc (PHA-E/Fc) was significantly higher in the caAMR and aAMR groups compared to controls, and significantly higher in caAMR compared to aAMR patients (Figure 2A). Core fucose (LCA/Fc) was significantly higher in both caAMR and aAMR groups compared to controls (Figure 2B). Mannose (ConA/Fc) was significantly higher in caAMR than in controls (Figure 2C). There was no statistical difference among the 3 groups in the α 2,6-sialylation (SNA/Fc) levels (Figure 2D). When gathering aAMR and caAMR, we found higher level of bisecting GlcNAc ($p < 0.001$), of fucosylation ($p < 0.001$), and of Mannose ($p < 0.001$) in rejection patients compared to controls while sialylation levels were similar ($p = 0.41$).

Associations between IgG glycosylation and the severity of rejection

We then compared the amount of each glycoform with the severity of AMR using each of the AMR parameters listed in the Banff score (0–3), i.e., g-, cg-, and ptc- scores and C4d deposition intensity (0–3). We used cg-score to quantify the chronicity of lesions. We found no association between bisecting GlcNAc (PHA-E/Fc) and g-score increasing severity (Figure 3A). Core fucose (LCA/Fc), mannose (ConA/Fc) and α 2,6-sialic acids (SNA/Fc) levels in IgG were increased in samples from patients with the highest g-scores compared to those with lower scores (Figures 3B–D). Within AMR cases, core fucosylation was increasing with the severity of glomerulitis (Figure 3B). We also found that patients

with highest cg-score had increased bisecting GlcNAc (PHA-E/Fc), core fucose (LCA/Fc), mannose (ConA/Fc), and sialylation (SNA/Fc; Supplementary Figure 3) levels. Finally, there was a correlation between ptc-score severity and IgG glycan levels (Supplementary Figure 4).

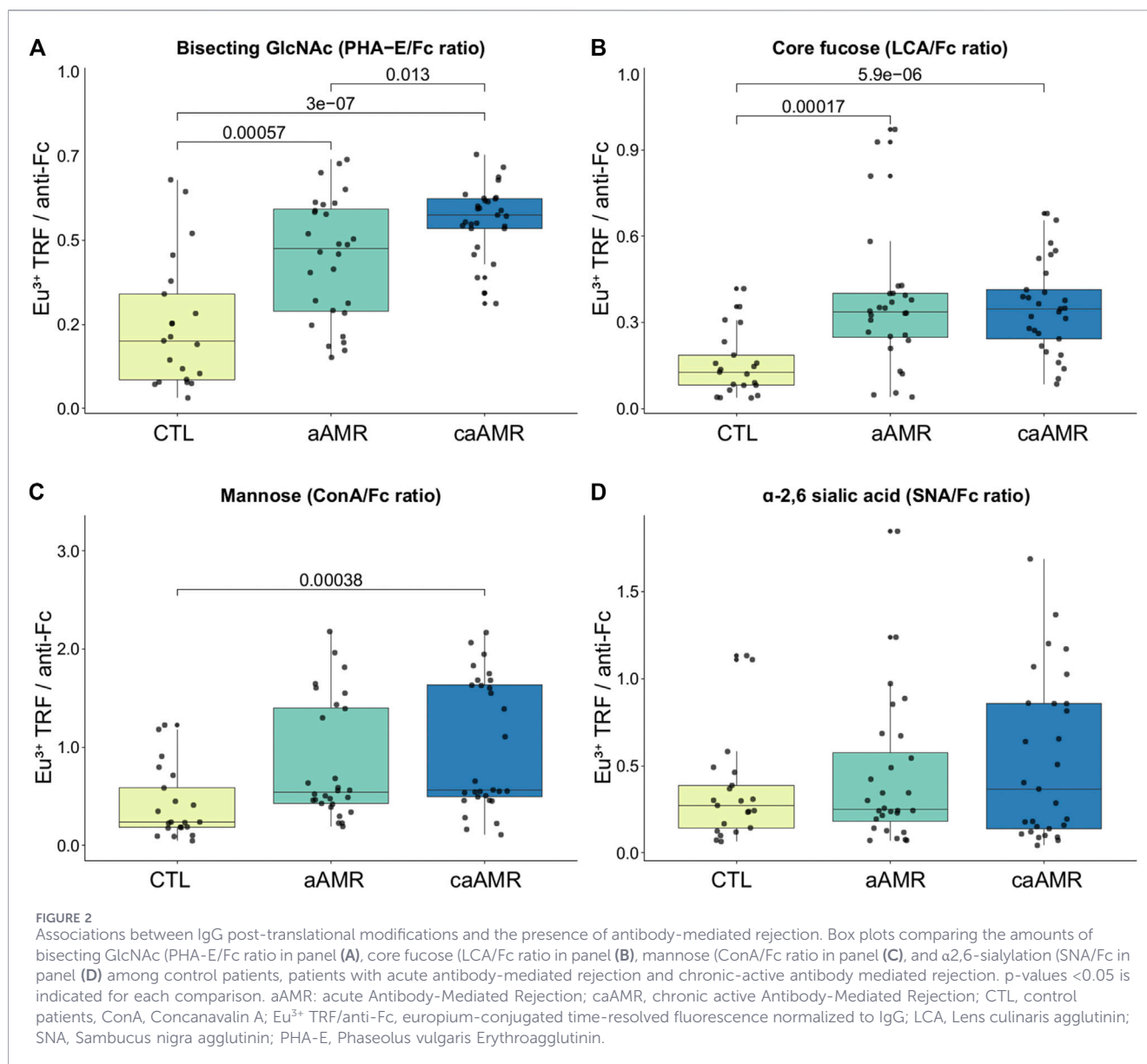
Association between IgG glycosylation and DSA

Not all patients in our cohorts had DSA. Therefore, we tested the association between IgG glycosylation and DSA. Among the different glycosylation tested, we found no significant correlation between total IgG glycosylation and the presence of DSA in patients with AMR (Supplementary Figure 5A–D). In particular, the level of bisecting GlcNAc was not different between aAMR and caAMR patients with or without DSA (Supplementary Figure 5A).

We repeated the analyses comparing glycoform levels in controls, aAMR, and caAMR cohorts, by stratifying patients based on the presence of DSA. In DSA negative patients, caAMR remained significantly higher than controls for bisecting GlcNAc, total mannose and core fucose while aAMR remained significantly associated with total mannose and core fucose (Figures 4A–C). In DSA positive patients, bisecting GlcNAc was higher in caAMR compared to aAMR patients (Figure 4A). α 2,6-sialylation was not significantly different between controls and AMR conditions, regardless of DSA status (Figure 4D).

Multivariable analysis of factors associated with histologic features of AMR

Finally, we tested whether the IgG glycosylation and AMR severity are independently associated.



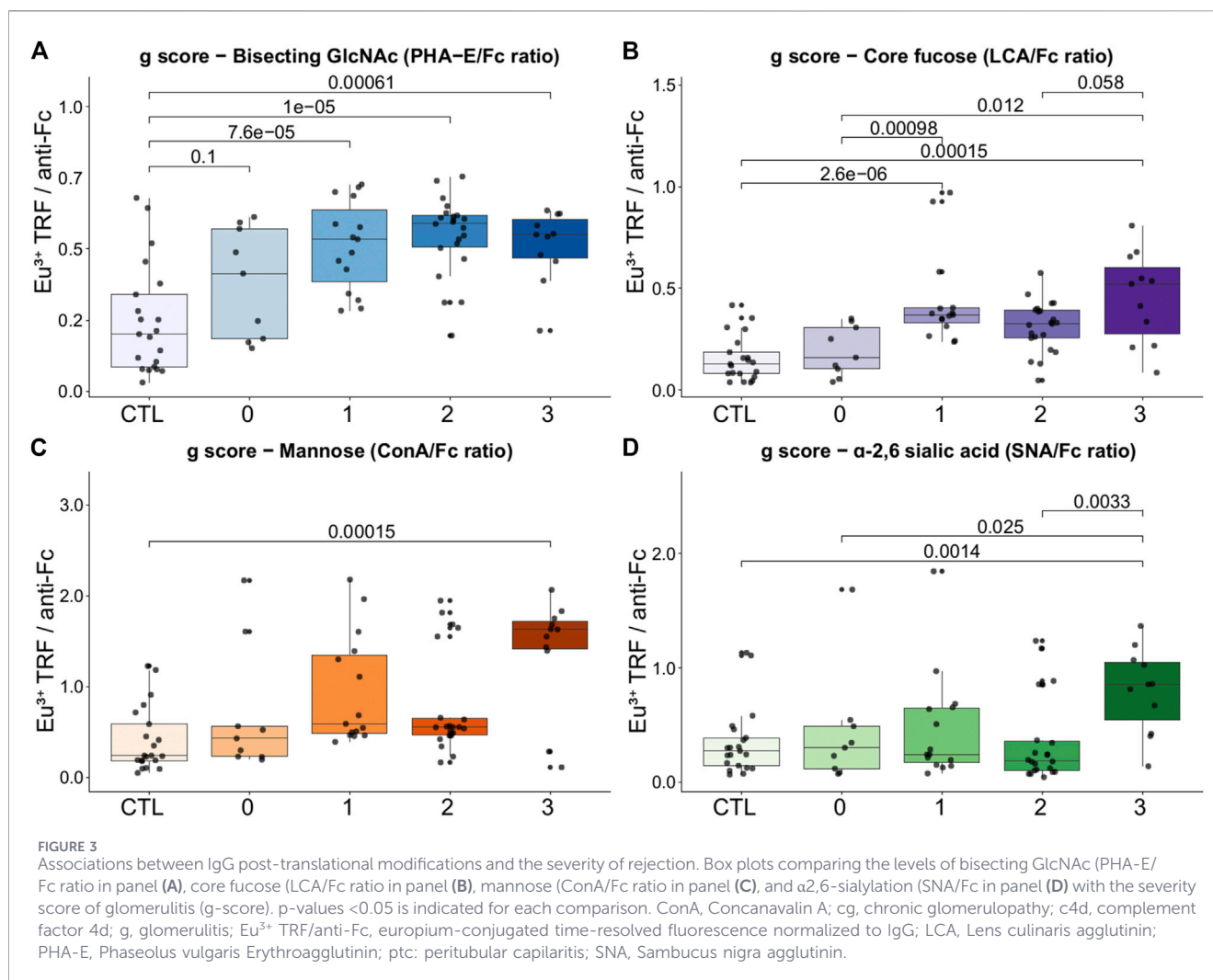
We found that bisecting GlcNAc (PHA-E/Fc) was significantly associated with both higher g- and gc-scores (in ordinal models, OR = 2.1 [95% CI: 1.2–4.1; $p = 0.017$], and OR = 2.0 [95% CI: 1.1–4.3; $p = 0.046$], respectively). Mannose level (ConA/Fc) was significantly associated with higher g-score (2.1 [1.1–4.2], $p = 0.017$) (Table 2). Core fucosylation and sialylation were not significantly associated with AMR severity in multivariable analyses. In Table 2 DSA might seem protective with respect to cg-score (OR less than 1), but this multivariable finding is artifactual as shown by the visual inspection of the predicted probability from the ordinal regression model (Figure 5). In DSA-negative patients, high bisecting GlcNAc predicted severe glomerulopathy (cg = 3), while low levels were protective (cg = 0). This dose-response relationship was attenuated in DSA-positive patients, where high bisecting GlcNAc barely influenced glomerulopathy severity. The apparent protective effect of DSA in multivariable models is therefore artifactual, likely reflecting DSA's dominant role that masks glycosylation-mediated injury. Bisecting GlcNAc and

DSA appear to represent independent but competing pathogenic mechanisms.

Discussion

In this study, we found an association between total IgG core-fucosylation, bisecting GlcNAc, and detectable mannose, and the presence of biopsy-proven AMR in kidney transplant recipients. In contrast, IgG α2,6-sialylation was not associated with AMR in our cohort. Moreover, bisecting GlcNAcylation was significantly associated with both glomerulitis (g-score) and chronic glomerulopathy (cg-score). Interestingly, this association appeared independent of DSA status and may represent a competing pathogenic mechanism.

An inverse association between DSA presence and cg-score emerged in the multivariable model. Exploratory interaction analyses suggest that this finding likely reflects effect-modification

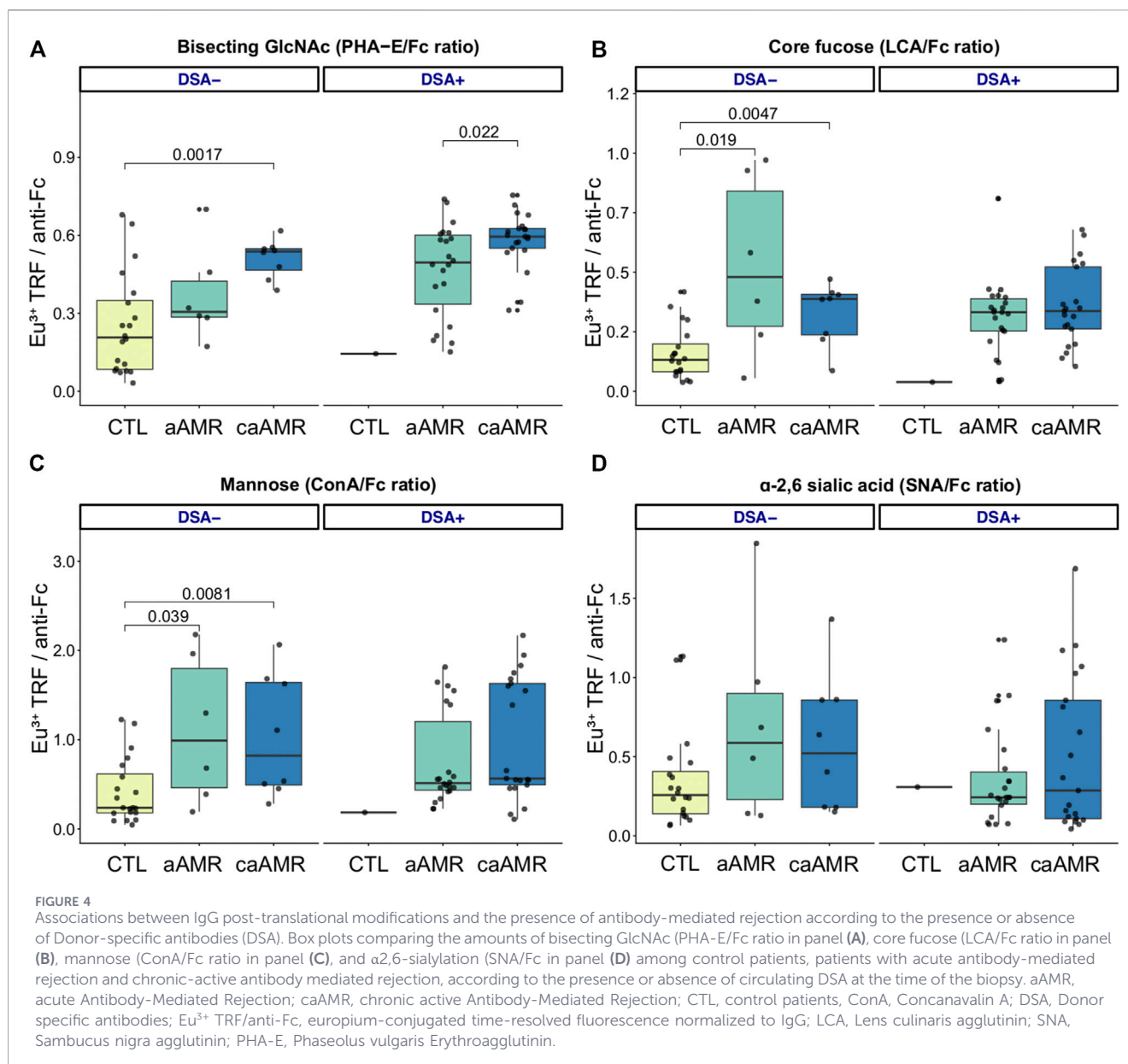


rather than a protective role of DSA. The association between higher bisecting GlcNAc levels and severe chronic glomerulopathy was mainly observed in DSA-negative patients, whereas in DSA-positive individuals, glycosylation levels did not substantially alter the predicted probability of severe lesions. These findings suggest that the association between IgG glycosylation and chronic glomerulopathy may be primarily observable in the absence of detectable DSA, whereas in DSA-positive patients, the pathogenic signal associated with DSA may dominate and attenuate the relative contribution of glycosylation levels.

Our findings confirm and expand results from prior smaller studies. Pernin et al. performed mass spectrometry on isolated IgG3 *de novo* DSA and showed that higher bisecting GlcNAc glycosylation was higher in AMR patients overall compared to patients DSA+, but without histological AMR [16]. If mass spectrometry allows a precise characterization of glycoforms, it can also exhibit higher technical variability compared to other methods, potentially affecting reproducibility [20]. ELISA, on the contrary, is well-suited for large-scale studies due to its simplicity and ability to process many samples simultaneously, is generally less expensive and requires less specialized equipment compared to mass spectrometry, and can be standardized across different laboratories, enhancing reproducibility [21].

Our findings are in line with the literature about bisecting GlcNAc. Bisecting GlcNAcylation levels can modify the Fc activity of IgG by influencing its binding affinity to FcγRs. Specifically, the presence of a bisecting GlcNAc moiety has been shown to inhibit Fc core-fucosylation and enhance the binding of Fc to FcγRIIIa, an activating Fcγ receptor [22, 23]. This enhancement in binding affinity can lead to increased ADCC activity, which conceivably results in increased glomerular injury induced by HLA and non-HLA antibodies.

In the study by Pernin et al., sialylation of IgG3 DSA was found to be associated with a reduced risk of AMR. Sialylation of total IgG assessed at the time of transplantation was also associated with reduced AMR risk during the follow-up in another study [24]. In our cohort, we did not find any correlation between IgG sialylation and the presence of AMR. Together, these data suggest a reduced activity of HLA and non-HLA antibodies in kidney transplantation when associated with a higher sialylation. This hypothesis is supported by the literature, as sialylation of the IgG Fc domain has been shown to impair complement-dependent cytotoxicity (CDC) by reducing the binding of C1q [25], decreasing ADCC (in the presence of core fucosylation) by reducing the binding affinity of IgG to FcγRIIIa [26], and exhibiting anti-inflammatory effects [27].



The significance of our data extends to individuals with autoimmune diseases, where alterations in IgG glycosylation have been well-documented. For instance, in rheumatoid arthritis, there is a notable decrease in galactosylation and sialylation, and increased fucosylation in IgG during active phases of the disease [28], suggesting that these glycosylation changes can serve as biomarkers for disease activity and therapeutic response. Similarly, systemic lupus erythematosus patients exhibit decreased IgG galactosylation and sialylation, increased bisecting GlcNAc, and decreased core fucose [29, 30]. These glycosylation changes are associated with disease activity and specific clinical manifestations, such as nephritis and the onset of antinuclear antibodies. IgG glycosylation patterns, including sialylation, bisecting GlcNAcylation, fucosylation, and mannosylation, are significantly altered in various other autoimmune and inflammatory diseases, and these changes have been proposed as potential biomarkers for disease activity and severity [31, 32].

One limitation of our study is that we analyzed total IgG glycosylation, instead of DSA-specific IgG glycosylation, as they may theoretically differ. Of note, one single patient in the control group had DSA, further limiting the power of our analyses to infer the impact of total IgG glycosylation, DSA and AMR severity. However, Sonneveld et al. demonstrated that the Fc-glycosylation profiles of total serum IgG1 and IgG3 were highly similar to those of antigen-specific anti-K antibodies [33]. In that study, the authors analyzed the Fc-tail glycosylation of both total serum and antigen-specific IgG1 and IgG3 using mass spectrometry and found that the glycosylation patterns were consistent across both types, with little individual variability. Using an integrated method for simultaneous protein quantitation and glycosylation profiling of antigen-specific IgG1, Gijze et al. also found that the glycosylation profiles of antigen-specific IgG1 are comparable to those of total IgG1 in a large coronavirus vaccination cohort [21]. Of note, we found that the differences in bisecting

TABLE 2 Multivariable ordinal regression analysis of the association between clinical variable, IgG post-translational modifications, and AMR severity scores in the overall cohort.

Variables	g-score		ptc-score		cg-score	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Presence of DSA						
Bisecting GlcNAc	2.1 (1.2–4.1)	0.017			2.0 (1.1–4.3)	0.046
Core fucose						
α2,6 sialic acid						
Mannose	.1 (1.1–4.2)	0.017				
Recipient age (years)						
Recipient gender (male)			4.1 (1.2–15.7)	0.027	3.7 (1.1–13.8) ^{§,ε,§}	0.038
eGFR (ml/min/1.73m ²)*					0.9 (0.9–0.9) ^{§,ε,ε}	0.02
Delay post-transplant (year)					1.1 (1.0–1.2) ^{§,ε,ε}	0.009
DSA						

Bisecting GlcNAc (PHA-E/Fc ratio); Core fucose (LCA/Fc ratio); α2,6 sialic acid (SNA/Fc ratio); mannose (ConA/Fc ratio), DSA: Donor specific Antibody; eGFR: estimated Glomerular Filtration Rate. *At rejection. [§]Model with bisecting GlcNAc. ^εModel with Core fucosylation. [§]Model with Sialylation. ^εModel with Mannose.

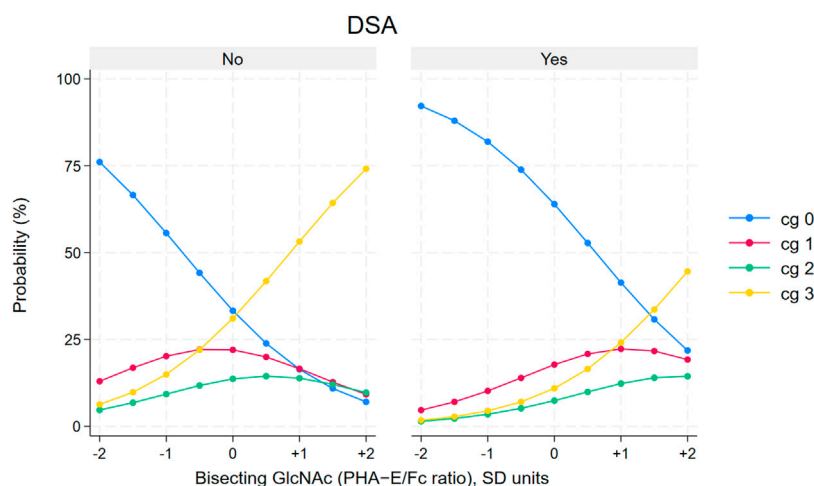


FIGURE 5

Visual representation of the multivariable ordinal logistic regression for chronic glomerulopathy severity (cg score), including both DSA and bisecting GlcNAc. Predicted probabilities of each cg-score (0–3) derived from the fitted multivariable ordinal logistic regression including bisecting GlcNAc (PHA-E/Fc ratio) and DSA. The x-axis represents standardized bisecting GlcNAc levels. The left panel shows DSA-negative patients; the right panel shows DSA-positive patients. Lines represent predicted probabilities of cg = 0 (blue), cg = 1 (red), cg = 2 (green), and cg = 3 (yellow). In DSA negative patients (left panel), high bisecting GlcNAc values (+2 SD units) are associated with a large difference between the probability of cg = 3 and cg = 0; this difference vanishes in DSA positive patients (right panel).

GlcNAcylation persist in patients with and in those without DSA, suggesting that although DSA may have a unique glycosylation profile, some key immunomodulatory glycoforms are probably shared across antibody specificities. However, *ad hoc* studies are needed to formally test this working model. Glycosylation of total IgG may capture information from non-HLA anti-donor antibodies that are hard to capture in routine clinical testing, or it may reflect a more systemic state of inflammation. Further studies are necessary to confirm and investigate these hypotheses.

In conclusion, we found a significant, independent correlation between total IgG glycosylation and AMR in kidney transplant recipients. More specifically, we showed the association of IgG bisecting GlcNAc regardless of the presence of DSA, with glomerulitis and chronic glomerulopathy in graft biopsy. Therefore, our findings provide the rationale for larger studies testing the hypothesis that total IgG glycosylation can be used as a biomarker for the risk of aAMR and caAMR. Strategies to target IgG glycosylation may also be leveraged therapeutically to improve the outcomes of transplant recipients.

Data availability statement

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

Ethics statement

The studies involving humans were approved by CNIL approval number 1987785v0 and Institutional Review of Barcelona, project 2020/9117/L. 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

PC and BC designed the study, JN, LG, AI, and GC performed the experiments. JN wrote the manuscript. CD and AB collected the samples. UM performed statistical analysis. All authors contributed to the article and approved the submitted version.

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References

1. Lefaucheur C, Loupy A. Antibody-mediated rejection of solid-organ allografts. *N Engl J Med* (2018) 379(26):2580–2. doi:10.1056/NEJMc1813976
2. Valenzuela NM, Mulder A, Reed EF. HLA class I antibodies trigger increased adherence of monocytes to endothelial cells by eliciting an increase in endothelial P-selectin and, depending on subclass, by engaging FcγRs. *J Immunol* (2013) 190(12):6635–50. doi:10.4049/jimmunol.1201434
3. Bailly E, Macedo C, Ossart J, Louis K, Gu X, Ramaswami B, et al. Interleukin-21 promotes Type-1 activation and cytotoxicity of CD56dimCD16bright natural killer cells during kidney allograft antibody-mediated rejection showing a new link between adaptive and innate humoral allo-immunity. *Kidney Int* (2023) 104(4):707–23. doi:10.1016/j.kint.2023.04.024
4. Nevarez-Mejia J, Jin YP, Pickering H, Parmar R, Valenzuela NM, Sosa RA, et al. Human leukocyte antigen class I antibody-activated endothelium promotes CD206+ M2 macrophage polarization and MMP9 secretion through TLR4 signaling and P-selectin in a model of antibody-mediated rejection and allograft vasculopathy. *Am J Transpl* (2024) 24(3):406–18. doi:10.1016/j.ajt.2023.10.020
5. Charreau B. Cellular and molecular crosstalk of graft endothelial cells during AMR: effector functions and mechanisms. *Transplantation* (2021) 105(11):e156–67. doi:10.1097/TP.00000000000003741
6. Koenig A, Mezaache S, Callemeyn J, Barba T, Mathias V, Sicard A, et al. Missing self-induced activation of NK cells combines with non-complement-fixing donor-specific antibodies to accelerate kidney transplant loss in chronic antibody-mediated rejection. *J Am Soc Nephrol* (2021) 32(2):479–94. doi:10.1681/ASN.2020040433
7. Chambon M, Koenig A. NK cells: not just followers but also initiators of chronic vascular rejection. *Transpl Int* (2024) 37:13318. doi:10.3389/ti.2024.13318
8. Charmetant X, Pettigrew GJ, Thauat O. Allorecognition unveiled: integrating recent breakthroughs into the current paradigm. *Transpl Int* (2024) 37:13523. doi:10.3389/ti.2024.13523
9. Koenig A, Chen CC, Marçais A, Barba T, Mathias V, Sicard A, et al. Missing self triggers NK cell-mediated chronic vascular rejection of solid organ transplants. *Nat Commun* (2019) 10(1):5350. doi:10.1038/s41467-019-13113-5
10. van de Bovenkamp FS, Hafkenscheid L, Rispens T, Rombouts Y. The emerging importance of IgG Fab glycosylation in immunity. *J Immunol* (2016) 196(4):1435–41. doi:10.4049/jimmunol.1502136
11. Krištić J, Lauc G. The importance of IgG glycosylation-what did we learn after analyzing over 100,000 individuals. *Immunol Rev* (2024) 328(1):143–70. doi:10.1111/imr.13407
12. Quast I, Peschke B, Lünemann JD. Regulation of antibody effector functions through IgG Fc N-glycosylation. *Cell Mol Life Sci* (2017) 74(5):837–47. doi:10.1007/s00018-016-2366-z
13. Cobb BA. The history of IgG glycosylation and where we are now. *Glycobiology* (2020) 30(4):202–13. doi:10.1093/glycob/cwz065
14. Golay J, Andrea AE, Cattaneo I. Role of Fc core fucosylation in the effector function of IgG1 antibodies. *Front Immunol* (2022) 13:929895. doi:10.3389/fimmu.2022.929895
15. Plomp R, Ruhaak LR, Uh HW, Reiding KR, Selman M, Houwing-Duistermaat JJ, et al. Subclass-specific IgG glycosylation is associated with markers of inflammation and metabolic health. *Sci Rep* (2017) 7(1):12325. doi:10.1038/s41598-017-12495-0

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2026.16461/full#supplementary-material>

16. Pernin V, Bec N, Beyze A, Bourgeois A, Szwarc I, Champion C, et al. IgG3 donor-specific antibodies with a proinflammatory glycosylation profile may be associated with the risk of antibody-mediated rejection after kidney transplantation. *Am J Transpl* (2022) 22(3):865–75. doi:10.1111/ajt.16904
17. Naesens M, Roufosse C, Haas M, Lefacheur C, Mannon RB, Adam BA, et al. The Banff 2022 kidney meeting report: reappraisal of microvascular inflammation and the role of biopsy-based transcript diagnostics. *Am J Transpl* (2024) 24(3):338–49. doi:10.1016/j.ajt.2023.10.016
18. Wu Y, Zhang Z, Chen L, Sun S. Immunoglobulin G glycosylation and its alterations in aging-related diseases. *Acta Biochim Biophys Sin (Shanghai)* (2024) 56(8):1221–33. doi:10.3724/abbs.2024137
19. Baković MP, Selman MHJ, Hoffmann M, Rudan I, Campbell H, Deelder AM, et al. High-throughput IgG Fc N-glycosylation profiling by mass spectrometry of glycopeptides. *J Proteome Res* (2013) 12(2):821–31. doi:10.1021/pr300887z
20. Reiding KR, Bondt A, Hennig R, Gardner RA, O'Flaherty R, Trbojević-Akmačić I, et al. High-throughput serum N-Glycomics: Method comparison and application to study rheumatoid arthritis and pregnancy-associated changes. *Mol Cell Proteomics* (2019) 18(1):3–15. doi:10.1074/mcp.RA117.000454
21. Gijze S, Wasynczuk A, van Leeuwen L, Grobbsen M, van Gils MJ, Nouta J, et al. Simultaneous protein quantitation and glycosylation profiling of antigen-specific immunoglobulin G1 in large clinical studies. *J Proteome Res* (2024) 23(12):5600–5. doi:10.1021/acs.jproteome.4c00538
22. Zou G, Ochiai H, Huang W, Yang Q, Li C, Wang LX. Chemoenzymatic synthesis and Fcγ receptor binding of homogeneous glycoforms of antibody Fc domain. Presence of a bisecting sugar moiety enhances the affinity of Fc to FcγIIIa receptor. *J Am Chem Soc* (2011) 133(46):18975–91. doi:10.1021/ja208390n
23. Bosman GP, Stoof ID, Bastiaansen HP, Quarles van Ufford L, Dobruchowska JM, Langenbach JWH, et al. Bisecting N-Acetylglucosamine of the N-Glycan of immunoglobulin G does not affect binding to Fc gamma receptors. *ACS Chem Biol* (2025) 20(3):680–9. doi:10.1021/acscchembio.4c00807
24. Malard-Castagnet S, Dugast E, Degauque N, Pallier A, Souillou JP, Cesbron A, et al. Sialylation of antibodies in kidney recipients with *de novo* donor specific antibody, with or without antibody mediated rejection. *Hum Immunol* (2016) 77(11):1076–83. doi:10.1016/j.humimm.2015.10.021
25. Quast I, Keller CW, Maurer MA, Giddens JP, Tackenberg B, Wang LX, et al. Sialylation of IgG Fc domain impairs complement-dependent cytotoxicity. *J Clin Invest* (2015) 125(11):4160–70. doi:10.1172/JCI82695
26. Li T, DiLillo DJ, Bournazos S, Giddens JP, Ravetch JV, Wang LX. Modulating IgG effector function by Fc glycan engineering. *Proc Natl Acad Sci U S A* (2017) 114(13):3485–90. doi:10.1073/pnas.1702173114
27. Ohmi Y, Ise W, Harazono A, Takakura D, Fukuyama H, Baba Y, et al. Sialylation converts arthritogenic IgG into inhibitors of collagen-induced arthritis. *Nat Commun* (2016) 7:11205. doi:10.1038/ncomms11205
28. Su Z, Xie Q, Wang Y, Li Y. Abberant immunoglobulin G glycosylation in rheumatoid arthritis by LTQ-ESI-MS. *Int J Mol Sci* (2020) 21(6):2045. doi:10.3390/ijms21062045
29. Vučković F, Krištić J, Gudelj I, Teruel M, Keser T, Pezer M, et al. Association of systemic lupus erythematosus with decreased immunosuppressive potential of the IgG glycome. *Arthritis Rheumatol* (2015) 67(11):2978–89. doi:10.1002/art.39273
30. Ramos-Martínez I, Ramos-Martínez E, Cerbón M, Pérez-Torres A, Pérez-Campos Mayoral L, Hernández-Huerta MT, et al. The role of B cell and T cell glycosylation in systemic lupus erythematosus. *Int J Mol Sci* (2023) 24(1):863. doi:10.3390/ijms24010863
31. Šimurina M, de Haan N, Vučković F, Kennedy NA, Štambuk J, Falck D, et al. Glycosylation of immunoglobulin G associates with clinical features of inflammatory bowel diseases. *Gastroenterology* (2018) 154(5):1320–33.e10. doi:10.1053/j.gastro.2018.01.002
32. Ząbczyńska M, Link-Lenczowski P, Pocheć E. Glycosylation in autoimmune diseases. *Adv Exp Med Biol* (2021) 1325:205–18. doi:10.1007/978-3-030-70115-4_10
33. Sonneveld ME, Koeleman CAM, Plomp HR, Wührer M, van der Schoot CE, Vidarsson G. Fc-Glycosylation in human IgG1 and IgG3 is similar for both total and anti-red-blood cell Anti-K antibodies. *Front Immunol* (2018) 9:129. doi:10.3389/fimmu.2018.00129



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Impact of pre-transplantation exposure to immunosuppressive agents on lung transplant outcomes in interstitial lung disease

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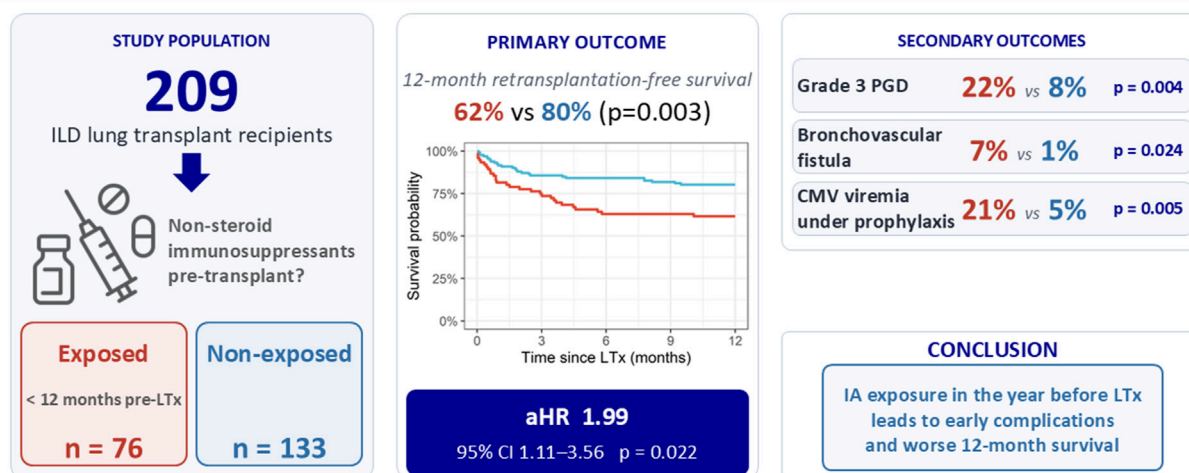
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Fibrosing interstitial lung diseases (ILDs) are the leading cause of lung transplantation (LTx), with worse results than other indications. We hypothesised that exposure to non-steroidal immunosuppressive agents (IAs) in the year preceding LTx for ILD may result in poorer early outcomes. We retrospectively analysed adults who underwent LTx for ILD from April 2011 to June 2024 in our institution and compared patients who received IA within 1 year before LTx to those who did not (systemic steroids exposure was used to adjust analysis). The primary outcome was 12-month retransplantation-free survival. Among 209 patients included, 76 (36%) had received IA within 1 year of LTx, and these patients had significantly worse 12-month retransplantation-free survival on multivariate analysis (62% vs. 80%; HR 1.99, 95%CI [1.11–3.56], $p = 0.022$); IA exposure increased the odds of grade 3 PGD (OR 3.20 [1.42–7.45], $p = 0.005$), bronchovascular fistula (OR 9.43, 95% CI [1.48–183], $p = 0.042$), pneumonia episodes in the first 6 months (median 2 [IQR 1–4] vs. 1 [0.5–2.5], $p = 0.001$), cytomegalovirus viremia under prophylaxis (21% vs. 5.2%, $p = 0.005$) and incidence of ganciclovir-resistant cytomegalovirus (14% vs. 3.2%, $p = 0.021$). In conclusion, IA exposure in the year before LTx leads to early complications and worse 12-month survival.

KEYWORDS

corticosteroid therapy, immunosuppressants, interstitial lung disease, lung transplantation, selection criteria

Impact of pre-transplantation exposure to immunosuppressive agents on lung transplant outcomes in interstitial lung disease



GRAPHICAL ABSTRACT

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Introduction

Fibrosing interstitial lung diseases (ILDs) are a heterogeneous group of respiratory diseases that can progress to terminal respiratory failure. Although they now represent the most frequent indication for lung transplantation (LTx) worldwide, they have the poorest prognosis, with a median survival of approximately 5 years after LTx [1]. Therefore, improving outcomes in this population is a critical unmet need.

The outcomes of LTx highly depend on the perioperative period, when severe complications can occur. Because this period can be affected by the preoperative characteristics of recipients, transplant teams strive to optimize candidate selection criteria. Treatment for patients with ILD before LTx may include corticosteroids, immunosuppressive agents (IAs, e.g., mycophenolate mofetil, rituximab, and cyclophosphamide), and antifibrotic agents [2, 3]. Retrospective studies have shown that corticosteroid treatment on the day of LTx is associated with increased infectious complications and poor survival [4], and many LTx centres now contraindicate LTx in patients receiving >20 mg prednisone equivalent per day [5]. The effect of antifibrotic therapies (pirfenidone, nintedanib) on LTx has also become a research focus [6, 7]. However, the impact of exposure to IA has not been specifically investigated. Given this lack of data, no specific guidelines have been established for IA use in the context of LTx [8].

Evidence suggests that the IA effects can persist beyond withdrawal, with varying intervals and severity depending on the treatment modalities [9, 10]. Hence, we hypothesized that prior treatment with IA may deepen the immunodeficiency of patients with ILD undergoing LTx, thereby increasing the risk of adverse outcomes after LTx, especially infectious complications.

This study aimed to determine whether early outcomes were worse for patients with ILD who underwent LTx after IA exposure in the year preceding LTx as compared with patients not exposed.

Materials and methods

Design

This was a single-centre retrospective study performed at the Lung Transplantation Centre of Bichat Hospital, Paris, France. We included all consecutive adults (>18 years old) who underwent LTx for chronic fibrosing ILD from April 2011 to June 2024. We excluded patients with sarcoidosis, interstitial diseases not classified as chronic fibrosing ILD (pulmonary alveolar proteinosis, hemosiderosis, acute respiratory disease syndrome), lung retransplantation for restrictive allograft syndrome (RAS), and multiple organ transplantations or if essential data were missing.

Data collection and definitions

We relied on a local prospective database of all patients undergoing LTx and the electronic health records system at our institution. Data collected included patient demographics and pre- and post-LTx information.

Recent IA exposure was defined as IA treatment within the 12 months before LTx, as a dichotomous variable. IAs included biologic IAs (rituximab, tocilizumab, abatacept, anti-tumour necrosis factor) and non-biologic IAs (mycophenolate mofetil, azathioprine, cyclophosphamide, methotrexate, tofacitinib or tacrolimus, leflunomide, anti-JAK therapy). Systemic

corticosteroids were not considered as IA but were recorded separately. The 12-month threshold was chosen to capture the clinically relevant peri-transplant immunosuppressive burden, encompassing agents with prolonged biological effects such as rituximab (B-cell depletion persisting months after the last infusion) while excluding remote exposures unlikely to affect peri-operative immunity.

Several variables were defined as follows: severe pulmonary hypertension (PH) as mean pulmonary artery pressure >20 mmHg and pulmonary vascular resistance (PVR) ≥ 5 Wood units (WU), or in the absence of PVR measurement, cardiac index <2 L/min/m²; pneumonia episode as a clinical suspicion of respiratory infection and evidence of new pulmonary infiltrates on chest imaging (chest X-ray or CT-scan), which was reviewed by the investigator; bronchovascular fistula as an abnormal connection between the bronchial lumen and adjacent thoracic blood vessels on bronchoscopy and CT-scan; acute cellular rejection (ACR) by a clinical suspicion supported by histological evidence or a favourable clinical and functional response to corticosteroid pulse therapy; allo-immunization as *de novo* donor-specific antibody detection with mean fluorescence intensity >1000 ; and acute antibody-mediated rejection (AMR), chronic lung allograft dysfunction (CLAD), bronchiolitis obliterans syndrome (BOS), RAS and primary graft dysfunction (PGD) as consensus reported [11, 12]. We defined grade 3 PGD as persistent PaO₂/FiO₂ ratio <200 or extracorporeal membrane oxygenation requirement (ECMO) for 72 h after LTx or death occurring earlier than 72 h with grade 3 PGD at the time of death. The LTx protocol at our institution is presented succinctly in Supplementary Methods, including bronchoscopy surveillance, maintenance immunosuppression and antimicrobial prophylaxis.

Clinical outcomes

The primary outcome was 12-month all-cause mortality or retransplantation. Retransplantation was included in the composite primary endpoint as an event equivalent to death, representing definitive graft failure. Secondary outcomes were the occurrence of grade 3 PGD, occurrence of bronchovascular fistula, number of pneumonia episodes in the first 6 months, number of antibiotics courses in the first 6 months, invasive fungal infection in the first 6 months, occurrence of cytomegalovirus (CMV) viremia under prophylaxis and after prophylaxis withdrawal, occurrence of ganciclovir-resistant CMV, ACR, acute AMR within the first 12 months, forced expiratory volume in 1 s (FEV1) trajectory within the first 24 months, and all-cause mortality and CLAD-free survival at 36 months.

To evaluate infectious events during the first 6 months, only patients followed for at least 6 months were analysed. CMV donor-negative and recipient-negative (D-/R-) patients were excluded from CMV outcomes analysis. CMV viremia was defined as DNAemia over 3 log units/mL, which is the threshold for beginning curative treatment at our centre. FEV1 trajectory was analysed at each time point according to procedure laterality (single/double LTx).

Statistical analysis

Variables are reported as median with interquartile range (IQR). We compared means for quantitative variables by unpaired Student's t-test or Mann Whitney U test depending on the normality of the data distribution and chi-squared test or Fisher's exact test for categorical variables (the normal distribution of variables was assessed with the Shapiro-Wilk test and by examining QQ plots). For multiple comparisons, we used ANOVA or the Kruskal-Wallis rank sum test. Survival analysis involved the log-rank test, Kaplan Meier curves and a Cox model for multivariate analysis. For multivariate analysis, covariables were selected according to clinical relevance, statistical significance ($p < 0.1$) and number of events to avoid overfitting. Preoperative patient characteristics were prioritized for survival analysis adjustment considering the focus of our study on pre-LTx risk factors and the expected collinearity between post-operative outcomes and early death. As a sensitivity analysis to assess the robustness of the primary finding to group imbalance, an inverse probability of treatment weighting (IPTW) analysis was performed. A propensity score for IA exposure was estimated using a logistic regression model including ILT subtype, severe pulmonary hypertension, acute exacerbation of ILT in the preceding 12 months, high emergency transplantation status, annual corticosteroid dose, age, and BMI. Stabilised average treatment effect (ATE) weights were derived and applied to a weighted Cox model with robust sandwich standard errors.

We conducted univariate logistic regression to assess the risk associated with early complications of LTx such as PGD and bronchovascular fistula. Competitive risk analysis was performed to analyse acute rejection risk and CMV viremia occurrence (with death as a competing risk), calculating cumulative incidence functions to describe the probability of events, with univariate regression analyses involving the Fine and Gray proportional-hazards model, estimating hazard ratios (HRs) and 95% confidence intervals (CIs). A multistate model was constructed, and transition probabilities were calculated for the analysis of CLAD-free survival at 36 months. A Wald-type Z-test was used to determine the statistical significance of differences in probability of events. Linear mixed models were fitted with FEV1 as a dependent variable; time (continuous), type of LTx (single/double) and optionally IA exposure as fixed effects; and individual as a random effect. Comparing these models allowed for assessing the effect of IA exposure on FEV1 trajectories. Correlation tests (Pearson or Spearman) were used to assess correlation between continuous variables. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using the R environment R v4.2.0, The R Project for Statistical Computing.¹

Ethics

This observational study was designed, conducted, and written following the STROBE guidelines, and it received ethical approval from the French society of respiratory medicine (*Société de*

¹ <http://www.r-project.org/>

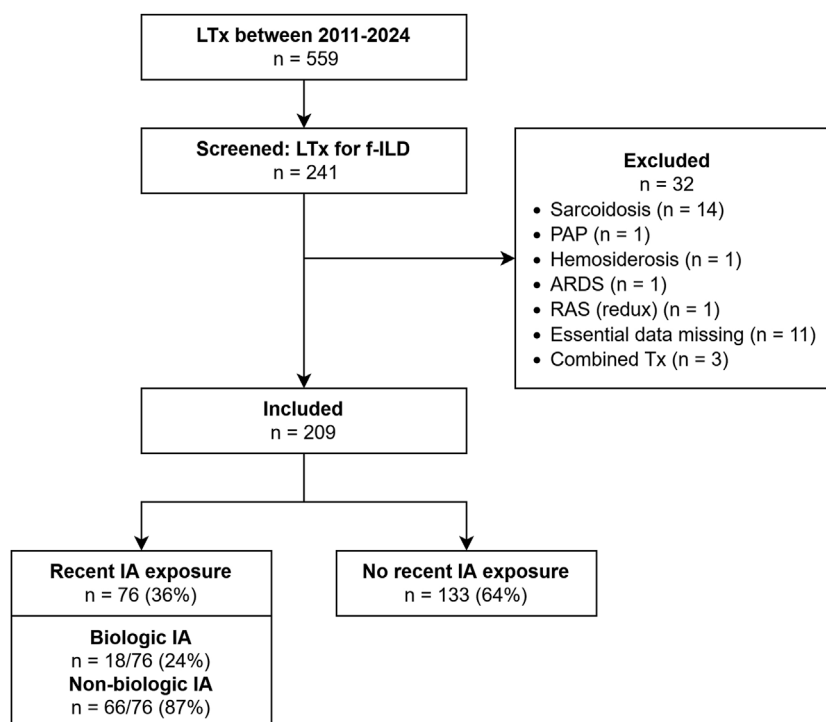


FIGURE 1

Flow chart of the study population. Essential missing data: related to immunosuppressive exposure and the time between their discontinuation and LTx. Biologic and non-biologic IA numbers do not add to 76 because more than one IA type was used in some patients. IA, immunosuppressive agent; f-ILD, fibrosing interstitial lung disease; PAP, pulmonary alveolar proteinosis; ARDS, acute respiratory distress syndrome; LTx, lung transplantation; RAS, restrictive allograft syndrome; recent IA exposure, last IA exposure <12 months before LTx; no recent IA exposure, no exposure or last IA exposure >12 months.

Pneumologie de Langue Française) review board (CEPRO no. 2025-006).

Patient and public involvement

Patients or members of the public were not involved in the design, conduct, reporting, or dissemination plans of this research.

Results

Population baseline characteristics

From April 2011 to June 2024, 559 LTx procedures were performed; 241 (43%) were for ILD. A total of 209 patients were included in this study; 76 (36%) had received IAs within 1 year of LTx (Figure 1). The most frequent IAs administered were cyclophosphamide ($n = 25$, 33%), mycophenolate mofetil ($n = 33$, 43%), azathioprine ($n = 15$, 20%), and rituximab ($n = 15$, 20%), with 17 (22%) patients having received two or more of these treatments during this interval (Supplementary Table S1). In the IA group, 49 (62%) patients were in the inter-cure phase, meaning they were within 6 months of their last dose of rituximab ($n = 9$, 12%), 28 days of cyclophosphamide ($n = 4$, 5%), and 2 weeks of tocilizumab ($n = 2$, 3%) and/or were receiving mycophenolate mofetil ($n = 26$, 34%), azathioprine ($n = 10$, 13%), tacrolimus ($n = 2$,

3%), tofacitinib ($n = 2$, 3%) or abatacept ($n = 1$, 1%) until the week of transplantation.

Pre-LTx baseline characteristics are summarized in Table 1. Patients with recent IA exposure were younger, more frequently women and had a higher body mass index. They more frequently had a diagnosis of connective tissue disease-ILD (CTD-ILD) or idiopathic non-specific interstitial pneumonia as opposed to idiopathic pulmonary fibrosis. They also had higher cumulative 12-month corticosteroid exposure (median 3.67 g [IQR 3.27–5.47] vs. 0 g [0–3.65], $p < 0.001$) and less frequently had received antifibrotics (28% vs. 62%, $p < 0.001$).

LTx procedures

More than half of procedures were bilateral (55%) (Table 1). LTx was performed with the French high emergency allocation protocol for 64 (31%) patients. Patients with recent IA exposure more frequently had bilateral LTx (66% vs. 48%, $p = 0.014$), had high emergency transplantation (41% vs. 25%, $p = 0.016$) (Table 1) and received more packed red blood cells intraoperatively (median 3 packs [1–5] vs. 2 [0–3], $p < 0.001$) than those not exposed (Table 2).

Survival analysis

Overall median survival was 43 months (95%CI [33.7–64.4]), and 12-month retransplantation-free survival was 74%

TABLE 1 Baseline and lung transplantation (LTx) characteristics by immunosuppressive agent (IA) exposure during the 12 months before LTx.

Characteristics	Total (N=209)	Recent IA (N=76)	No recent IA (N=133)	p-value
Age at transplantation (years)	58 (51–63)	56 (50–60)	59 (54–64)	0.001
Male sex, n (%)	159 (76)	50 (66)	109 (82)	0.008
BMI (kg/m ²)	25.9 (23.4–28.7)	27.0 (23.5–29.7)	25.4 (23.4–28.1)	0.04
Ever-smoker, n (%)	139 (67)	46 (61)	93 (70)	0.2
Tobacco exposure (pack-years)	10 (0–25)	9 (0–25)	12 (0–25)	0.4
Comorbidities, n (%)				
Diabetes	28 (13)	12 (16)	16 (12)	0.4
Coronary disease	16 (7.7)	3 (3.9)	13 (9.8)	0.13
Arterial hypertension	42 (20)	14 (18)	28 (21)	0.6
GFR <60 (mL/min/m ²)	7 (3.6)	5 (7.2)	2 (1.6)	0.1
ILD etiology, n (%)				<0.001
IPF	86 (41)	10 (13)	76 (57)	
f-HP	31 (15)	12 (16)	19 (14)	
CTD-ILD	37 (18)	31 (41)	6 (4.5)	
f-NSIP	21 (10)	11 (14)	10 (7.5)	
uILD	16 (7.7)	9 (12)	7 (5.3)	
Other	18 (8.6)	3 (3.9)	15 (11)	
Pre-LTx treatment				
Corticosteroids cumulative dose during the last 12 months (g)	3.00 (0.00–4.50)	3.67 (3.27–5.47)	0.00 (0.00–3.65)	<0.001
Corticosteroids treatment at transplantation	120 (57)	67 (88)	53 (40)	<0.001
Corticosteroids dosage at transplantation (mg)	5 (0–10)	10 (8–16)	0 (0–10)	<0.001
Any antifibrotics	103 (49)	21 (28)	82 (62)	<0.001
Antifibrotics treatment ongoing at transplantation	84 (40)	18 (24)	66 (50)	<0.001
Pre-transplantation lung function				
Baseline FVC (%predicted)	44 (34–56)	41 (31–54)	46 (36–56)	0.1
Baseline DLCO (%predicted)	24 (18–32)	23 (15–28)	26 (20–33)	0.009
6-min walking test distance (m)	330 (240–435)	335 (230–420)	330 (240–450)	0.4
Right heart catheterization				
Pre-capillary PH	108 (52)	36 (48)	72 (55)	0.4
Severe PH	14 (6.9)	7 (9.6)	7 (5.3)	0.3
PAPm (mmHg)	23 (18–29)	23 (17–30)	24 (18–29)	>0.9
Type of lung transplantation				0.014
Double	114 (55)	50 (66)	64 (48)	
Single	95 (45)	26 (34)	69 (52)	
High emergency transplantation	64 (31%)	31 (41)	33 (25)	0.016
Acute exacerbation before LTx				
History of ILD exacerbation	72 (34)	31 (41)	41 (31)	0.14
Time since last exacerbation at transplant (days)	197 (34–343)	197 (27–338)	197 (40–359)	0.8
Donor age (years)	54 (42–64)	54 (43–64)	53 (41–63)	0.9

(Continued)

TABLE 1 Continued

Characteristics	Total (N=209)	Recent IA (N=76)	No recent IA (N=133)	p-value
Preformed donor-specific antibodies	48 (23)	14 (19)	34 (27)	0.3
CMV mismatch	24 (12)	9 (12)	15 (12)	>0.9

Data are n (%) or median (interquartile range).

IA, immunosuppressive agents; BMI, body mass index; GFR, glomerular filtration rate; ILD, interstitial lung diseases; IPF, idiopathic pulmonary fibrosis; f-HP, fibrotic hypersensitivity pneumonia; CTD-ILD, connective tissue disease associated interstitial lung disease; f-NSIP, fibrotic non-specific interstitial pneumonia; uILD, unclassified interstitial lung disease; PH, pulmonary hypertension; MMF, mycophenolate mofetil; AZA, azathioprine; FVC, forced vital capacity; DLCO, diffusing capacity of the lung for carbon monoxide; WU, Wood unit.

*Other ILD etiology: pleuroparenchymal fibroelastosis, silicosis, desquamative interstitial pneumonia.

Bolded values represents $p < 0.05$.

TABLE 2 Immediate perioperative outcomes by immunosuppressive agent (IA) exposure.

Characteristics	Total (N=209)	Recent IA (N=76)	No recent IA (N=133)	p-value
Number of RBC pack transfusions	2.0 (0.0–4.0)	3.0 (1.0–5.0)	2.0 (0.0–3.0)	<0.001
ECMO				
Intraoperative ECMO	163 (78)	62 (82)	101 (76)	0.3
Preoperative VV-ECMO	12 (5.7)	6 (7.9)	6 (4.5)	0.4
Intraoperative VV-ECMO	8 (3.8)	5 (6.6)	3 (2.3)	0.14
Postoperative VV-ECMO	37 (18)	18 (24)	19 (14)	0.087
Preoperative VA-ECMO	6 (2.9)	4 (5.3)	2 (1.5)	0.2
Intraoperative VA-ECMO	160 (77)	59 (78)	101 (76)	0.8
Postoperative VA-ECMO	43 (21)	22 (29)	21 (16)	0.024
Time on mechanical ventilation (days)	5 (1–20)	9 (1–25)	4 (1–15)	0.059
Tracheotomy	55 (26)	26 (35)	29 (22)	0.043
PGD grade 3*	28 (13)	17 (22)	11 (8.3)	0.004
Airways complications				
Bronchovascular fistula	6 (2.9)	5 (6.7)	1 (0.8)	0.024
Bronchial dilatation procedures	52 (25)	18 (24)	34 (26)	0.8
Acute kidney injury in ICU	63 (30)	31 (41)	32 (24)	0.011
Dialysis in ICU	36 (17)	18 (24)	18 (14)	0.062
Re-transplantation	4 (1.9)	2 (2.7)	2 (1.5)	>0.9
Re-transplantation <12 months	3 (0.1)	1 (1.4)	2 (1.5)	>0.9

Data are n (%) or median (interquartile range).

ECMO, extracorporeal membrane oxygenation; VV, venovenous; VA, venoarterial; PGD, primary graft dysfunction; ICU, intensive care unit; RBC, red blood cell.

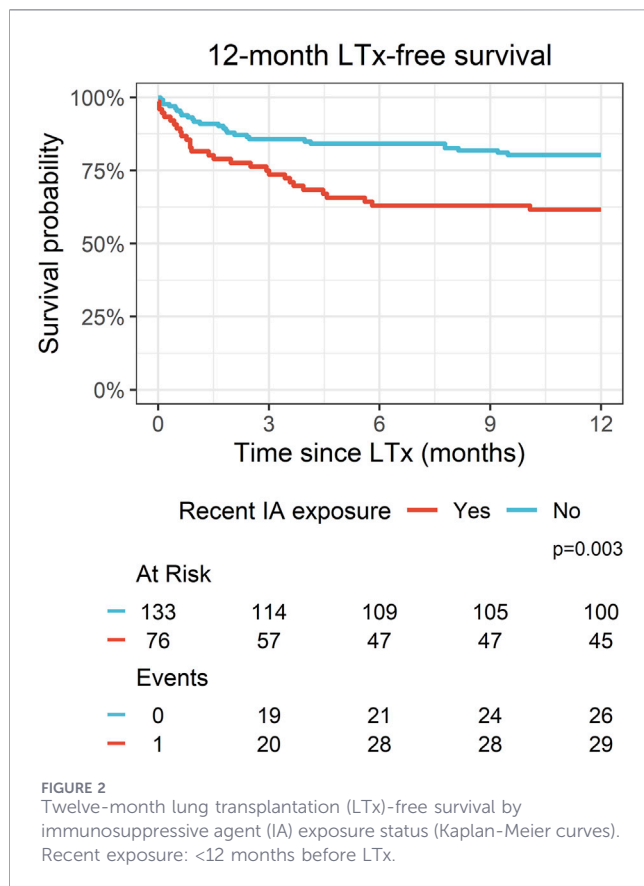
*Defined as a persistent PaO₂/FiO₂ ratio <200 or ECMO, requirement within 48–72 h after LTx, or death occurring within the first 48 h with grade 3 PGD. Bolded values represents $p < 0.05$.

(Supplementary Table S2). Four patients (1.9%) underwent re-transplantation, 3 (1.4%) within the first 12 months after LTx (1 in the IA group).

Twelve-month re-transplantation-free survival was reduced with recent IA exposure on univariate analysis (62% vs. 80%, HR 2.21, 95% CI [1.30–3.76], $p = 0.003$) (Figure 2) and multivariate analysis (HR 1.99 [1.11–3.56], $p = 0.022$) after adjusting for severe PH, cumulative dose of corticosteroids, high emergency status and recent ILD acute exacerbation (Table 3). Preoperative ECMO was not included in the multivariable model, as it is collinear with high emergency status. In the IPTW sensitivity analysis ($n = 204$ with

complete covariate data; 10 of 12 covariates achieved standardised mean differences <0.10 after weighting; Supplementary Table S3), the weighted HR of IA exposure for 12-month retransplantation-free survival was 2.44 (95% CI 1.27–4.70, $p = 0.007$), consistent with the primary multivariable estimate.

Notably, patients exposed to a non-biologic IA alone ($n = 56$) versus a biologic IA alone ($n = 10$) in the year before LTx did not differ in 12-month re-transplantation-free survival on multivariate analysis ($n = 58$) (HR 1.53 [0.57–4.14], $p = 0.398$) (Supplementary Table S4; Supplementary Figure S1). Considering the span of our study included the recent start of a systematic immunological



induction protocol at our centre, we performed a sensitivity analysis excluding the patients who had received induction (Basiliximab $n = 8$, anti-thymocyte globulin $n = 2$), and found a similar impact of IA exposure on survival (HR 2.29 [1.22–4.30], multivariate $p = 0.010$). To account for other potential changes in practice over the years, we performed univariate Cox regressions stratified on LTx era. The prognostic effect of recent IA exposure was preserved in both early (2011–2018, $n = 97$, HR 2.38 [1.08–5.22], $p = 0.031$) and recent eras (2019–2024, $n = 122$, HR 2.08 [1.01–4.26], $p = 0.046$).

Finally, longer-term analysis using multi-state modelling incorporating CLAD risk demonstrated a higher probability of death at 36 months for patients with recent IA exposure before LTx (48% vs. 35.5%, $p = 0.034$) (Supplementary Table S5; Supplementary Figure S2).

Immediate perioperative outcomes, PGD

Patients with recent IA exposure more frequently required postoperative veno-arterial ECMO (VA-ECMO) after surgery (29% vs. 16%, $p = 0.024$) and had grade 3 PGD (22% vs. 8.3%, $p = 0.004$) and acute kidney injury (41% vs. 24%, $p = 0.011$) (Table 2). Univariate logistic regression analysis revealed the following risk factors for grade 3 PGD: recent IA exposure (OR 3.20, 95% CI [1.42–7.45], $p = 0.005$), perioperative blood transfusion volume (OR 1.19, 95% CI [1.07–1.35], $p = 0.003$), and double LTx (OR 4.55 [1.78–1.04], $p = 0.003$) (Supplementary Table S6).

Airway complications

Bronchovascular fistula was diagnosed in 6 patients (2.9%), mainly in the IA group (5/76 (6.7%) versus 1/133 (0.8%), $p = 0.024$) and recent IA exposure was the only factor associated with this severe complication in univariate analysis (OR 9.43, 95% CI [1.48–183], univariate $p = 0.042$) (Supplementary Table S7). Conversely, there were similar rates of bronchial stenosis requiring dilatation during the 12 months after LTx in both groups (Table 2).

Early infectious outcomes

We included 156 patients with follow-up data for at least 6 months in the analysis. IA exposure was associated with increased number of pneumonia infections (median 2 [1–4] vs. 1 [0.5–2.5], $p = 0.001$) and antibiotics courses (4 [3–5] vs. 3 [1–5], $p = 0.010$) within 6 months post-LTx (Supplementary Table S8). The groups did not differ in *Clostridium difficile*, *Pseudomonas aeruginosa* or invasive fungal infections.

CMV complications

Overall, 20 of 150 recipient-positive (R+) and donor-positive recipient-negative (D + R-) patients (13% of those at risk) experienced CMV viremia under prophylaxis and 60 (40%) after prophylaxis withdrawal. In a survival analysis with death as a competing risk, the cumulative incidence of CMV viremia under prophylaxis during the first 12 months after LTx was higher for patients with IA exposure (21% vs. 5.2%, $p = 0.005$) (Supplementary Figure S3; Supplementary Table S9). Also, ganciclovir-resistant CMV was more frequent in patients with recent IA exposure (14% vs. 3.2%, $p = 0.021$) (Supplementary Table S10).

Immunological outcomes and allograft dysfunction

Preformed DSA was present in 14 patients (18.7%) in the IA group versus 34 (26.8%) in the non-IA group ($p = 0.3$), while 35 (46%) patients in the IA group (50%) exhibited allo-immunization after LTx vs. 70 (53%) ($p = 0.4$), and acute AMR and ACR affected 22 (11%) and 62 patients (30%) in the first year, with no difference between the 2 groups (Supplementary Figure S4). CLAD, mainly BOS (74% of all CLAD diagnoses), was diagnosed in 48 patients (23%) (Supplementary Tables S11–S13). Multi-state modelling did not demonstrate a difference in probability of CLAD occurrence between the 2 groups at 36 months (Supplementary Figure S2).

FEV1 trajectories

Patients reached their best median FEV1% of 76 (IQR 62–88) % predicted after a median of 177 (87–405) days post-LTx. On time point analysis, FEV1% during the first 3 months was lower for patients with recent IA exposure (Supplementary Figure S5), and linear-mixed modelling of FEV1 during the first year confirmed that IA exposure negatively affected FEV1 evolution over time (effect

TABLE 3 Univariate and multivariate analysis of 12-month re-transplantation-free survival.

Characteristics	Univariate analysis				Multivariate analysis		
	N	HR	95% CI	p-value	HR	95% CI	p-value
IA exposure <12 months	209	2.21	1.30–3.76	0.003	1.99	1.11–3.56	0.022
Corticosteroids cumulative dose (last 12 months) (g)	200	1.08	1.01–1.17	0.029	1.02	0.92–1.13	0.7
Corticosteroids at transplantation	209	1.33	0.77–2.31	0.3			
Corticosteroids dosage at transplantation (mg)	209	1.01	1.00–1.02	0.14			
Antifibrotics at transplantation	209	0.85	0.49–1.47	0.6			
Male sex	209	1.77	0.87–3.62	0.12			
Age at transplantation (years)	209	1.0	0.97–1.02	0.7			
BMI ≥ 30 (kg/m ²)	209	1.28	0.64–2.54	0.5			
Severe pulmonary hypertension	204	2.30	1.04–5.10	0.040	1.77	0.77–4.07	0.2
ILD etiology	204						
IPF		—	—				
CTD-ILD		1.40	0.64–3.06	0.4			
f-HP		1.34	0.58–3.10	0.5			
f-NSIP		2.11	0.91–4.89	0.081			
Other		1.14	0.38–3.39	0.8			
uILD		3.45	1.49–8.01	0.004			
ILD exacerbation <12 months	209	1.87	1.10–3.17	0.021	1.56	0.85–2.87	0.2
Type of LTx	209						
Single		—	—	—			
Double		1.09	0.64–1.86	0.8			
High emergency transplantation	209	1.68	0.98–2.88	0.061	1.16	0.62–2.17	0.6

IA, immunosuppressive agent; CI, confidence interval; HR, hazard ratio; ILD, interstitial lung diseases; IPF, idiopathic pulmonary fibrosis; f-HP: fibrotic hypersensitivity pneumonia; CTD-ILD, connective tissue disease-associated interstitial lung disease; f-NSIP, fibrotic non-specific interstitial pneumonia; uILD, unclassified interstitial lung disease; LTx, lung transplantation. Bolded values represents $p < 0.05$.

size: 0.77, 95%CI [0.63–0.96], random effect standard deviation: 0.04). ANOVA showed that including IA exposure status as a fixed effect provided improved the model performance ($p = 0.027$).

To explain this difference in early evolution in FEV1, we sought to analyse the relation between perioperative infections and respiratory function. The number of pneumonia episodes in the first 6 months was negatively correlated with FEV1 at 6, 12, and 24 months ($r = -0.45$ to -0.57 , $p < 0.001$ for all) (Supplementary Figure S6).

Discussion

To date and to our knowledge, this is the first study to demonstrate that patients with ILD who received non-steroidal IA during the year before LTx exhibited increased mortality in the first 12 months after the procedure. These patients had more pneumonia episodes and were at increased risk of grade 3 PGD, bronchovascular fistulas, CMV viremia under prophylaxis and ganciclovir-resistant CMV. For patients with recent IA exposure, pulmonary function was significantly worse during the first months after LTx and remained

persistently negatively correlated over time with the number of pneumonia episodes experienced in the first 6 months.

The main findings of our study align with previous research suggesting a detrimental effect of pre-LTx IA on post-LTx outcomes in patients with ILD. In a cohort of CTD-ILD patients undergoing LTx, postoperative infections were more numerous and earlier for those exposed [13]. In another cohort of 286 patients with ILD, those who received corticosteroids or IA without antifibrotics experienced more intraoperative blood loss, longer mechanical ventilation and more frequent grade 3 PGD than those not exposed, but with no difference in mortality [14].

We believe that our selection criteria enabled us to better discriminate the specific effect of pre-LTx non-steroidal IA on outcomes. Although we chose to consider any IA exposure within 12 months of LTx as significant, because the effects of these treatments can persist even after their discontinuation [9, 10, 15], we separately analysed IA without systemic corticosteroids. Hence, we could quantify the yearly cumulative dose of corticosteroids patients received, which was used to adjust multivariate analyses, to better individualize these risk factors. This approach was applied successfully to assess corticosteroid-

related adverse events and immunodeficiency in rheumatological diseases [16] and asthma, with most adverse outcomes occurring at cumulative exposures of 1 g per year [17]. In our study, cumulative corticosteroid exposure was associated with increased mortality on univariate but not multivariate analysis, thereby suggesting a more serious relative impact of non-steroidal IA exposure.

The higher rates of high emergency transplantation and bilateral procedures in the IA group reflect the clinical trajectory of patients requiring escalated immunosuppression: acute exacerbation of CTD-ILD frequently necessitates high-dose pulse corticosteroids or rituximab as a bridge, and this same clinical deterioration drives listing prioritisation. Both high emergency status and acute exacerbation were included in the multivariable model; the persistence of the independent IA effect after their adjustment, and its confirmation in the IPTW analysis indicate that the association exceeds what can be attributed to measurable pre-LTx severity alone.

The two most common causes of early mortality after LTx are PGD and infection [1], two complications more frequently observed in patients with recent IA exposure in our study. PGD is a form of acute lung injury after LTx characterized by hypoxemia and infiltrates seen on chest radiography occurring within 72 h¹². Known recipient- and procedure-specific risk factors include obesity, PH, operating time, and surgical procedure complexity [18]. In our study, patients exposed to IA also had higher rates of intraoperative VA-ECMO and more red blood cell transfusions than those not exposed, which suggests more difficult procedures.

Several mechanisms may account for the higher rates of grade 3 PGD and bronchovascular fistula in IA-exposed patients. Regarding PGD: cytotoxic agents deplete myeloid progenitors and may impair the innate immune response required for alveolar repair after ischemia-reperfusion injury, even after clinical discontinuation. Systemic corticosteroids, at significantly higher cumulative doses in the IA group, cause protein catabolism, sarcopenia, and impaired tissue repair capacity through suppression of fibroblast activity and angiogenesis, compromising both post-reperfusion recovery and anastomotic wound healing [19–21]. Regarding bronchovascular fistula: anastomotic healing depends on fibroblast proliferation, collagen synthesis, and bronchial arterial neovascularisation, all of which are inhibited by corticosteroids and cytotoxic agents [21]. An indirect pathway is equally plausible: severe PGD compromises bronchial arterial reconstitution, creating ischaemic anastomotic conditions, while the higher burden of peri-operative infections in the IA group may further weaken anastomotic integrity.

Longitudinal analysis showed that patients with IA exposure had significantly worse pulmonary function in the first months after LTx than those not exposed and that FEV1 remained negatively correlated with the number of pneumonia episodes during the first 6 months at 6, 12, and 24 months. Considering the prognostic value of early FEV1 values after LTx [22], these patients may exhibit increased respiratory vulnerability at a critical phase of their clinical course.

The overall breakthrough viremia rate of 13% in the CMV-at-risk population is consistent with recently published rates in the CTOT-20 prospective cohort [23]. The higher rate in the IA group (21% vs. 5.2%) likely reflects entry into transplantation with impaired CMV-specific T-cell immunity following immunosuppressive therapy. The enrichment of ganciclovir-

resistant CMV in the IA group (14% of CMV-at-risk IA-exposed patients) is mechanistically consistent: impaired T-cell control permits higher viral burdens at breakthrough, increasing selective pressure for UL97 kinase mutations; prolonged prophylaxis duration in high-risk patients further compounds this [24, 25].

Despite the higher intraoperative transfusion burden in the IA group, a recognised driver of HLA sensitization, preformed DSA prevalence and allo-immunization rates were comparable between groups, numerically lower in the IA group, potentially consistent with the B-cell suppressive effects of rituximab and cytotoxic agents. These findings argue against differential HLA sensitisation as a primary explanation for the observed outcome differences.

Finally, we found no significant association between IA exposure and allo-immunization, ACR, AHR or CLAD occurrence, probably because these treatments do not increase immunoreactivity and had no link to risk factors for these complications.

One limitation of our study is its monocentric and retrospective design, although this allowed us to compare a more homogeneous population in terms of follow-up and post-transplant management in a field in which therapeutic approaches are highly centre-dependent. Overall survival was lower than in some other cohorts and international registry data [1, 26, 27], but was in line with the most recent statement from the French *Agence de Biomédecine* (2023 report) which stated a similar nation-wide 1-year survival rate of 71% and a median survival of 55 months for patients undergoing LTx for pulmonary fibrosis. The slightly lower median survival in our cohort may be linked to the high rate of high-emergency and single LTx procedures, reflecting more severe ILD, challenging conditions for LTx, and a high proportion of older patients with comorbidities—however, these factors were used in adjusting multivariate analysis to individualize IA-related risk. The Lung Allocation Score was not available as an adjusting covariate, as this cohort was managed within the French allocation system; a standardised international severity comparator is therefore lacking.

The higher prevalence of CTD-ILD in the IA group is a direct reflection of clinical practice: IAs are the established disease-modifying therapy for most CTD-ILD subtypes, and their use is therefore intrinsic to the management of this diagnosis. Importantly, CTD-ILD was not an independent predictor of survival in the univariate Cox model (HR 1.40, 95% CI 0.64–3.06, $p = 0.40$ vs. IPF), and ILD subtype was not retained in the multivariable model, a decision which was supported by the results of the IPTW analysis. Residual confounding from CTD-specific comorbidities not captured in the dataset, such as cardiac involvement in systemic sclerosis or oesophageal dysmotility, cannot be excluded. Finally, the IA group was exposed to agents with markedly different mechanisms of action, pharmacokinetic profiles, and durations of immunosuppressive effect; the pooled analysis captures the clinical phenotype of recent IA exposure but precludes drug class-specific conclusions. The duration of individual exposures and the potential additive immunosuppressive effects of combination regimens (present in 22% of IA-exposed patients) could also not be formally quantified.

Overall, our results support considering IA exposure during the selection of LTx candidates among patients with ILD, to limit exposure whenever possible. Raising the awareness of clinicians and researchers to this risk factor may help inform management decisions at multiple levels: 1) in the context of worsening disease,

the decision to escalate to IA should be carefully considered, especially for diseases for which these drugs have weaker evidence (e.g., idiopathic interstitial pneumonias) [28, 29]; 2) during the pre-LTx evaluation process, considering IA exposure may help stratify risk in candidates; and 3) among patients with an active LTx project, reducing or stopping IA may be discussed, particularly if the patient could benefit or already benefits from an antifibrotic agent in that antifibrotic therapies have now demonstrated their efficacy on the disease course and exacerbations in progressive pulmonary fibrosis beyond IPF [30, 31] and show no evidence of increasing post-LTx adverse outcomes [6, 7]. However, the use of IA in an eligible patient should not be wholly restricted because responders may find their LTx project delayed or even cancelled.

Conclusion

IA exposure in the year preceding LTx was an independent risk factor for 12-month mortality among patients with ILD and predisposed them to early complications, including severe PGD, bronchovascular fistulas, pneumonia and the emergence of ganciclovir-resistant CMV. Considering IA exposure during pre-LTx assessment process may improve risk stratification in these patients and guide therapeutic decisions. Further prospective and multicentric studies are needed to confirm these findings.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#), further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the French society of respiratory medicine (Société de Pneumologie de Langue Française) review board (CEPRO no. 2025-006). 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

KT: Conceptualization, Methodology, Data collection, Writing (original draft). VB: Conceptualization, Project administration, Supervision, Writing (review and editing). HM: Conceptualization, Investigation, Writing (review and editing). TG: Investigation, Writing (review and editing). PH: Investigation, Writing (review and editing). LM: Investigation, Writing (review and editing). DM: Investigation, Writing (review and editing). MS: Investigation, Writing (review and editing). AS: Investigation, Writing (review and editing). GW: Investigation,

Writing (review and editing). PhM: Investigation, Writing (review and editing). EA: Investigation, Writing (review and editing). ST: Investigation, Writing (review and editing). JF: Investigation, Writing (review and editing). LH: Investigation, Writing (review and editing). YC: Investigation, Writing (review and editing). PiM: Investigation, Writing (review and editing). BC: Investigation, Writing (review and editing). RB: Conceptualization, Methodology, Writing (review and editing). KH: Conceptualization, Methodology, Data curation, Formal analysis, Supervision, Writing (review and editing). All authors contributed to the article and approved the submitted version.

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Conflict of interest

RB reports consulting fees from Boehringer Ingelheim, Ferrer, Sanofi, support for attending meetings from Boehringer Ingelheim. VB reports consulting fees from Takeda, CSL Behring, Sanofi, LFB, support for attending meetings from Takeda, Grifols and LFB.

The remaining author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Correction note

This article has been corrected with minor changes. These changes do not impact the scientific content of the article.

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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.2026.16549/full#supplementary-material>

References

- Chambers DC, Cherikh WS, Harhay MO, Hayes D, Jr, Hsich E, Khush KK, et al. The international thoracic organ transplant registry of the international society for heart and lung transplantation: thirty-Sixth adult lung and heart-lung transplantation Report—2019; focus theme: donor and recipient size match. *J Heart Lung Transpl* (2019) 38(10):1042–55. doi:10.1016/j.healun.2019.08.001
- Raghu G, Remy-Jardin M, Richeldi L, Thomson CC, Inoue Y, Johkoh T, et al. Idiopathic pulmonary fibrosis (an update) and progressive pulmonary fibrosis in adults: an official ATS/ERS/JRS/ALAT clinical practice guideline. *Am J Respir Crit Care Med* (2022) 205(9):e18–e47. doi:10.1164/rccm.202202-0399ST
- Johnson SR, Bernstein EJ, Bolster MB, Chung JH, Danoff SK, George MD, et al. 2023 American college of rheumatology (ACR)/american college of chest physicians (CHEST) guideline for the treatment of interstitial lung disease in people with systemic autoimmune rheumatic diseases. *Arthritis Rheumatol Hoboken NJ* (2024) 76(8):1182–200. doi:10.1002/art.42861
- McAnally KJ, Valentine VG, LaPlace SG, McFadden PM, Seoane L, Taylor DE. Effect of pre-transplantation prednisone on survival after lung transplantation. *J Heart Lung Transpl* (2006) 25(1):67–74. doi:10.1016/j.healun.2005.07.012
- Kapnadak SG, Raghu G. Lung transplantation for interstitial lung disease. *Eur Respir Rev* (2021) 30(161):210017. doi:10.1183/16000617.0017-2021
- Mackintosh JA, Munsif M, Ranzanbacher L, Thomson C, Musk M, Snell G, et al. Risk of anastomotic dehiscence in patients with pulmonary fibrosis transplanted while receiving anti-fibrotics: experience of the Australian lung transplant collaborative. *J Heart Lung Transpl* (2019) 38(5):553–9. doi:10.1016/j.healun.2019.02.005
- Moncombe E, Weisenburger G, Picard C, Dégot T, Reynaud-Gaubert M, Nieves A, et al. Effect of antifibrotic agents on postoperative complications after lung transplantation for idiopathic pulmonary fibrosis. *Respirology* (2024) 29(1):71–9. doi:10.1111/resp.14605
- Leard LE, Holm AM, Valapour M, Glanville AR, Attawar S, Aversa M, et al. Consensus document for the selection of lung transplant candidates: an update from the international society for heart and lung transplantation. *J Heart Lung Transpl* (2021) 40(11):1349–79. doi:10.1016/j.healun.2021.07.005
- Faurschou M, Mellemkjaer L, Voss A, Keller KK, Hansen IT, Baslund B. Prolonged risk of specific malignancies following cyclophosphamide therapy among patients with granulomatosis with polyangiitis. *Rheumatology (Oxford)* (2015) 54(8):1345–50. doi:10.1093/rheumatology/keu372
- Kridin K, Mruwat N, Amber KT, Ludwig RJ. Risk of infections in patients with pemphigus treated with rituximab vs. azathioprine or mycophenolate mofetil: a large-scale global cohort study. *Br J Dermatol* (2023) 188(4):499–505. doi:10.1093/bjd/ljac118
- Verleden GM, Glanville AR, Lease ED, Fisher AJ, Calabrese F, Corris PA, et al. Chronic lung allograft dysfunction: definition, diagnostic criteria, and approaches to treatment—A consensus report from the pulmonary council of the ISHLT. *J Heart Lung Transpl* (2019) 38(5):493–503. doi:10.1016/j.healun.2019.03.009
- Snell GI, Yusef RD, Weill D, Strueber M, Garrity E, Reed A, et al. Report of the ISHLT working group on primary lung graft dysfunction, part I: definition and grading—A 2016 consensus group statement of the international society for heart and lung transplantation. *J Heart Lung Transpl* (2017) 36(10):1097–103. doi:10.1016/j.healun.2017.07.021
- Fitzgerald LJ, Lyu DM, Lin J, Chan KM. Impact of pre-transplant immunosuppression in CT-ILD recipients. *ISHLT 42nd Annu Meet Sci Sess* (2022) 41(4, Suppl. ment):S414–S415. doi:10.1016/j.healun.2022.01.1044
- Munker D, Arnold P, Leuschner G, Irlbeck M, Michel S, Kauke T, et al. Impact of ILD-specific therapies on perioperative course in patients with progressive interstitial lung disease undergoing lung transplantation. *J Clin Med* (2023) 12(15):4996. doi:10.3390/jcm12154996
- Veeken LD, Opdam MAA, Verhoef LM, Popa C, van Crevel R, den Broeder AA. Infection incidence, timing and dose dependency in rheumatoid arthritis patients treated with rituximab: a retrospective cohort study. *Rheumatology* (2024) 63(5):1246–50. doi:10.1093/rheumatology/kead328
- Sweeney J, Patterson CC, Menzies-Gow A, Niven RM, Mansur AH, Bucknall C, et al. Comorbidity in severe asthma requiring systemic corticosteroid therapy: cross-sectional data from the optimum patient care research database and the British thoracic difficult asthma registry. *Thorax* (2016) 71(4):339–46. doi:10.1136/thoraxjnl-2015-207630
- Price D, Castro M, Bourdin A, Fucile S, Altman P. Short-course systemic corticosteroids in asthma: striking the balance between efficacy and safety. *Eur Respir Rev* (2020) 29(155):190151. doi:10.1183/16000617.0151-2019
- Diamond JM, Lee JC, Kawut SM, Shah RJ, Localio AR, Bellamy SL, et al. Clinical risk factors for primary graft dysfunction after lung transplantation. *Am Journal Respiratory Critical Care Medicine* (2013) 187(5):527–34. doi:10.1164/rccm.201210-1865OC
- Chamogeorgakis T, Mason DP, Murthy SC, Thuita L, Raymond DP, Pettersson GB, et al. Impact of nutritional state on lung transplant outcomes. *J Heart Lung Transpl* (2013) 32(7):693–700. doi:10.1016/j.healun.2013.04.005
- Crespo MM, McCarthy DP, Hopkins PM, Clark SC, Budev M, Bermudez CA, et al. ISHLT consensus statement on adult and pediatric airway complications after lung transplantation: definitions, grading system, and therapeutics. *J Heart Lung Transpl* (2018) 37(5):548–63. doi:10.1016/j.healun.2018.01.1309
- Anstead GM. Steroids, retinoids, and wound healing. *Adv Wound Care J Prev Heal* (1998) 11(6):277–85.
- Liu J, Jackson K, Weinkauff J, Kapasi A, Hirji A, Meyer S, et al. Baseline lung allograft dysfunction is associated with impaired survival after double-lung transplantation. *J Heart Lung Transpl* (2018) 37(7):895–902. doi:10.1016/j.healun.2018.02.014
- Snyder LD, Weber JM, Avery R, Neely M, Robien M, Schaenman J, et al. CMV incidence, risk factors and breakthrough infections in a multicenter lung transplant cohort. *J Heart Lung Transpl* (2025) 44(4):S107. doi:10.1016/j.healun.2025.02.218
- Chou S. Cytomegalovirus UL97 mutations in the era of ganciclovir and maribavir. *Rev Med Virol* (2008) 18(4):233–46. doi:10.1002/rmv.574
- Limaye AP, Raghu G, Koelle DM, Ferrenberg J, Huang ML, Boeckh M. High incidence of ganciclovir-resistant cytomegalovirus infection among lung transplant recipients receiving preemptive therapy. *J Infect Dis* (2002) 185(1):20–7. doi:10.1086/338143
- Leong SW, Bos S, Lordan JL, Nair A, Fisher AJ, Meachery G. Lung transplantation for interstitial lung disease: evolution over three decades. *BMJ Open Respir Res* (2023) 10(1):e001387. doi:10.1136/bmjresp-2022-001387
- Hartert M, Senbaklavaci O, Gohrbandt B, Fischer BM, Buhl R, Vahl CF. Lung transplantation. *Dtsch Arztebl Int* (2014) 111(7):107–16. doi:10.3238/arztebl.2014.0107
- Morisset J, Johansson KA, Vittinghoff E, Aravena C, Elicker BM, Jones KD, et al. Use of mycophenolate mofetil or azathioprine for the management of chronic hypersensitivity pneumonitis. *Chest* (2017) 151(3):619–25. doi:10.1016/j.chest.2016.10.029
- Adegunsoye A, Oldham JM, Fernández Pérez ER, Hamblin M, Patel N, Tener M, et al. Outcomes of immunosuppressive therapy in chronic hypersensitivity pneumonitis. *ERJ Open Res* (2017) 3(3):00016–2017. doi:10.1183/23120541.00016-2017
- Flaherty KR, Wells AU, Cottin V, Devaraj A, Walsh SLF, Inoue Y, et al. Nintedanib in progressive fibrosing interstitial lung diseases. *N Engl J Med* (2019) 381(18):1718–27. doi:10.1056/NEJMoa1908681
- Distler O, Highland KB, Gahlemann M, Azuma A, Fischer A, Mayes MD, et al. Nintedanib for systemic sclerosis-associated interstitial lung disease. *N Engl J Med* (2019) 380(26):2518–28. doi:10.1056/NEJMoa1903076



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Resilience of lung grafts to warm ischemia: assessment by *ex vivo* perfusion using functional and molecular analysis

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To address the shortage of donor lungs, donation after circulatory death with extended warm ischemia (WI) is increasingly used. Normothermic *ex vivo* lung perfusion (EVLP) allows assessment and rehabilitation of WI lungs. However, the safety limits of WI duration remain unclear due to conflicting preclinical data and variations in clinical protocols across countries. Using a clinically-relevant pig model, we compared paired right (control) and left (WI) lungs from five donors. WI lungs experienced 2-h WI followed by 1-h cold preservation and 6-h EVLP, while control lungs underwent the same protocol without WI. Initial 2-h WI impaired compliance by 32% and increased vascular resistance by 25%, but both parameters normalized during EVLP. Oxidative and mitochondrial stress markers, cytokine release, histological injury, and edema showed no significant difference between control and WI lungs. During EVLP, both control and WI lungs exhibited similar transcriptomic responses in terms of the number of regulated genes (69/96) and their expression levels. Overall, EVLP reversed initial WI-associated functional impairments and led to convergent molecular profiles in WI and control lungs. These findings support the possibility of extending acceptable WI duration thresholds in lung transplantation.

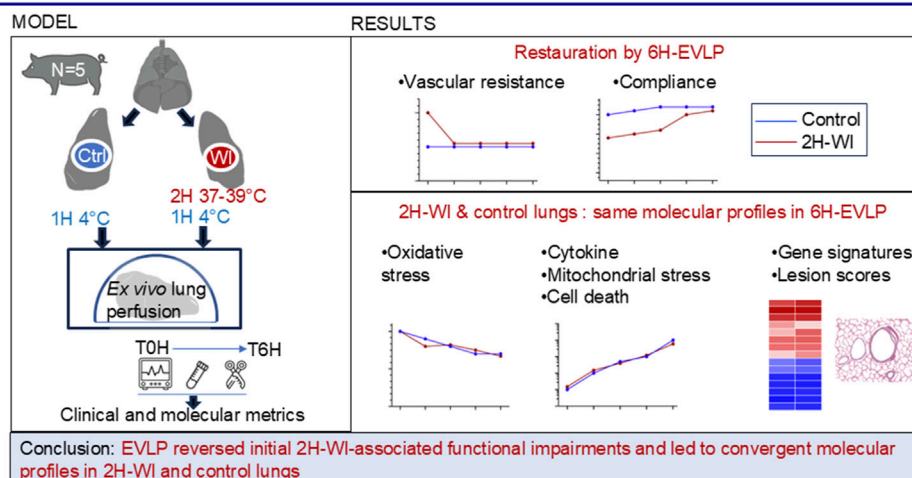
KEYWORDS

ischemia-reperfusion injuries, lung, pig model, transplantation, warm ischemia

Introduction

Due to the persistent shortage of donor lungs, donation after circulatory death (DCD) and prolonged warm ischemia (WI) are increasingly tolerated for transplantation, despite elevated risk of ischemia-reperfusion injury (IRI) [1]. To mitigate this risk, normothermic *ex vivo* lung perfusion (EVLP) is used to assess and rehabilitate these grafts. In countries with formal legal frameworks (e.g., France and Italy), EVLP is mandatory for DCD lungs, whereas in other

Resilience of Lung Grafts to Warm Ischemia: Assessment by Ex Vivo Perfusion using Functional and Molecular Analysis



GRAPHICAL ABSTRACT

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countries, its use is guided by clinical judgment or institutional protocols. There is no consensus on the safe duration of WI after DCD. Clinical limits vary and are based on inconsistent preclinical and clinical results. Indeed, some studies reported that WI beyond 60 min exacerbated inflammatory responses and impaired respiratory function during EVLP [2–4]. In contrast, retrospective clinical analyses have shown that DCD lungs with a mean WI duration of up to 208 min exhibited inflammatory profiles during EVLP and early post-transplant outcomes similar to those of marginal lungs from brain-dead donors [5]. Interpretation of preclinical data is complicated by inconsistencies in EVLP protocols, particularly due to the variable inclusion of erythrocytes and a lack of corticosteroids, the latter being standardly used in clinical settings. In this context, national guidelines differ widely in their maximum permitted WI durations: 90 min in France [6] and Australia [7], 120 min in the UK [8], 150 min in Spain [9], and up to 180 min in Italy [5].

We hypothesized that lungs subjected to an extended 2-h WI period may be rehabilitated by EVLP. The objective of this report was to assess the impact of a 2-h WI period on lung responses to a clinically EVLP relevant protocol (i.e., the Toronto technique with corticosteroids), in a porcine lung model, widely recognized as the most translationally relevant model. We performed a comprehensive comparison of paired right and left lungs undergoing EVLP under control conditions (control lungs, right) and after a 2-h WI (WI lungs, left) using a broad range of metrics.

Materials and Methods

Ethics

The experiments were performed in compliance with the EU guidelines and the French regulations (DIRECTIVE 2010/63/EU,

2010; Code rural, 2018; Décret n°2013-118, 2013). The procedures received authorization of the Ministry of Higher Education and Research and the experimental protocols were approved by the COMETHEA ethic committee under the number APAFIS# DAP2022120510144946 v3. The surgery was carried out at the Medical Imaging in Animal platform (accreditation B78-322-2, doi.org/10.15454/1.5572348210007727E12).

Animals, and EVLP procedures

Lungs were harvested from five Large-White x Landrace x Pietrain pigs (48–55 kg). Animals were anesthetized with intramuscular ketamine (20 mg·kg⁻¹, Imalgene®) and xylazine (2 mg·kg⁻¹, Rompun®). Orotracheal intubation was performed after deepening anesthesia with 3%–5% isoflurane using mask, and intravenous injection of fentanyl (20 µg·kg⁻¹) and diazepam (0.1 mg·kg⁻¹). Anesthesia was maintained with 2%–3% inhaled isoflurane and continuous propofol infusion at 5 mg·kg⁻¹·h⁻¹ (Propofur®). Mechanical ventilation was initiated with a tidal volume (Vt) of 7 mL·kg⁻¹, a positive end-expiratory pressure (PEEP) of 5 cmH₂O, and a respiratory rate of 15 cycles·min⁻¹. Upon chest opening, a biopsy from the azygos lobe was harvested (designated as the “Time of death” time point). The animal was then euthanized by aortic clamping and exsanguination following intravenous administration of 25,000 IU of heparin. Euthanasia marked the onset of WI. After inflation, each lung was isolated. The right lung (control group) was immediately flushed with 2 L Perfadex® at 4 °C (XVIVO Perfusion, Gothenburg, Sweden) and stored in the refrigerator at 6 °C for 1 h. The left lung (WI group) remained inflated *in situ* for 2 h, before being similarly flushed and stored at 6 °C for 1 h. Each lung was then connected to an EVLP circuit following the Toronto protocol [10]. The circuit was

primed with 1 L of Steen® solution supplemented with 0.5 g methylprednisolone, 0.75 g cefuroxime, and 3750 ui heparin. The lung size differences were taken into account to determine the flow of 16% (left) and 24% (right) of the theoretical cardiac output. The system reached 37 °C after approximately 30 min of perfusion. Ventilation settings were adjusted to a Vt of 3.5 mL/kg for the right lung and 2.5 mL/kg for the left lung, with a fraction of inspired oxygen FiO₂ of 0.21, positive and expiratory pressure (PEEP) of 5 cmH₂O and a respiratory rate of 15 breaths per minute. The perfusate was deoxygenated using a gas mixture containing 8% CO₂, 86% N₂O, and 6% O₂. EVLP was maintained for a total duration of 6 h.

EVLP monitoring, sample collections

Pulmonary gas exchange capacity was evaluated using PaO₂ and PaCO₂ measured in the perfusate at an FiO₂ of 0.21, using i-STAT cartridges (Abbott, Chicago, USA). Respiratory mechanics and mechanical ventilation were assessed by measuring plateau pressure (Pplat), driving pressure ($\Delta P = P_{\text{plat}} - \text{PEEP}$), pulmonary compliance (Vt/ ΔP) and recruitment (compliance change during a PEEP test performed at 2 cmH₂O and 8 cmH₂O). The pulmonary strain was estimated as stress divided by specific elastance, with stress approximated by Pplat for isolated lungs, and specific elastance estimated in pigs at 6 cmH₂O [11]. Hemodynamics were evaluated by calculating pulmonary vascular resistance ($\text{PVR} = [(m\text{PAP} - 0)/\text{ECMO flow}] \times 80$). Perfusates were collected at hourly intervals and frozen at -80 °C for lactate dehydrogenase (LDH), mitochondrial DNA and cytokine detection. Lung tissue samples (about 100 mg) were collected using a lung stapler from the azygos lobe just before exsanguination, from the superior lobe at the end of the cold ischemic phase and after 2-h perfusion, and from the inferior lobe after 4- and 6-h perfusion. These samples preserved in RNAlater (Merck, Darmstadt, Germany) or directly frozen in liquid nitrogen and kept at -70 °C. Additionally, a 100 mg lung tissue sample collected at 6 h was fixed in 4% buffered paraformaldehyde for histological analysis, then rinsed after 24 h and stored in 70% ethanol. Bronchoalveolar lavages were performed with 2 × 20 mL cold PBS, and samples were kept frozen at -70 °C.

Biological dosages and histology

Detailed histology protocols, the commercial kits employed (LDH, cytokine assays, carbonylated proteins, oxidized DNA), and mitochondrial DNA quantification by PCR (ND1 and ND4 genes) are described in [Supplementary Material 1](#).

Transcriptomic analyses

Total RNA was extracted from lung tissue using TRIzol reagent with bead homogenization (Precellys 24 bead grinder) and purified with the NucleoSpin RNA kit including DNase treatment. RNA quantity and integrity were assessed using the Qubit RNA assay kit and Agilent 2100 Bioanalyzer. Reverse transcription (1.2 µg RNA) was performed using the PrimeScript RT reagent kit. Gene expression was measured using custom TaqMan Array Cards (96 genes, 4 housekeeping genes, [Supplementary Material 2](#)) on a

QuantStudio 7 Flex Real-Time PCR System. Data were analyzed using the $2^{-\Delta\Delta\text{CT}}$ method normalized to RPS24, RPLP2, and GAPDH; genes with Ct > 32 were excluded. Principal component analysis was performed (mixOmics). Differentially expressed genes (DEGs) versus “Time of death” were identified using paired one-way tests with Benjamini–Hochberg correction (adjusted p < 0.1). Fold changes were calculated relative to “Time of death”. Comparisons between all conditions and the 2-h WI at time zero used paired non-parametric tests with multiple testing correction (adjusted p < 0.2). More details are provided in [Supplementary Material 1](#).

Statistical analyses

The statistics of gene expression is described in the TAC array design and analysis. For the physiological and biological parameters, a Linear Mixed Model with fixed effects for group, time, and their interaction, and a random intercept for pig subject, was used to analyze repeated measures using R Studio2 v2024.09.0 + 375; the fixed effects were tested with a type III ANOVA. For the *post hoc* comparisons between lung groups, a Tukey test was performed (paired data), using estimated marginal means, and provided adjusted p-values. For histology and percent weight gain, the data were statistically compared using a paired t-test, after checking normal distribution (Shapiro-Wilk). [Supplementary Material 3](#) reports the raw data, means, standard deviation as well as the statistical results (F-val, p-val).

Results

The effects of 2-h WI on pig lung functions are corrected by EVLP

Lungs were explanted from five pig donors (see [Supplementary Material 1](#)). The azygos lobe was sampled immediately post-mortem (“Time of death”). Each lung block was divided into right and left lungs to allow paired analysis. The right lung (control group) was kept at 6 °C for 1 hour and immediately subjected to 6-h EVLP. The left lung (WI group) remained for 2 h in the thoracic cavity, before undergoing the same cold preservation and EVLP protocol ([Figure 1](#)). At the time of surgery, the animals’ body temperatures were within the physiological range (38.5 °C–39 °C) and decreased to 35 °C–37 °C 2 hours after death. During EVLP, oxygenation was maintained in both groups, with PaO₂ values consistently exceeding 150 mmHg ([Figure 2A](#), see [Supplementary Material 3](#) for the raw data of [Figures 2–5](#)). Initial lung compliance was lower in the WI group than in the control group (32% lower, p-val = 0.02), and improved over EVLP time (time effect p-val = 0.008 for both groups, [Figure 2B](#)). The strain ratios were below 2.5 and not different between groups ([Figure 2C](#)). The initial vascular resistance was higher in the WI than in the control group (25.6% higher, p-val = 0.007), and then the difference became non-significant during EVLP ([Figure 2D](#)). At 6 h, weight gain was limited and comparable between groups (percent weight gain: 7.89 ± 11.76 in WI and 14.61 ± 12.23 in control group, [Figure 2E](#)). So overall, although 2-h WI initially impaired lung functions

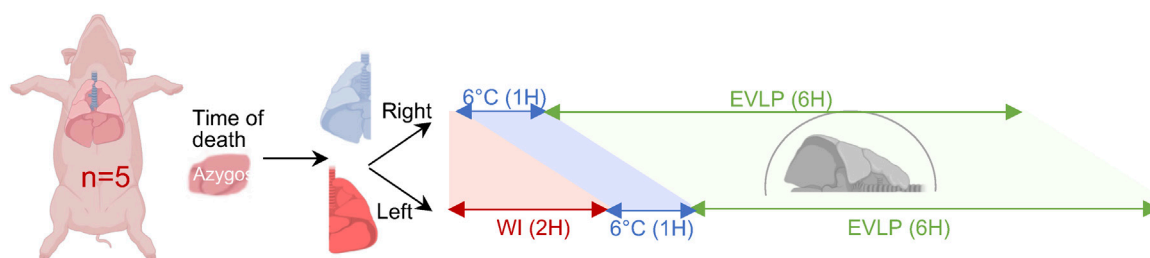


FIGURE 1

Experimental scheme. Lungs were obtained from 5 pigs. After general anesthesia, the azygos lobe was removed and used as a reference lung tissue sample designated as “Time of death” in the whole paper. After subsequent euthanasia by exsanguination, the right and left lungs were separated. The right (Control) was immediately flushed with Perfadex, kept at 6 °C for 1-h, and subjected to EVLP using the Toronto protocol, during 6 h. The left lung (warm ischemia, WI) was placed in the thoracic cavity for 2-h, and then similarly processed as the right lung.

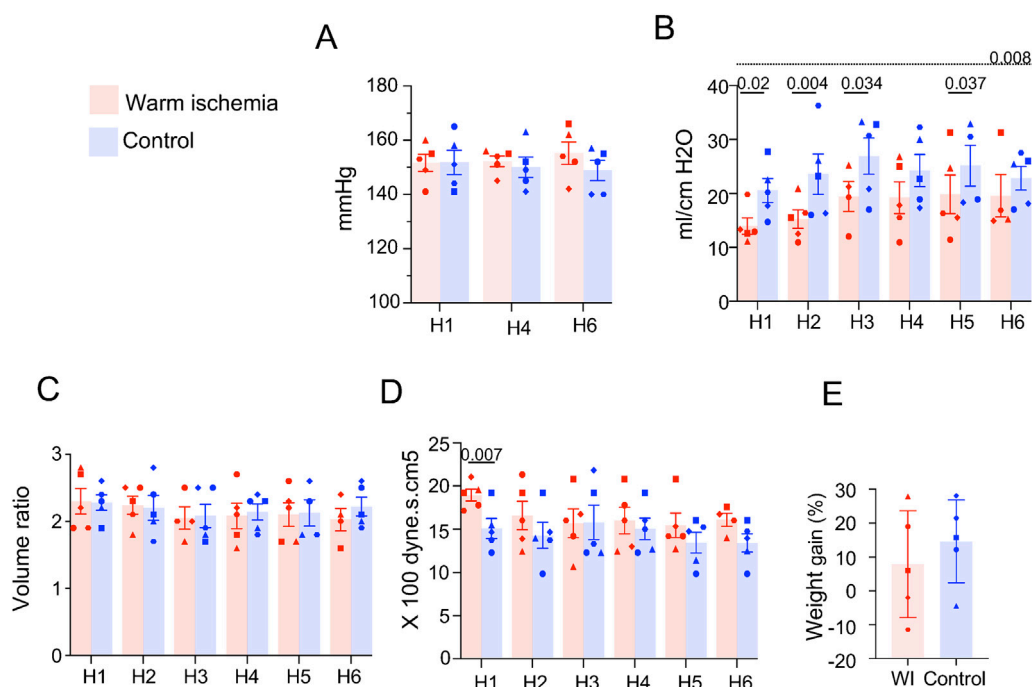


FIGURE 2

Physiological respiratory parameters. (A) PaO₂, Partial pressure of oxygen in arterial blood. (B) Lung compliance. (C) Strain (change in lung volume divided by functional residual capacity). (D) Vascular resistance. (E) Weight gain at 6H versus 0H (% increase). For (A–D), a linear mixed model with fixed effects for group, time, and their interaction, and a random intercept for pig subject, was used to analyze repeated measures. Type III ANOVA was performed, followed by Tukey-adjusted *post hoc* comparisons using estimated marginal means. Statistical significance is indicated as i) adjusted p-val between groups at specific time points, and ii) as the time effect p-val for both groups over the dashed line. For E, the data were statistically compared using a paired t-test, following a test for assessing the normal distribution of the data (Shapiro-Wilk). Data from the control group are in blue and data from the WI group are in red. Each pig is labelled with the same symbol in all figures (triangle, square, diamond, circle, hexagon).

(reducing compliance and increasing vascular resistance), these effects were corrected by a 6-h EVLP.

Extended WI in pig lung has little impact on the IRI response during EVLP

Biological markers associated with ischemia–reperfusion injury were measured during EVLP (Figures 3–5). At baseline (“Time of death”), carbonylated protein levels in lung tissue reached mean values of 2.5 mmol/mg protein (Figure 3A). These levels decreased

over time during EVLP (time effect $p = 0.01$), with no difference between groups at any time point. Similarly to carbonylated protein levels, oxidative DNA levels showed a temporal decrease in both groups (Figure 3B). Mitochondrial DNA concentrations in the perfusate increased over time (time effect $p = 0.031$), without intergroup differences (Figure 3C). LDH was progressively liberated in the perfusion liquid (time effect, $p\text{-val} < 0.001$), without difference between groups (Figure 3D). The metabolic response reflected by lactate production increased over time ($p\text{-val}$ in both groups < 0.001) and showed higher values in the

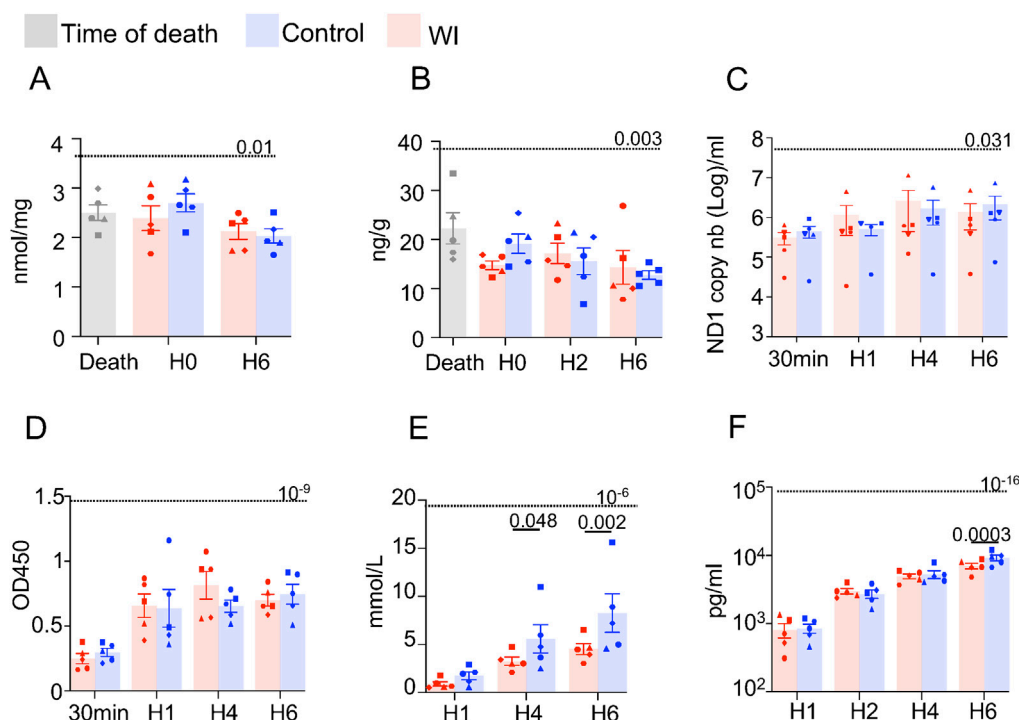


FIGURE 3

Cellular stress and injury parameters. (A) Carbonylated proteins were measured by an ELISA test using frozen lung tissue samples and the results were expressed as ng carbonylated proteins per mg total proteins. (B) The 8-hydroxy-2'-deoxyguanosine in DNA was measured by an ELISA test using frozen lung tissue samples and the results were expressed as ng oxidated DNA per Gram of tissue. (C) The mitochondrial DNA levels were evaluated by quantifying the pig mitochondrial ND1 gene in perfusion liquids using quantitative PCR. The results are expressed as ND1 gene copy numbers per ml. (D) The lactate dehydrogenase (LDH) liberated in the perfusion liquid was measured by a colorimetric assay and OD values minus background are reported. (E) Lactate levels were measured with iStat cartridge from fresh perfusion liquid. (F) PECAM shedding was measured with a pig luminex discovery assay. A linear mixed model with fixed effects for group, time, and their interaction, and a random intercept for pig subject, was used to analyze repeated measures. Type III ANOVA was performed, followed by Tukey-adjusted *post hoc* comparisons using estimated marginal means. Statistical significance is indicated as adjusted p-val between groups at specific time points, and as the time effect p-val over a dashed line for both groups. Data from the control group are in blue and data from the WI group are in red. Each pig is labelled with the same symbol in all figures (triangle, square, diamond, circle, hexagon).

control group as compared to in the WI group particularly at 6 h (p-val = 0.002, Figure 3E). Finally, the levels of PECAM release also increased over time with higher values in the control group at 6 h (p-val in both groups = 0.0003, Figure 3F). Cytokine concentrations in the perfusate increased over time for TNF α , IL-6, and IL-10, with no differences between groups (Figure 4A). Similar findings were observed in bronchoalveolar lavage samples (Figure 4B). Histological assessment at 6 h showed low injury scores in both groups, with no significant difference (WI: 0.59 ± 0.19 vs. control: 0.42 ± 0.12 ; $p = 0.145$; Figures 5A–C).

Extended WI in pig lung primes but does not escalate the EVLP-associated gene expression

We designed a 96 PCR-array using genes previously shown by us and others to be modulated by EVLP ([12–18], Supplementary Material 2). We also incorporated the PDK4 and FKBP5 genes that are upregulated by corticosteroids. Among the 96 genes, 84 genes were retained for analysis based on expression thresholds ($C_q < 32$; see Methods). Principal component analysis of $2^{-\Delta\Delta C_T}$ values showed overlap between samples obtained at “Time of death”

and those from the control group at EVLP initiation (0 hour-EVLP) (Figures 6A,B). During EVLP, transcriptomic profiles from the control and WI groups followed similar trajectories and converged at 6 h. Interestingly, at 0-h EVLP, the WI group samples were located in the EVLP trajectory.

For each condition (group and time), a list of differentially expressed genes relative to the “Time of death” (DEGs, p-val < 0.1) was established, revealing 69 genes that were differentially expressed at least once (Figure 7, Supplementary Material 4). Among these DEGs, genes related to inflammation (IL6, CXCL2, CXCL8, CCL2, IL17F, TGFA), IRI (CD14, TLR4, MyD88, STAT3) and oxidative stress (GADD45, MT1A, SOD2) were upregulated, whereas genes related to immune activation (such as CD1E, TCRD, XCL1, IFNG) and cytoskeleton signaling and cell adhesion/migration (ACTG1, ACTB, Myo5C, ITGA4, ITGA6, CX3CR1) were downmodulated. PDK4 and FKBP5 were upregulated only during EVLP, reflecting effective response to corticosteroids. Figure 7 shows that among these 69 DEGs, 29 were significantly modulated by WI *per se* at 0-h EVLP whereas none were found in the control condition at 0-h EVLP. Thereafter during EVLP, there was no significant difference in the gene expression fold changes between the WI and control group at any time point. Furthermore at 2-

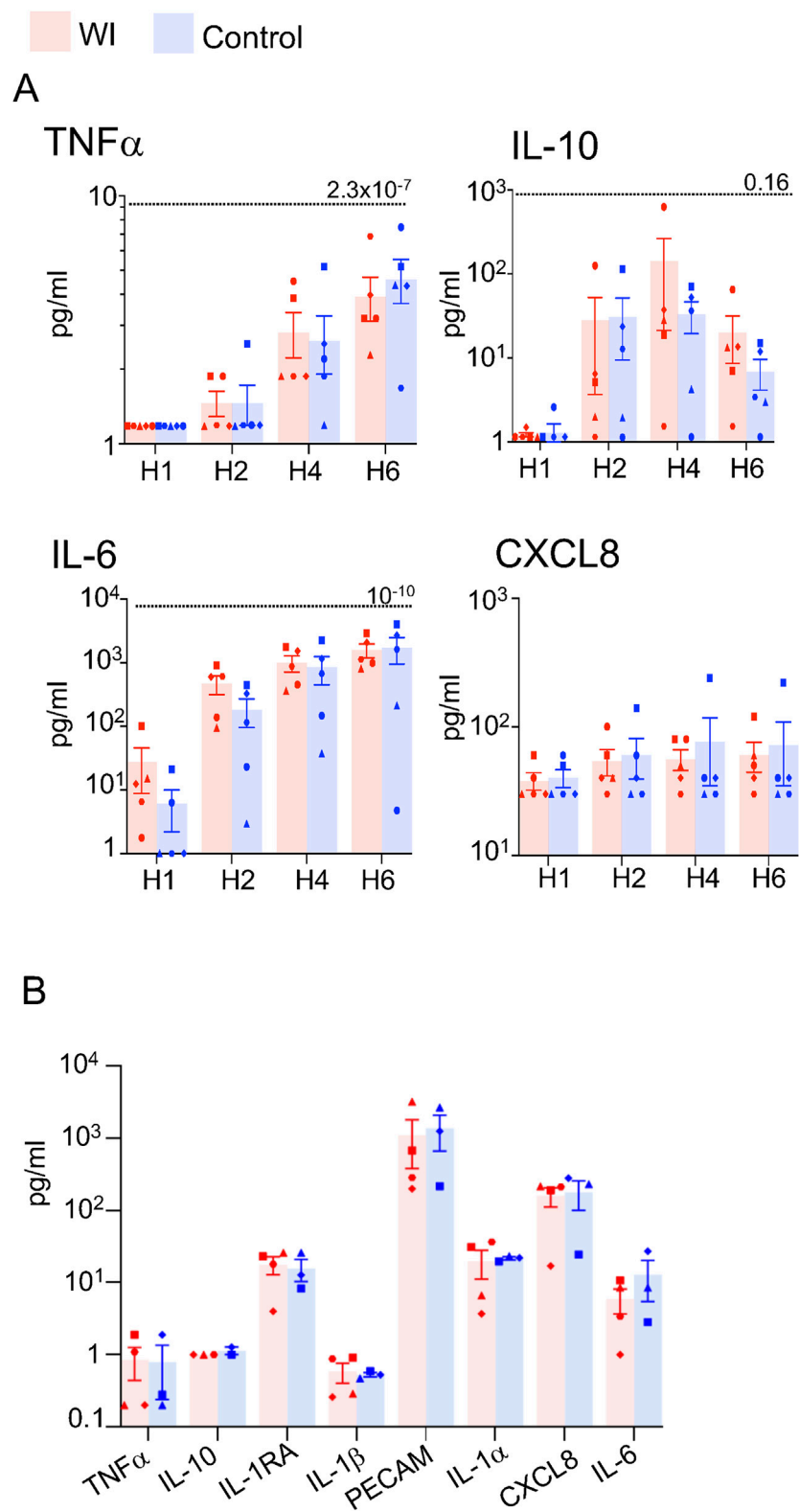
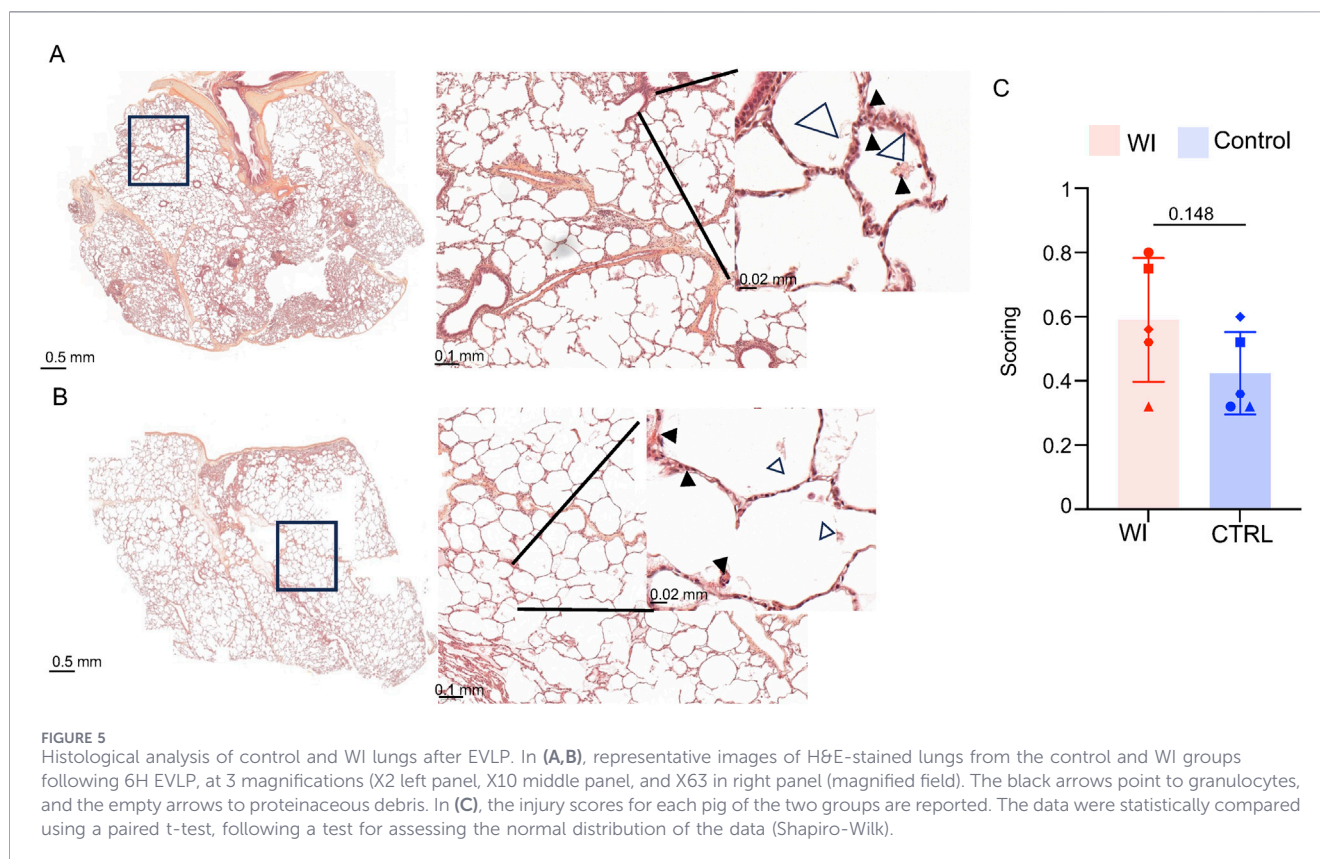


FIGURE 4
Cytokine release in the perfusion liquid at different timings (A) and in the bronchoalveolar lavage at 6 h (B). Cytokine levels were measured with a Porcine Luminex Discovery Assay. In perfusion liquids, IL-1 α , IL-1 β , IL1RA levels were below the detection threshold and not represented. A linear mixed model with fixed effects for group, time, and their interaction, and a random intercept for pig subject, was used to analyze repeated measures. Type III ANOVA was performed, followed by Tukey-adjusted *post hoc* comparisons using estimated marginal means. Statistical significance is indicated as the time effect p-val over a dashed line for both groups. There was no statistically significant differences between the control and WI groups. Data from the control group are in blue and data from the WI group are in red. Each pig is labelled with the same symbol in all figures (triangle, square, diamond, circle, hexagon).



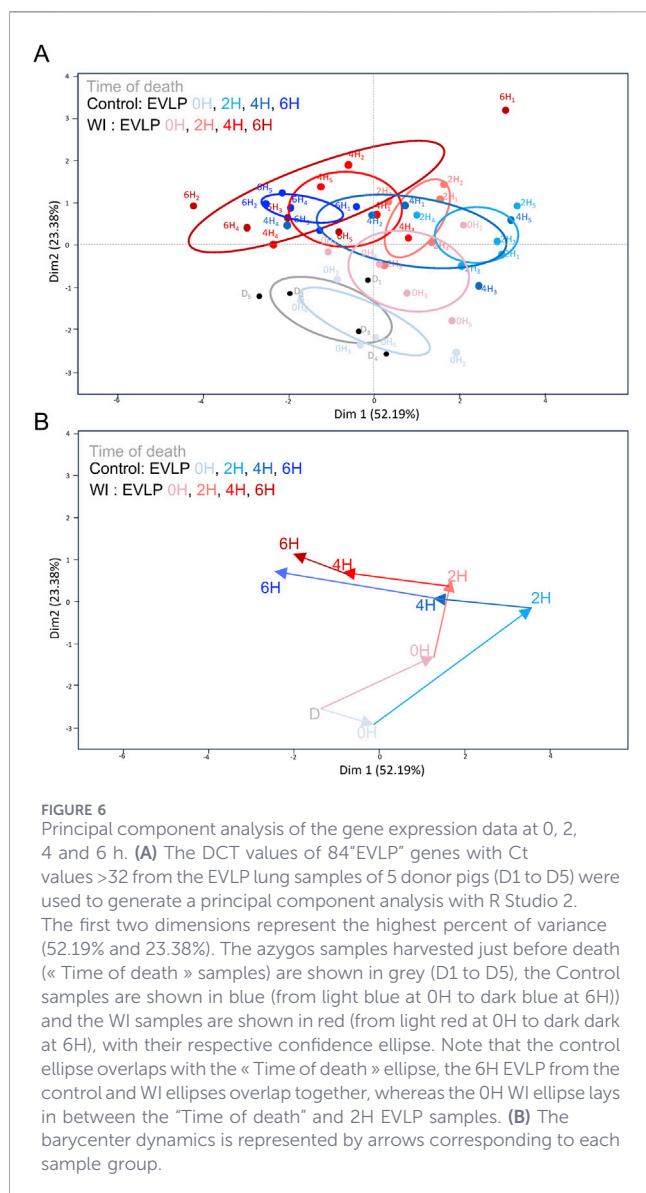
and 4-h EVLP, the WI and control groups presented a majority of DEGs in common (40 out of 52 DEGs at 2 h, and 42 out of 58 DEGs at 4 h). At 6 h, the WI group showed a high dispersion of the FC values between pig lungs, leading to less DEGs in the WI group than in the control group (51 in the control group, 27 in the WI group, and 23 in common). Finally, when comparing gene expression during EVLP conditions to that of WI lungs at 0-h EVLP, we found that the absolute mean FC of most DEGs (42 out of 69) tended to be higher (p-val <0.2, [Supplementary Material 4](#)).

Discussion

The present study evaluated the impact of 2-h WI on donor lungs using a paired porcine EVLP model. Two-hour WI initially increased vascular resistance and reduced compliance; interestingly, both parameters normalized after 6 h of perfusion. Physical recruitment of atelectasis may also have contributed to the normalization. Compliance and resistance values were consistent with published reports, taking into account known differences between single- and double-lung procedures [16, 19, 20]. Overall, biological measurements obtained during 6-h EVLP did not indicate increased cellular stress or inflammation in the 2-h WI group compared with the control group. Markers of oxidative stress in tissue (protein carbonylation and oxidized DNA), mitochondrial DNA and LDH release, inflammatory cytokines in perfusate and BAL, and PECAM shedding in the perfusate—a marker of

endothelial stress—were all comparable between the two groups. Transcriptomic analysis further showed that EVLP induced similar gene expression changes in both groups (compared to “Time of death”), involving pathways related to inflammatory responses, ischemia–reperfusion injury, oxidative stress, cell death, immune activation, cell adhesion/migration, and cytoskeletal signaling, consistent with previous reports [15, 21]. No significant differences in gene expression were detected between the WI and control groups at any EVLP time point. Notably, transcriptomic changes induced by WI alone (at 0-h EVLP) overlapped with the EVLP-driven gene expression trajectory, and profiles converged between groups by the end of perfusion. Taken together, these physiological and molecular findings suggest that lungs subjected to 2-h WI can be effectively recovered by 6-h EVLP, supporting the clinical expansion of WI tolerance thresholds.

As a consequence of the disruption of oxygen supply and impaired ATP production in cells, WI generally induces IRI through ROS production, mitochondrial stress, and changes in energetic metabolism, cell death and the release of inflammatory cytokines [16, 22]. However, the lung appears to show resilience to WI, possibly due to lower metabolic demands and higher oxygen storage capacity, as compared to other organs such as the liver and kidney that withstand less than 30 min WI [5, 23]. For instance, in the pig model, the respiratory functions after transplantation were similar in lung grafts from non-heart-beating donors (with up to 90 min WI) and heart-beating donors [24]. Furthermore, in a clinical study, extension of WI beyond 60 min was not associated with higher mortality nor with worse outcomes following lung



transplantation [23]. Indeed, in a retrospective Spanish study, the short and mid-term outcomes were comparable between uncontrolled circulatory death and brain death donors [25].

In contrast with our results, several experimental studies reported that prolonged WI (1–2 h) promoted the inflammatory responses upon EVLP in pig and rat models [2, 4, 26]. A main difference of these studies with our model resides in the use of a high dose of methylprednisolone during EVLP in our case, as done in the clinical settings. Indeed, we found that this treatment induced the genomic response of corticosteroid response genes (PDK4 and FKBP5). This treatment may also explain the reduced expression of genes involved in immune activation, cytoskeleton, and adhesion/migration pathways. However, this is unlikely the case for the *ACT*, *TCRB*, and *ITGA6* genes that were shown in other systems to be unaffected or even upregulated by dexamethasone [27–29]. The high dose methylprednisolone may also have reduced ROS production, which could account for the low levels of carbonylated proteins and oxidized DNA.

Surprisingly, we observed higher levels of lactate and PECAM in the perfusion fluids of the control group compared with the WI group. One possibility is that the 2-h WI period may have primed lung cells to better tolerate the subsequent EVLP stress. Indeed, for instance, anti-oxidant gene expression pathways were found to be induced by 2-h WI, as reflected by increased levels of *MT1A*, *GPX4* and *SOD2* transcripts (Figure 7). The anti-oxidant pathway may reduce the glycolytic metabolism in the mitochondria and partially reduce the vascular stress induced by EVLP, resulting in lower levels of lactate and PECAM release.

Our study presents several limitations. The resilience to WI found here in our pig model may not be translatable to other species, and may vary between pig genetic strains [30]. We used inflated lungs during WI; however, outcomes may differ under rhythmic ventilation. Although the sample size was limited to five pigs, the split-lung design enabled paired intra-animal comparisons, providing greater robustness than independent group analyses. Nevertheless, variability within this small cohort may have masked subtle differences between conditions.

Critically, additional experiments with full transplantation following extended WI periods and EVLP should be conducted to better assess functional recovery; indeed, the biological metrics used here likely do not capture the full complexity of tissue repair and recovery. In particular, our analyses focused largely on transcriptomics, which are limited to RNA-level changes. WI followed by EVLP may also additionally affect translational and post-translational processes, metabolism, enzymatic activities, and cell death pathways -including apoptosis, ferroptosis and pyroptosis- which were not assessed here. Notably, no differences in cytokine expression were detected at the protein level between control and WI groups during EVLP. Additional studies using unbiased bulk RNA-seq would strengthen the conclusions or alternatively, may reveal differential expression modulation specific to the 2-h WI group. Extending EVLP beyond 6 h may also uncover delayed biological effects. Finally, while our model focuses on a 2-h WI duration in a controlled experimental setting, the circulatory death in real life involves additional phases of hypoperfusion and inter-organ signaling responses induced by WI. Despite these limitations, our results provide a reference point, i.e., 2-h WI, at which lungs appear amenable to rehabilitation by EVLP.

Donation after circulatory death is highly considered for the future of organ donation. Furthermore, the end-of-life pathways are increasingly linked to protocols of organ donation, therefore, knowledge regarding the impact of WI duration is of highest importance. Future research should systematically extend WI duration followed by EVLP to identify measurable biomarkers defining lung tolerance limits to WI. Prolonged WI duration in experimental models may reveal transcriptomic, metabolic or protein expression patterns during EVLP that diverge from control conditions, signaling impaired recovery. While a ceiling effect in the EVLP response may occur even in control conditions, such models are worth pursuing, as the identification of a non-recovery signature could help determine which WI-exposed lungs are suitable or not for transplantation and may also unravel therapeutic targets.

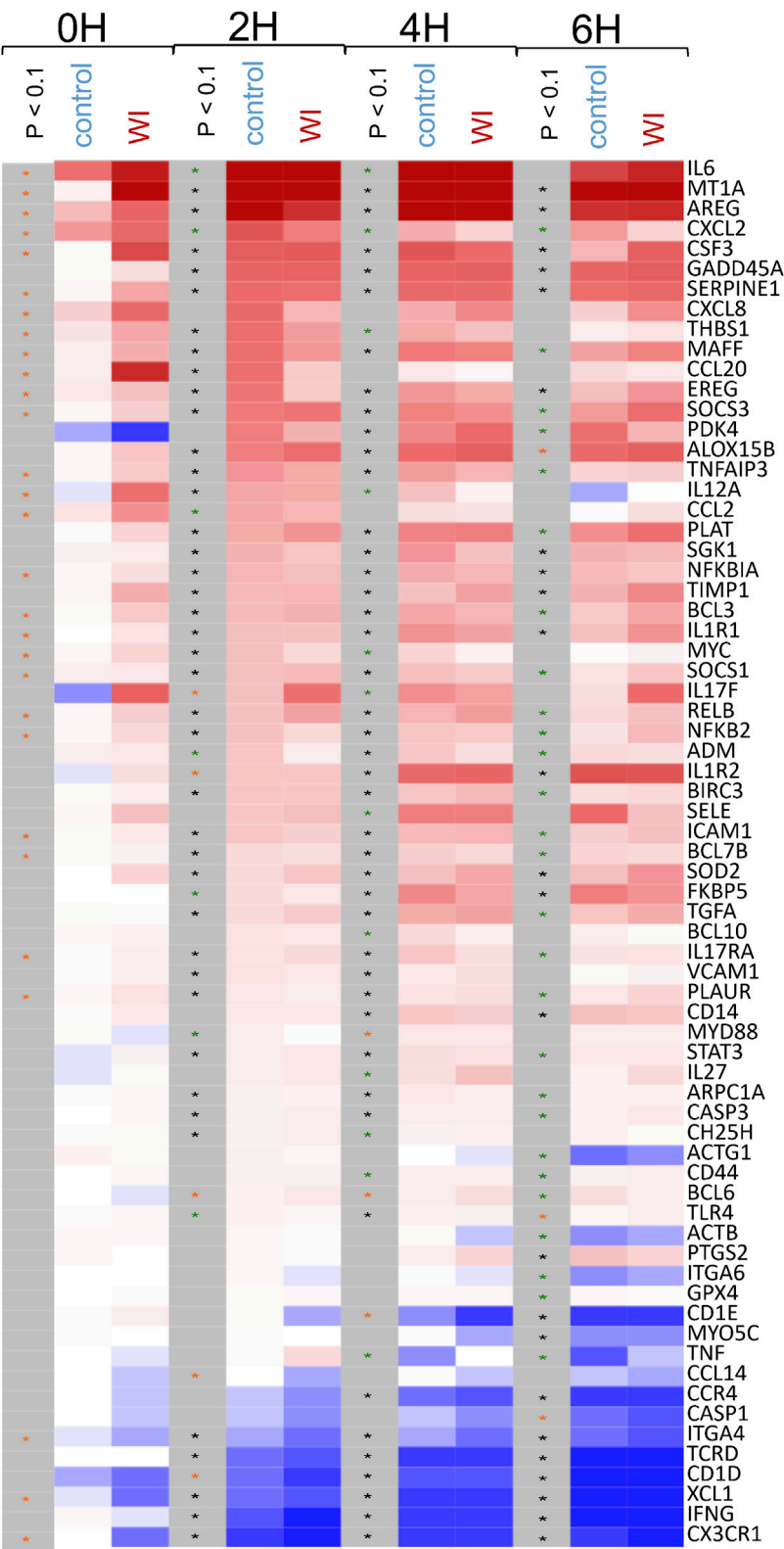


FIGURE 7
Heat map of the differentially expressed genes versus "Time of death". Fold changes (FC) in gene expression were calculated at each time point (0H, 2H, 4H, 6H EVLP) relative to the Time of death. Mean FCs were computed per group. Genes showing differential expression (adjusted $p < 0.1$) at any time point compared to "Time of death" were selected for illustration. Black star: genes significantly modulated in both Control and WI groups at a given timing. Orange star: genes significantly modulated only in the WI group. Green star: genes significantly modulated only in the Control group.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/[Supplementary Material](#). The PCR array data were deposited on ZENODO under DOI [10.5281/zenodo.18389866](https://doi.org/10.5281/zenodo.18389866).

Ethics statement

The experiments were performed in compliance with the EU guidelines and the French regulations (DIRECTIVE 2010/63/EU, 2010; Code rural, 2018; Décret n°2013-118, 2013). The procedures received authorization of the Ministry of Higher Education and Research and the experimental protocols were approved by the COMETHEA ethic committee under the number APAFIS# DAP2022120510144946 v3. The surgery was carried out at the Medical Imaging in Animal platform (accreditation B78-322-2, doi.org/10.15454/1.5572348210007727E12). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

AP: Investigation, Formal Analysis, Visualization, Funding acquisition, Writing – review and editing. FP: Investigation, Formal Analysis, Data curation, Writing – review and editing. CM: Investigation, Funding acquisition, Writing – review and editing. LJ: Formal Analysis, Visualization, Writing – review and editing. SJ: Investigation, Writing – review and editing. CR: Investigation, Resources, Writing – review and editing. VG: Investigation, Resources, Writing – review and editing. CB: Formal Analysis, Investigation, Writing – review and editing. JL: Investigation, Investigation, Writing – review and editing. JR: Investigation, Investigation, Writing – review and editing. MG: Investigation, Investigation, Writing – review and editing. MLG: Investigation, Validation, Resources, Writing – review and editing. IS-C: Validation, Formal Analysis, Visualization, Resources, Writing – original draft, Funding acquisition, Writing – review and editing. ES: Investigation, Validation, Resources, Funding acquisition, Writing – review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2026.16314/full#supplementary-material>

References

- Charles EJ, Mehaffey JH, Sharma AK, Zhao Y, Stoler MH, Isbell JM, et al. Lungs donated after circulatory death and prolonged warm ischemia are transplanted successfully after enhanced *ex vivo* lung perfusion using adenosine A2B receptor antagonism. *J Thorac Cardiovasc Surg* (2017) 154:1811–20. doi:10.1016/j.jtcvs.2017.02.072

2. Hebsgaard M, Kissow H, B Lilleør N, S Lorenzen U, A Chemtob R, Sørensen H, et al. Tolerable duration of warm ischaemia after circulatory death is safe for at least one hour in porcine lungs: functional assessment with *Ex Vivo* lung perfusion. *Heart Surg Forum* (2022) 25:E048–E052. doi:10.1532/hsf.4347
3. Spratt JR, Mattison LM, Iaizzo PA, Brown RZ, Helms H, Iles TL, et al. An experimental study of the recovery of injured porcine lungs with prolonged normothermic cellular *ex vivo* lung perfusion following donation after circulatory death. *Transpl Int* (2017) 30:932–44. doi:10.1111/tri.12981
4. Parapanov R, Wang X, Wang Y, Debonneville A, Lugin J, Liaudet L, et al. Experimental models of ischemic lung damage for the study of therapeutic reconditioning during *Ex Vivo* lung perfusion. *Transpl Direct* (2022) 8:e1337. doi:10.1097/TXD.0000000000001337
5. Gori F, Fumagalli J, Lonati C, Carlin A, Leonardi P, Biancolilli O, et al. Lung biomolecular profile and function of grafts from donors after cardiocirculatory death with prolonged donor warm ischemia time. *J Clin Med* (2022) 11:3066. doi:10.3390/jcm11113066
6. Antoine C, BR, Silleran-Chassany J. *Conditions À Respecter Pour Réaliser Des Prélèvements D'Organes Sur Des Donneurs Décédés Après Arrêt Circulatoire De La Catégorie Iii De Maastricht Dans Un Etablissement De Santé, V10_Guide_Ddac_Miii_Decembre2023* (2023).
7. Levvey BJ, Westall GP, Kotsimbos T, Williams TJ, Snell GI. Definitions of warm ischemic time when using controlled donation after cardiac death lung donors. *Transplantation* (2008) 86:1702–6. doi:10.1097/TP.0b013e3181901f24
8. Barbero C, Messer S, Ali A, Jenkins DP, Dunning J, Tsui S, et al. Lung donation after circulatory determined death: a single-centre experience. *Eur J Cardiothorac Surg* (2019) 55:309–15. doi:10.1093/ejcts/ezy254
9. Valdivia D, Gomez de Antonio D, Hoyos L, Campo-Canaveral de la Cruz JL, Romero A, Varela de Ugarte A. Expanding the horizons: uncontrolled donors after circulatory death for lung transplantation—first comparison with brain death donors. *Clin Transpl* (2019) 33:e13561. doi:10.1111/ctr.13561
10. Cypel M, Yeung JC, Hirayama S, Rubacha M, Fischer S, Anraku M, et al. Technique for prolonged normothermic *ex vivo* lung perfusion. *J Heart Lung Transpl* (2008) 27:1319–25. doi:10.1016/j.healun.2008.09.003
11. Chiumello D, Carlesso E, Cadringer P, Caironi P, Valenza F, Polli F, et al. Lung stress and strain during mechanical ventilation for acute respiratory distress syndrome. *Am J Respir Crit Care Med* (2008) 178:346–55. doi:10.1164/rccm.200710-1589OC
12. De Wolf J, Glorion M, Jouneau L, Estephan J, Leplat JJ, Blanc F, et al. Challenging the *Ex Vivo* lung perfusion procedure with continuous dialysis in a pig model. *Transplantation* (2022) 106:979–87. doi:10.1097/TP.0000000000003931
13. De Wolf J, Gouin C, Jouneau L, Glorion M, Premachandra A, Pascale F, et al. Prolonged dialysis during *ex vivo* lung perfusion promotes inflammatory responses. *Front Immunol* (2024) 15:1365964. doi:10.3389/fimmu.2024.1365964
14. Gouin C, Vu Manh TP, Jouneau L, Bevilacqua C, De Wolf J, Glorion M, et al. Cell type- and time-dependent biological responses in *ex vivo* perfused lung grafts. *Front Immunol* (2023) 14:1142228. doi:10.3389/fimmu.2023.1142228
15. Lonati C, Bassani GA, Brambilla D, Leonardi P, Carlin A, Favarsani A, et al. Influence of *ex vivo* perfusion on the biomolecular profile of rat lungs. *FASEB J* (2018) 32:5532–49. doi:10.1096/fj.201701255R
16. Ali A, Wang A, Ribeiro RVP, Beroncal EL, Baciuc C, Galasso M, et al. Static lung storage at 10 degrees C maintains mitochondrial health and preserves donor organ function. *Sci Transl Med* (2021) 13:eabf7601. doi:10.1126/scitranslmed.abf7601
17. Baciuc C, Sage A, Zamel R, Shin J, Bai XH, Hough O, et al. Transcriptomic investigation reveals donor-specific gene signatures in human lung transplants. *Eur Respir J* (2021) 57, 2000327. doi:10.1183/13993003.00327-2020
18. Movahed M, Brockie S, Hong J, Fehlings MG. Transcriptomic hallmarks of ischemia-reperfusion injury. *Cells* (2021) 10:1838. doi:10.3390/cells10071838
19. Niikawa H, Okamoto T, Ayyat KS, Itoda Y, Hata JS, McCurry KR. Significant parameters in the evaluation of donor lungs in single-lung cellular *ex vivo* lung perfusion. *Interact Cardiovasc Thorac Surg* (2019) 28:767–74. doi:10.1093/icvts/ivy327
20. Niikawa H, Okamoto T, Ayyat KS, Itoda Y, Farver CF, McCurry KR. The protective effect of prone lung position on ischemia-reperfusion injury and lung function in an *ex vivo* porcine lung model. *J Thorac Cardiovasc Surg* (2019) 157:425–33. doi:10.1016/j.jtcvs.2018.08.101
21. Jeon JE, Rajapaksa Y, Keshavjee S, Liu M. Applications of transcriptomics in ischemia reperfusion research in lung transplantation. *J Heart Lung Transpl* (2024) 43:1501–13. doi:10.1016/j.healun.2024.03.006
22. Schauer RJ, Gerbes AL, Vonier D, Meissner H, Michl P, Leiderer R, et al. Glutathione protects the rat liver against reperfusion injury after prolonged warm ischemia. *Ann Surg* (2004) 239:220–31. doi:10.1097/01.sla.0000110321.64275.95
23. Alzahrani A, Noda K, Chan EG, Ryan JP, Furukawa M, Sanchez PG. The length of the warm ischemic interval in lung donation after circulatory death does not impact post-transplantation outcomes. *JHLT Open* (2025) 8:100244. doi:10.1016/j.jhlto.2025.100244
24. Greco R, Cordovilla G, Sanz E, Benito J, Criado A, Gonzalez M, et al. Warm ischemic time tolerance after ventilated non-heart-beating lung donation in piglets. *Eur J Cardiothorac Surg* (1998) 14:319–25. doi:10.1016/s1010-7940(98)00181-x
25. Campo-Canaveral de la Cruz JL, Crowley Carrasco S, Tanaka S, Romero Roman A, Hoyos Mejia L, Gil Barturen M, et al. Lung transplantation from uncontrolled and controlled donation after circulatory death: similar outcomes to brain death donors. *Transpl Int* (2021) 34:2609–19. doi:10.1111/tri.14120
26. Renne J, Gutberlet M, Voskrebenezv A, Kern A, Kaireit T, Hinrichs JB, et al. Functional pulmonary magnetic resonance imaging for detection of ischemic injury in a porcine *Ex-Vivo* lung perfusion system prior to transplantation. *Acad Radiol* (2019) 26:170–8. doi:10.1016/j.acra.2018.05.006
27. Weiner FR, Czaja MJ, Jefferson DM, Giambrone MA, Tur-Kaspa R, Reid LM, et al. The effects of dexamethasone on *in vitro* collagen gene expression. *J Biol Chem* (1987) 262:6955–8.
28. Migliorati G, Bartoli A, Nocentini G, Ronchetti S, Moraca R, Riccardi C. Effect of dexamethasone on T-cell receptor/CD3 expression. *Mol Cell Biochem* (1997) 167:135–44. doi:10.1023/a:1006829421509
29. Robinson JM, Turkington S, Abey SA, Kenea N, Henderson WA. Differential gene expression and gene-set enrichment analysis in Caco-2 monolayers during a 30-day timeline with dexamethasone exposure. *Tissue Barriers* (2019) 7:e1651597. doi:10.1080/21688370.2019.1651597
30. Miyamoto E, Ohsumi A, Hwang D, Boonstra K, Cypel M, Martinu T, et al. *Ex vivo* lung perfusion-to-lung transplant rat survival model with reproducible development of acute lung injury and graft rejection. *JHLT Open* (2025) 9:100299. doi:10.1016/j.jhlto.2025.100299



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Elevated filling pressures are associated with poor long-term graft survival after pediatric heart transplantation

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Long-term survival has improved in the current era of pediatric heart transplantation (HT). The impact of elevated filling pressures [EFP; defined as pulmonary capillary wedge pressure (PCWP) > 15 mmHg and/or right atrial pressure (RAP) > 12 mmHg in the absence of biopsy-confirmed rejection] on long-term outcomes beyond 10 years remains poorly characterized. We assessed whether EFP during the early years after HT are associated with poor graft survival and cardiovascular adverse events (AE). We retrospectively analyzed 114 pediatric HT grafts (1986–2020) with available PCWP and/or RAP measurements 7 months to 5 years post-transplant (grouping period), representing a landmark cohort of 5-year survivors. Associations of EFP with graft survival and AE were evaluated. Fourteen grafts (12%) had EFP during the grouping period. Grafts with EFP had significantly worse long-term survival (44% vs. 85% at 10 years; log-rank $p < 0.001$), and higher risk of graft loss (overall HR 6.04, 95% CI [2.01–16.85]). The incidence of AE was numerically higher in grafts with EFP (26.6 [15.2–43.2] vs. 11.9 [9.4–14.9] per 100 person-years), but should be interpreted as exploratory. EFP within the early years post-transplant are associated with poor graft survival and may indicate cardiovascular complications.

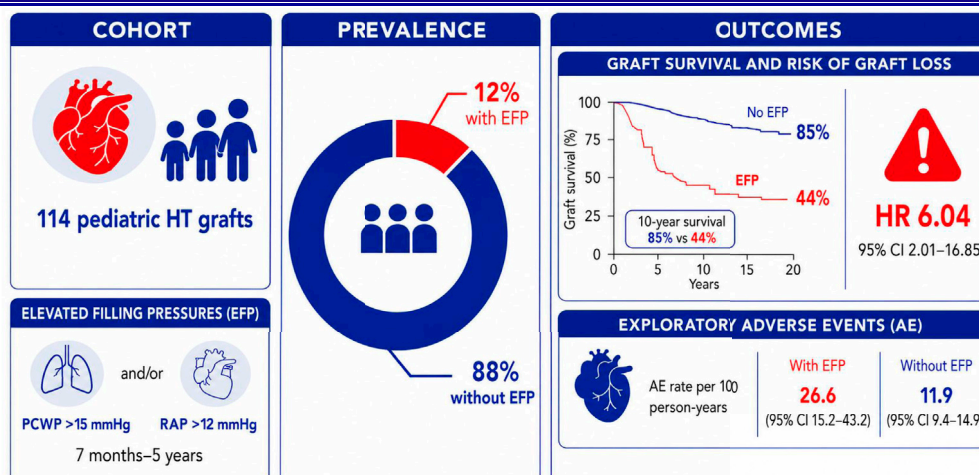
KEYWORDS

cardiovascular outcomes, coronary allograft vasculopathy, filling pressures, heart transplantation, pediatrics, pulmonary capillary wedge pressure, right atrial pressure

Introduction

Heart transplantation (HT) is an established and effective therapy for children with end-stage heart failure. Approximately 650 pediatric transplantations are performed annually, with more than 14,000 recipients worldwide [1]. Advances in surgical techniques and immunosuppression have resulted in excellent short-term outcomes, with first-year survival rates approaching 90%, and long-term survival beyond 10 years became increasingly common in the current pediatric HT era, with median post-transplant survival of approximately 15 years across all pediatric age groups [2]. Nevertheless, overall graft

Elevated Filling Pressures Are Associated With Poor Long-Term Graft Survival After Pediatric Heart Transplantation



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GRAPHICAL ABSTRACT

longevity remains limited, and late graft failure continues to represent a major clinical challenge [2, 3].

Coronary allograft vasculopathy (CAV) is a well-established contributor to late graft failure and morbidity [2]. It is characterized by diffuse intimal proliferation and maladaptive vascular remodeling affecting both the epicardial coronary arteries and the microvasculature, ultimately leading to graft dysfunction and failure [4–13]. However, CAV alone does not fully explain the heterogeneity of long-term outcomes among recipients who survive in the late post-transplant period. In particular, restrictive physiology and microvascular dysfunction have been proposed as additional contributing factors and have been associated with adverse outcomes [8, 12–14].

According to the current ISHLT guidelines, cardiac catheterization with coronary angiography remains the gold standard for surveillance of epicardial CAV, yet reliable clinical tools to assess microvascular function are lacking [15, 16]. While elevated filling pressures (EFP), reflected by increased pulmonary capillary wedge pressure (PCWP) and right atrial pressure (RAP), may represent a hemodynamic correlate of underlying graft pathology, their relationship with microvascular remains incompletely understood, primarily associative and inferential

[15]. Current guidelines address markedly elevated pressures (PCWP >25 mmHg and/or RAP >12 mmHg), while the 2023 update incorporates pediatric data from Kindel et al. suggesting that EFP defined by PCWP >15 mmHg and/or RAP >12 mmHg may already be clinically relevant [15–17]. Despite these observations, the prognostic significance of EFP for long-term outcomes after HT and their relationship with survival beyond epicardial CAV, remain poorly explored in pediatric recipients. Therefore, we investigated whether EFP during the early years after pediatric HT are associated with poor long-term survival and cardiovascular adverse events (AE).

Materials and methods

Study population and design

We screened all pediatric heart recipients who underwent primary HT and/or re-transplantation (re-HT) before 18 years of age between 1986 and 2020 at our institution and were followed thereafter. All patients underwent orthotopic HT using either biatrial or bicaval technique with ABO-compatible donor-recipient matching. Inclusion required at least one cardiac catheterization with invasive measurement of PCWP and/or RAP within the grouping period, defined as 7 months to 5 years after HT. Grafts without right-sided hemodynamic data during this period were excluded. Patients were excluded if they underwent multi-organ transplantation or if grafts failed to reach the 5-year landmark due to mortality or follow-up shorter than 5 years (Supplementary Figure S1). This study was approved by the Institutional ethics committee of Charité - Universitätsmedizin Berlin (EA2/055/23).

Abbreviations: ACR, Acute cellular rejection; AE, Adverse events; AMR, Antibody-mediated rejection; CAV, Cardiac allograft vasculopathy; CAV₁, Mild epicardial CAV; CAV₂, Moderate epicardial CAV; CAV₃, Severe epicardial CAV; EFP, Elevated filling pressures; EMB, Endomyocardial biopsy; HT, Heart transplantation; ISHLT, International Society for Heart and Lung Transplantation; LHC, Left heart catheterization; LVEF, Left ventricular ejection fraction; PCWP, Pulmonary capillary wedge pressure; re-HT, re-transplantation; RAP, Right atrial pressure; RHC, Right heart catheterization.

All patients received induction therapy with methylprednisolone combined with either a polyclonal lymphocyte or thymocytic antibody or basiliximab. Presensitized recipients additionally underwent plasmapheresis. Until 2000, maintenance immunosuppression consisted of a triple regimen with corticosteroids, cyclosporin A and azathioprine. From 2000 onward, recipients were treated with mycophenolate mofetil in combination with a calcineurin inhibitor, either cyclosporin A or tacrolimus, during the first year post-transplant. After the first year, immunosuppression typically consisted of a calcineurin inhibitor combined with either mycophenolate mofetil or everolimus.

This is a single-center retrospective cohort study. Data was analyzed between September 2024 and July 2025. The primary predictor was EFP defined as PCWP >15 mmHg and/or RAP >12 mmHg [17], excluding measurements during acute rejection episodes confirmed by concurrent endomyocardial biopsy (EMB) ($\geq 2R/3A$ acute cellular rejection [ACR] and/or antibody-mediated rejection [AMR]) according to ISHLT – 1990 and 2004 guidelines [18–20]. EFP status was assigned if present in at least one catheterization within the grouping period and may therefore have been influenced by variability in catheterization frequency and timing. Thresholds were applied to values from each examination without further adjudication. There was no averaging across catheterizations. Multiple measurements within a single catheterization were averaged. Grafts were classified into EFP and no-EFP groups accordingly and compared regarding survival and AE. AE were defined as a composite endpoint comprising moderate-severe epicardial CAV₂₋₃, myocardial infarction (MI) and/or coronary revascularization, arrhythmias, and non-rejection heart failure hospitalization. Graft loss was defined as death or re-HT due to graft dysfunction.

The 7-month lower bound of the grouping period was chosen to avoid perioperative confounding factors such as graft adaptation, cold ischemic time, and early rejection [21, 22]. Landmark approach was chosen because a uniform baseline assessment was not available and catheterizations varied by timing and content over the study period. In some examinations, only left heart catheterization (LHC) was performed, without right-sided hemodynamics (PCWP/RAP). Accordingly, grafts without any available PCWP/RAP within the grouping period were excluded. The 5-year upper bound was chosen because surveillance catheterizations were most consistently performed during this period, whereas beyond 5 years catheterizations were less frequent and mainly clinically indicated and a shorter time window yielded too few EFP events for stable estimates. This broad window enabled classification despite variability in right heart catheterization (RHC) timing. However, using a 5-year landmark conditioned analyses on graft survival, excluding early graft failures and defining a selected cohort with available hemodynamics.

Institutional graft surveillance

Invasive catheterization-based graft surveillance was performed as part of the institutional surveillance protocol including LHC with coronary angiography and RHC with EMB and measurement of PCWP/RAP beginning one-year post-transplant. Following the first year, LHC with coronary angiography and RHC with EMB was scheduled every 1–2 years during the first 5 years and at least every

2 years thereafter. Catheterization was also performed whenever clinically indicated. Procedures were almost exclusively performed under analgo-sedation with spontaneous breathing.

Coronary arteries were visualized by either aortography in children <10 kg or selective coronary angiography in children >10 kg. The presence of CAV was graded according to the 2010 ISHLT classification [15].

Until 2004, moderate and severe ACR were classified as grade 3A and $\geq 3B$ according to the ISHLT 1990 guideline [18, 20] from 2004 onward, they were classified as grade 2R and 3R according to the ISHLT 2004 guideline, respectively [19, 20]. AMR was considered when at least three of the following four ISHLT criteria were evident: histological or immunological evidence of antibody-mediated rejection, new-onset graft dysfunction, and donor-specific antibodies [23, 24]. After transition to adult HT care, the intervals between catheterizations were individually adjusted depending on individual risk profile, clinical status, graft performance, and time after HT.

Echocardiographic examinations were performed as part of non-invasive graft surveillance on the day of catheterization and during routine outpatient visits according to current echocardiographic guidelines [25, 26]. Left ventricular systolic function was assessed by calculating left ventricular ejection fraction (LVEF) using the biplane Simpson method, and two-dimensional-guided M-mode echocardiography when Simpson measurements were not available. LVEF was classified as preserved ($\geq 50\%$), mildly reduced (40%–49%), or reduced ($<40\%$) [25–28]. Advanced echocardiographic parameters (e.g., strain imaging) could not be included in the analysis, given their limited availability due to the long study period and evolving echocardiographic practice.

Statistical analysis

For practicability, grafts were treated as independent observations. Categorical variables were expressed as absolute and relative frequencies, and continuous variables as medians with interquartile ranges (IQRs). Demographic parameters were calculated over the whole study period, including pre-grouping (0–7 months), grouping (7 months–5 years), analysis (5–13.8 years), and post-analysis period (13.8–35 years). The post-analysis period begins once all grafts in one group have experienced graft loss or were censored. As no meaningful comparison is possible once one group is depleted, graft survival and AE analyses were restricted to the analysis period.

Exposure status (EFP vs. no-EFP) was determined based on whether filling pressures were elevated at least once within the grouping period. Survival analyses were conducted using a landmark approach, beginning 5 years post-transplant, so that both groups entered the analysis period uniformly. Some grafts initially classified as no-EFP subsequently developed EFP beyond 5 years post-transplant. Sensitivity analysis excluded these late converters, redefining the comparator as never-EFP group. Survival of grafts with EFP was then compared with that of grafts that never developed EFP. Grafts were administratively censored at 13.8 years, corresponding to the maximum graft survival time in the EFP group. Survival functions were estimated using the Kaplan–Meier method and compared using log-rank test. Raw graft lifespan up to 31 years were also reported. A Cox proportional

hazard model quantified effect size (hazard ratio) adjusting for transplantation era (≤ 1999 vs. ≥ 2000) and recipient age at HT. Confidence intervals of the main results were calculated by an accelerated bootstrap to adjust for overfitting. Multiple sensitivity analyses were performed to assess robustness to the proportional hazards assumption which was violated by main factor (EFP group) and covariates (transplantation era and recipient age). Two piecewise Cox models fitted to estimate hazard ratios for the 5–9 and 9–14 years post-transplant periods. Additionally, risk ratios were calculated from cumulative hazards for the timepoints 7 and 11 years. Recipient age at HT was dichotomized at three different cut-offs (2 years, 10 years, and 15 years).

AE during the analysis period were analyzed using incidence rates. Since observation time varied by graft, absolute event counts are misleading; therefore, event rates were standardized per 100 patient-years. Patient-years for a graft was calculated as time from the 5-year landmark until the last observation or censoring time (13.8 years), whichever came first. This approach incorporated recurrent events. Confidence intervals and explorative p-values were calculated based on the Poisson distribution. To assess robustness to overdispersion, rate ratios were additionally calculated using the negative binomial distribution. Moreover, a responder analysis compared the proportion of grafts with ≥ 1 AE during the grouping and analysis period. Grafts alive at study end, or lost to follow-up, were censored at the date of last patient contact.

All tests were two-sided and performed with R version 4.4.1, using the packages “survival” (3.6.4), “boot” (1.3.30), and “MASS” (7.3.64).

Results

Study population

Of 203 grafts from 193 patients, 43 grafts lacked digitized medical records available for review. Three grafts from patients who underwent re-HT after the age of 18, 24 grafts that failed to reach the 5-year landmark due to mortality or follow-up shorter than 5 years, and 19 grafts without RHC documenting PCWP and/or RAP during the grouping period were excluded (Supplementary Figure S1). A comparison of demographic and clinical characteristics between included and excluded grafts are provided as Supplementary Tables S1, S2. The final analysis cohort therefore included 114 grafts from 112 patients.

12% of the grafts (14/114) had ≥ 1 EFP within the grouping period (Figure 1). Over the entire follow-up period, EFP were diagnosed in 54% of the grafts (61/114) with a median onset of 6.8 years (IQR 2.0–10.6) post-transplant. Freedom from EFP was 87% at 2 years, 80% at 5 years, 61% at 10 years, and 47% at the end of the observation period.

The demographic characteristics are summarized in Table 1. Median age at HT for the cohort was 8.9 years with median follow-up time of 13.1 years. Compared with no-EFP, EFP recipients were slightly older at HT (median 12.2 vs. 8.8 years) however, age difference was not significant. Donor and recipient age difference was also not significantly different between both groups. Era distribution was similar across groups, with most transplantations performed in the most recent era 2000–2020. The EFP group had

more digitally available catheterizations than the no-EFP group (median 9.0 vs. 7.0). The time to first catheterization with available filling pressures did not differ significantly between groups (median 1.1 vs. 1.5 years, $p = 0.215$). A preserved LVEF ($\geq 50\%$) was present in 64% of EFP vs. 72% of no-EFP grafts, while a reduced systolic left ventricular function with an LVEF $< 40\%$ was present in 29% and 12% of grafts, respectively. Overall, 36% of grafts had ≥ 1 acute rejection ($\geq 2R/3A$ ACR and/or AMR), occurring at similar rates and comparably distributed in both groups, with most episodes arising after the first catheterization with available filling pressures.

Baseline characteristics were comparable between eras (Table 2). Follow-up time was longer in the earlier era (median 14.7 vs. 12.4 years, $p = 0.007$). Time to first catheterization with available filling pressures was not significantly different.

The proportion of catheterizations including PCWP and/or RAP measurements was lower in the earlier era (median 0.73 vs. 1.00), indicating more complete hemodynamic assessment in recent years.

Overall, graft loss occurred in 36% of the grafts (41/114), affecting 35% of the patients (39/112). Among 41 graft losses, causes were cardiac in 51% (21/41), non-cardiac in 7% (3/41), and unknown in 42% (17/41), respectively (Table 3).

Survival analysis

The comparison of graft survival over time is illustrated in Figure 2. Survival probability for grafts in the EFP group considerably decreased from year 6 onward. At 10 years post-transplant, graft survival was significantly lower in the EFP group (44%, 95% CI 16.8–68.4) compared with no-EFP group (85%, 95% CI 76.2–91.3, log-rank $p < 0.001$).

In a sensitivity analysis excluding grafts from the no-EFP group that developed EFP beyond 5 years post-transplant (redefined as never-EFP group), grafts with EFP showed particularly worse survival compared with those that never developed EFP (log-rank $p < 0.001$) (Figure 3). A comparison of grafts that never developed EFP and those with EFP, including demographics, clinical characteristics, and cardiovascular AE, is provided in Supplementary Tables S3, S4.

In Cox regression analyses, EFP were associated with a higher hazard of graft loss (overall HR = 6.04, 95% bootstrap-CI 2.01–16.85), representing a summary estimate across follow-up. This association remained consistent across stratified and piecewise Cox models, with higher effect estimates observed during later follow-up periods (9–14 years post-transplant HR = 10.15, 95% CI 2.19–46.93) (Table 4). Cumulative risk estimates supported this association, demonstrating a 5.5-fold higher risk of graft failure at 7 years (95% CI 1.4–16.6) and a 3.5-fold higher risk at 11 years (95% CI 1.4–6.9) in grafts with EFP.

Cardiovascular AE

The timing of EFP and AE occurrence is illustrated in Figure 1. During the grouping period, ≥ 1 AE occurred in 5/14 (36%) of EFP vs. 7/100 (7%) of no-EFP grafts ($p = 0.005$). Several component rates were higher in the EFP group: CAV₂₋₃ in 4/14 (29%) vs. 1/100 (1%), MI/revascularization in 4/14 (29%) vs. 2/100 (2%) (Table 5).

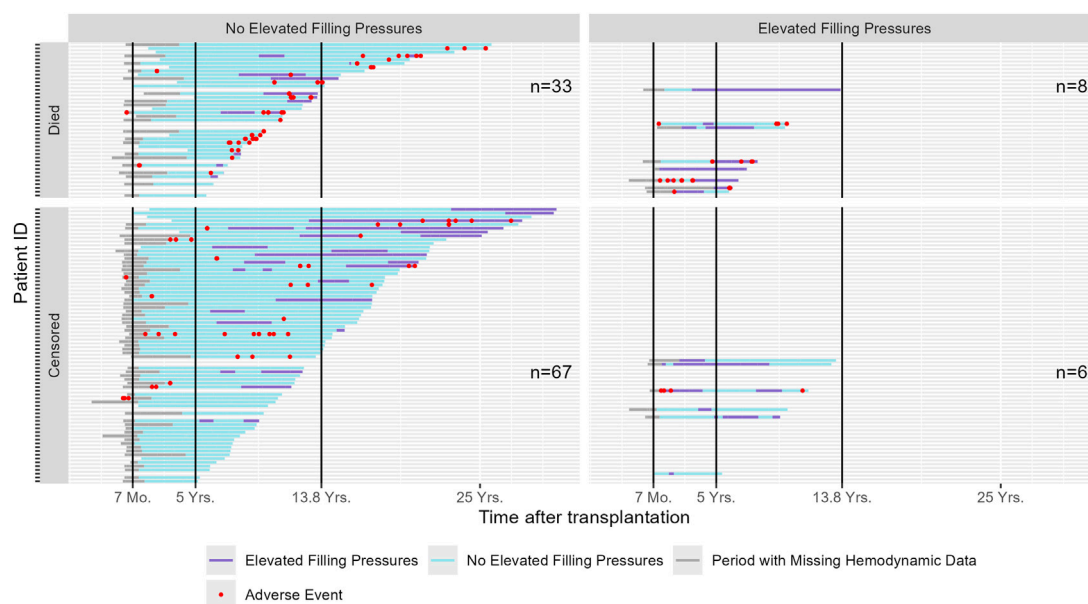


FIGURE 1

Swimmer plot showing individual graft follow-up trajectories from heart transplantation to graft loss or censoring, along with cardiovascular AE in both groups. Each horizontal bar represents a single graft. Color segments indicate periods with elevated filling pressures (EFP) (purple), no EFP (blue), or periods without available hemodynamic data (gray). The length and timing of these segments vary between grafts due to the retrospective nature of the cohort, reflecting differences in the timing and frequency of surveillance; early gray segments indicate periods prior to the first available hemodynamic assessment. Vertical lines mark key analysis time points: 7 months (start of grouping window), 5 years (landmark for survival analysis), and 13.8 years (end of analysis period). Red dots represent adverse events. Panels separate grafts that died from those censored, and cohorts are stratified by EFP vs. no-EFP status.

In the analysis period, 4/14 (29%) of the EFP grafts and 20/100 (20%) of the no-EFP grafts experienced ≥ 1 AE. EFP grafts showed numerically higher proportions of CAV₂₋₃ (3/14 [21%] vs. 14/100 [14%]) and MI/revascularization (3/14 [21%] vs. 9/100 [9%]), although this difference was not statistically significant. Arrhythmia (1/14 [7%] vs. 6/100 [6%]) and non-rejection heart failure hospitalization (1/14 [7%] vs. 6/100 [5%]) rates were similar.

The overall AE incidence was 26.6 events per 100 patient-years (95% CI 15.2–43.2) in the EFP group vs. 11.9 per 100 patient-years (95% CI 9.4–14.9) in the no-EFP group (Figure 4). Component-specific incidence rates with overlapping confidence intervals are presented in Figure 4. Sensitivity analyses using negative binomial models showed that these results are not robust to the overdispersion observed in the data and should therefore be considered exploratory (Supplementary Table S5). In these analyses, attenuation was most pronounced for CAV₂₋₃, MI/revascularization and non-rejection heart failure hospitalization, whereas findings for composite AE and arrhythmia were more consistent.

Discussion

In this single-center cohort of pediatric HT recipients, the presence of EFP within the first years post-transplant was strongly associated with poor long-term graft survival. The association remained significant after adjustment for transplantation era and recipient age at HT and was robust across sensitivity analyses. However, given the violation of the proportional hazards assumption, the hazard ratio

reflects a time-varying summary estimate. Despite potential residual confounding, these findings suggest that EFP is associated with increased long-term graft loss.

Multiple adult HT studies have demonstrated that adverse hemodynamic profiles characterized by elevated RAP and PCWP and reduced cardiac index are associated with mortality, graft failure, and CAV beyond angiographic findings [29–34]. In a recent cohort, patients with restrictive hemodynamics and heart-failure symptoms had worse survival than CAV₃ alone, underscoring the prognostic value of hemodynamics [34].

Our results are consistent with these previous findings, despite differences in applied thresholds and pediatric physiology. Pediatric data on this topic remain limited [17, 35]. Early work by Aiyagari et al. showed an association between higher filling pressures and graft loss, as well as with epicardial CAV [35]. In a large multicenter cohort of over 3,100 pediatric HT recipients, Kindel et al. proposed pediatric-specific thresholds and showed that children with CAV₁ and a single hemodynamic alteration had significantly worse survival [17].

Our study extends these findings by showing that EFP are associated with poor graft survival with follow-up spanning up to three decades and uniquely capturing outcomes from childhood through adulthood. While registry studies provide broader generalizability, they often lack data granularity. The single-center design allowed detailed procedural control, including exclusion of measurements obtained during biopsy-proven rejection, and enabled graft-level analysis within a prespecified exposure window. We acknowledge trade-offs, including a smaller sample size and limited generalizability however our data complement existing registry evidence by clarifying the timing,

TABLE 1 Demographic and clinical characteristics of grafts, stratified by EFP status.

Variables	Total (n = 114)	No-EFP group (n = 100)	EFP group (n = 14)	p-value
Recipients' age at HT/re-HT, years	8.9 (2.7–14.3)	8.8 (2.9–14.2)	12.2 (2.7–14.5)	0.769
Post-transplant follow up, years	13.1 (8.9–17.7)	13.9 (9.3–18.3)	9.7 (6.7–11.1)	0.002
Donors' age, years	9 (3–24)	9 (3–20.5)	12 (3–31.2)	0.861
Donor/recipient age difference, years	1.8 (–0.7–8.7)	1.8 (–0.8–7.1)	1.6 (–0.1–15.5)	0.583
Gender				0.312
Female	51 (45%)	47 (47%)	4 (29%)	
Number of re-HT	3 (3%)	2 (2%)	1 (7%)	0.815
Eras of transplantation:				0.674
• Early era (1986–1999)	34 (30%)	31 (31%)	3 (21%)	
• Late era (2000–2020)	80 (70%)	69 (69%)	11 (79%)	
Cardiac diagnosis prior HT:				0.217
• Cardiomyopathy	94 (83%)	82 (82%)	12 (86%)	
• Congenital heart disease	14 (12%)	14 (14%)	-	
• Others	3 (3%)	2 (2%)	1 (7%)	
• Re-HT	3 (3%)	2 (2%)	1 (7%)	
Time from HT to first catheterization with available FP, years	1.4 (1.0–2.9)	1.5 (1.0–2.9)	1.1 (1.0–1.9)	0.215
Number of catheterizations per graft	7 (5–9)	7 (5–9)	9 (7–15)	0.006
LVEF echocardiographic evaluation*				0.207
- Grafts with preserved pLVEF ≥50%	81 (71%)	72 (72%)	9 (64%)	
- Grafts with mildly reduced mrLVEF = 40–49%	17 (15%)	16 (16%)	1 (7%)	
- Grafts with reduced rLVEF <40%	16 (14%)	12 (12%)	4 (29%)	
Acute rejection episodes ≥ 1**	41 (36%)	36 (36%)	5 (36%)	1.000
- From HT to the first catheterization with FP	11 (10%)	10 (10%)	1 (7%)	
- Thereafter to the last follow-up or death/re-HT	30 (26%)	26 (26%)	4 (29%)	
PTLD	11 (10%)	11 (11%)	-	0.411

EFP, Elevated filling pressures; FP, Filling pressures HT, Heart transplantation; LVEF, Left ventricular ejection fraction. PTLD, Post-transplant lymphoproliferative disorder; re-HT, Re-transplantation.

*For LVEF, values indicate the number of grafts categorized according to their lowest recorded EF during follow-up: pLVEF: preserved, always ≥50%, mrLVEF: mildly reduced, at least once <50% but never <40%, rLVEF: reduced, at least once <40% over the whole study period.

**For acute rejection, values indicate the number of grafts that experienced ≥1 biopsy-proven episodes (≥2R/3A ACR and/or AMR).

measurement context, and clinical interpretation of EFP in pediatric HT.

Grafts with EFP showed a numerically higher incidence of AE. However, confidence intervals were wide, particularly in the EFP group due to the small sample size and results were sensitive to overdispersion in sensitivity analyses. Additionally, absolute and component-level differences were not statistically significant. Accordingly, these findings related to AE should be considered exploratory and hypothesis-generating. Despite the paucity of research explicitly correlating EFP to AE, our exploratory findings point in the same direction as adult data linking abnormal hemodynamics, particularly elevated PCWP and RAP during exercise, to adverse outcomes [36].

Our study encompasses a long period (1986–2020), during which substantial changes in immunosuppression, surveillance practices, and data availability may have influenced both EFP detection and outcomes. These changes raise the possibility of era-related ascertainment bias, with EFP potentially more frequently identified in recent eras with more consistent

hemodynamic assessment. Differences in surveillance intensity may have further influenced exposure classification. Grafts classified as EFP had more frequent catheterizations, increasing the possibility of detecting EFP, whereas grafts with fewer assessments may have had undetected EFP, introducing potential misclassification. Consistent with these considerations, EFP grafts were more frequently observed in recent eras, whereas many no-EFP grafts were transplanted in earlier eras, in which survival is generally poorer both at our center [37] and in registry reports [2]. Despite this distribution, survival remained consistently superior in the no-EFP group, arguing against era effects as the sole explanation for the observed association. To address this, we adjusted for transplantation era in Cox models. This adjustment, along with sensitivity analyses, did not alter the direction of the association between EFP and graft loss, suggesting that ascertainment differences alone are unlikely to fully explain the findings. As not all relevant confounders were included (graft function, CAV burden, rejection history, donor–recipient characteristics, changes in immunosuppressive regimens and surveillance intensity), residual

TABLE 2 Baseline characteristics and catheterization patterns by transplantation era.

Variables	1986–1999 (n = 34)	2000–2020 (n = 80)	Total (n = 114)	p-value
Recipients' age at HT/re-HT, years	10.5 (5.8–14.5)	8.7 (2.4–14)	8.9 (2.7–14.3)	0.227
Post-transplant follow up, years	14.7 (10.2–25.4)	12.4 (8.1–16.9)	13.1 (8.9–17.7)	0.007
Donors' age, years	9 (3.8–22.8)	9 (3–23.5)	9 (3–24)	0.588
Gender				1.000
Female	15 (44%)	36 (45%)	51 (45%)	
Number of re-HT	-	3 (4%)	3 (3%)	0.614
Number of catheterizations per graft	8 (4–9)	7 (5–9)	7 (5–9)	0.784
Time from HT to first catheterization with available FP, years	2.5 (1.5–3.8)	1.2 (1.0–1.9)	1.4 (1.0–2.9)	0.215
Time from HT to first catheterization, years	1.6 (0.4–3.1)	1.0 (0.2–1.3)	1.0 (0.3–1.7)	0.031
Proportion of catheterizations with available FP	0.73 (0.56–1.0)	1.0 (0.83–1.0)	1.0 (0.8–1.0)	<0.001
Number of grafts with EFP	3 (9%)	11 (14%)	14 (12%)	0.674

EFP, Elevated filling pressures; FP, Filling pressures; HT, Heart transplantation; re-HT, Re-transplantation.

TABLE 3 Distribution of deaths by cause and time interval after pediatric heart transplantation.

Characteristics	Total (n = 114)	No-EFP group (n = 100)	EFP group (n = 14)
Number of deaths during entire study period	41 (36%)	33 (33%)	8 (57%)
Cardiac deaths:			
• CAV and/or acute rejection	13 (11%)	12 (12%)	1 (7%)
• Heart failure	8 (7%)	7 (7%)	1 (7%)
Noncardiac deaths:			
• PTLD	1 (1%)	1 (1%)	-
• Infection/Sepsis	2 (2%)	1 (1%)	1 (7%)
Unknown	17 (15%)	12 (12%)	5 (36%)
Number of deaths within analysis period (5–13.8 years)	29 (25%)	21 (21%)	8 (57%)
Cardiac deaths:			
• CAV and/or acute rejection	11 (10%)	10 (10%)	1 (7%)
• Heart failure	6 (5%)	5 (5%)	1 (7%)
Noncardiac deaths:			
• PTLD	1 (1%)	1 (1%)	-
• Infection/Sepsis	2 (2%)	1 (1%)	1 (7%)
Unknown	9 (8%)	4 (4%)	5 (36%)
Number of deaths within post analysis period (after 13.8 years)	12 (11%)	12 (12%)	-
Cardiac deaths:			
• CAV and/or acute rejection	2 (2%)	2 (2%)	-
• Heart failure	2 (2%)	2 (2%)	-
Noncardiac deaths:			
• PTLD	-	-	-
• Infection/Sepsis	8 (7%)	8 (8%)	-
Unknown			

CAV, Coronary allograft vasculopathy; EFP, Elevated filling pressures; PTLD, Post transplant lymphoproliferative disorder.

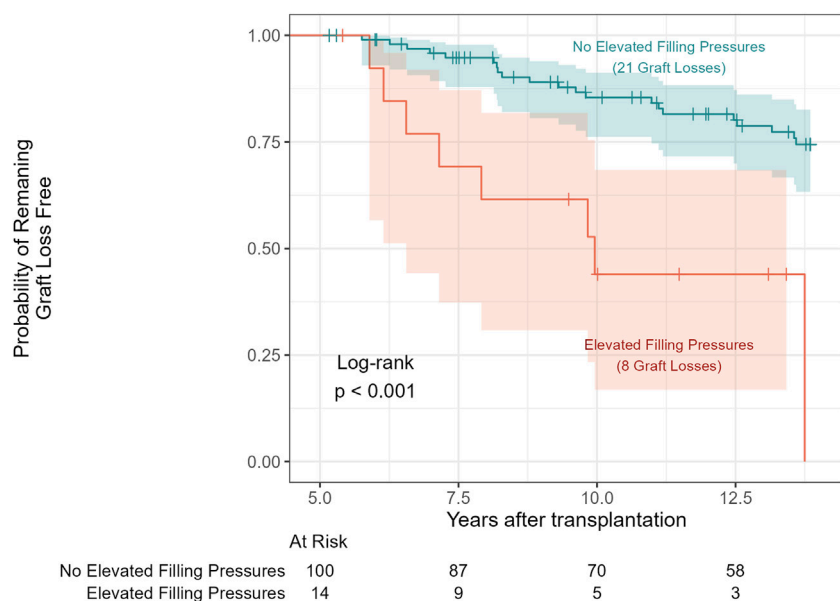


FIGURE 2
Graft survival comparing grafts with EFP to those without EFP during the analysis period. Kaplan–Meier curves estimate graft loss-free survival from the 5-year landmark to 13.8 years after transplantation, comparing grafts with elevated filling pressures (EFP) to those without EFP during the analysis period. Shaded areas represent 95% confidence intervals. Tick marks denote censoring. The log-rank test ($p < 0.001$) indicates significantly worse graft survival in the EFP group. Numbers at risk at selected time points are presented below the curves.

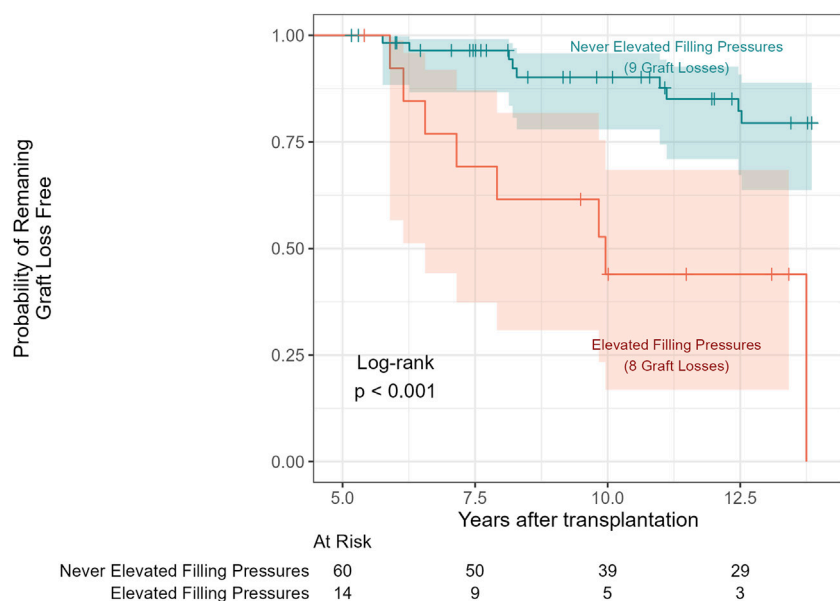


FIGURE 3
Sensitivity analysis of graft survival using a never-EFP comparator group. Kaplan–Meier curves estimate graft survival excluding grafts from the no-EFP group that developed elevated filling pressures (EFP) after the 5-year landmark (reclassified as never-EFP group). Shaded areas represent 95% confidence intervals. Tick marks denote censoring. Kaplan–Meier curves demonstrate persistent survival differences, with significantly worse graft survival in grafts EFP (log-rank $p < 0.001$). Numbers at risk at selected time points are presented below the curves.

confounding and ascertainment bias cannot be excluded. The observed associations should therefore be interpreted with appropriate caution as adjusted for selected covariates rather than fully independent.

The study design excluded the early post-transplant period to reduce perioperative confounding and minimize lead-time and immortal time bias and conditioned analyses on survival to the 5-year landmark, thereby excluding early graft

TABLE 4 Cox regression estimates with 95% confidence intervals.

Model	HR	CI
EFP-HR with Bootstrap-CI (main analysis)	6.04	[2.01, 16.85]
Sensitivity analyses		
EFP-HR with Wald-CI	6.04	[2.51, 14.54]
EFP-HR stratified by era, age ≥ 2	6.03	[2.48, 14.67]
EFP-HR stratified by era, age ≥ 10	6.08	[2.51, 14.73]
EFP-HR stratified by era, age ≥ 15	6.40	[2.62, 15.63]
EFP-HR piecewise, year 5–9	6.04	[2.51, 14.54]
EFP-HR piecewise, year 9–14	10.15	[2.19, 46.93]

CI, Confidence intervals; EFP, Elevated filling pressures HR, Hazard ratio.

failures. Grafts lacking qualifying hemodynamic assessments were also excluded. Accordingly, the cohort represents a selected population of 5-year survivors with available hemodynamic data, limiting generalizability to earlier post-transplant outcomes and the broader pediatric transplant population.

Although EFP may reflect underlying graft pathology including potential microvascular involvement, these interpretations remain speculative, as the mechanisms cannot be directly inferred from hemodynamic measurements and reliable tools to assess microvascular function are lacking. Overall, these findings suggest a potential clinical relevance of early recognition of EFP in pediatric recipients.

Systolic function was primarily assessed by LVEF using the Simpson method or, when unavailable, M-mode which may overestimate systolic function in transplanted hearts due to geometric assumptions. LVEF also has limited sensitivity for early myocardial dysfunction. Advanced parameters such as global longitudinal strain (GLS) and left atrial strain (LAS) may provide more sensitive assessment [38, 39] with LAS showing a superior correlation with PCWP [40–44]. These parameters were

not consistently available across the cohort, particularly in earlier eras, and therefore could not be included in the analysis. Their integration into future studies may improve mechanistic understanding and risk stratification.

Our study highlights the importance of structured long-term follow-up in pediatric heart transplant recipients, particularly during transition to adult care. The findings suggest that EFP, when assessed during routine evaluation, may help identify grafts at increased long-term risk. In this context, hemodynamic parameters may complement existing surveillance approaches, although their role in guiding follow-up strategies requires further investigation. Beyond surveillance, identifying the underlying substrates of EFP remains critical, as this may provide insight into graft pathophysiology. Future studies should aim to clarify the mechanisms contributing to EFP to enable more personalized long-term post-transplant management especially tailored to childhood-onset recipients.

Limitations

Taken together, this study has several important limitations. First, its single-center, retrospective design and the relatively small number of grafts with EFP limit generalizability, particularly for cardiovascular AE, which require confirmation in larger, adequately powered cohorts. Second, exclusion of grafts without retrievable hemodynamic data and conditioning on survival to the 5-year landmark may have introduced selection and ascertainment bias, enriching the cohort for later eras with more consistent surveillance. Practice patterns evolved over the 35-year study period, introducing potential heterogeneity in post-transplant surveillance. The retrospective design relied on routinely collected data, resulting in missing variables, and institutional follow-up may have led to under-capture of external events. A substantial proportion of graft losses were of unknown cause, limiting mechanistic attribution. A small number

TABLE 5 Cardiovascular adverse events within the grouping and analysis periods after pediatric heart transplantation, stratified by EFP status.

Variables:	Total (n = 114)	No-EFP group (n = 100)	EFP group (n = 14)	p-value**
Within grouping period (7 months - 5 years post-transplant)*				
Cardiovascular AE Composite Endpoint ≥ 1 :	12 (11%)	7 (7%)	5 (36%)	0.005
- Moderate - severe epicardial CAV _{2,3}	5 (4%)	1 (1%)	4 (29%)	<0.001
- Cardiac arrhythmia	5 (4%)	5 (5%)	-	0.874
- Non-rejection heart failure hospitalization	1 (1%)	0 (0%)	1 (7%)	0.248
- MI and/or coronary revascularization	6 (5%)	2 (2%)	4 (29%)	<0.001
Within analysis period (5–13.8 years post-transplant)*				
Cardiovascular AE Composite Endpoint ≥ 1 :	24 (21%)	20 (20%)	4 (29%)	0.699
- Moderate - severe epicardial CAV _{2,3}	17 (15%)	14 (14%)	3 (21%)	0.741
- Cardiac arrhythmia	7 (6%)	6 (6%)	1 (7%)	1.000
- Non-rejection heart failure hospitalization	6 (5%)	5 (5%)	1 (7%)	1.000
- MI and/or coronary revascularization	12 (11%)	9 (9%)	3 (21%)	0.340

AE, Adverse events; CAV, Coronary allograft vasculopathy EFP, Elevated filling pressures; MI, Myocardial infarction.

*Values represent the number of grafts (n) with at least one event, with percentages (%) referring to the respective group. Each graft is counted once per event category. Grafts may have experienced more than one event type, and multiple events within the same graft were possible.

**With event counts lower than 5, p-value violates assumption of chi-square test.

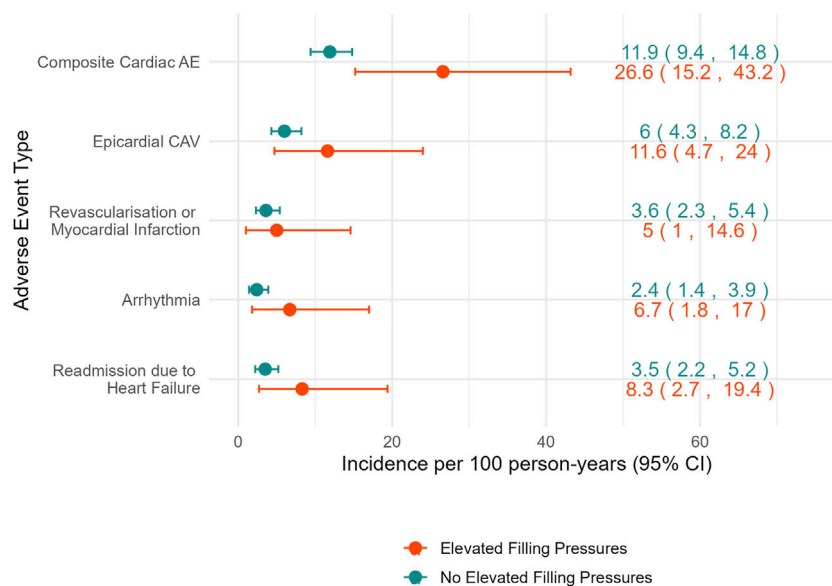


FIGURE 4

Comparison of incidence rates (per 100 person-years, 95% CI) of composite cardiovascular AE during the analysis period (5–13.8 years post-transplant) between grafts with EFP and those without. Point estimates and confidence intervals are shown for the composite cardiovascular adverse events (AE) endpoint and individual components: moderate - severe epicardial coronary allograft vasculopathy (CAV₂₋₃), myocardial infarction (MI) and/or revascularization, arrhythmia, and non-rejection heart failure hospitalization. Incidence rates account for varying follow-up time and include recurrent events.

of patients contributed more than one graft, analyzed as independent observations, which may underestimate within-patient correlation, although the impact is likely minimal. Advanced assessments of microvascular function, including index of microcirculatory resistance, coronary flow reserve, or biopsy-based techniques, were unavailable. Therefore, any association between EFP and microvascular CAV remains inferential. Collectively, these limitations indicate that the findings should be considered hypothesis-generating and warrant validation in larger multicenter, prospective studies.

Conclusion

The presence of EFP between 7 months and 5 years after pediatric HT is associated with significantly poorer long-term graft survival and potentially increased cardiovascular AE, although findings related to AE remain exploratory. These results suggest that EFP may help identify grafts at increased long-term risk. The clinical implications for surveillance strategies and therapeutic decision-making require further investigation. Future multicenter studies are needed to validate these findings, further elucidate underlying mechanisms, and determine their potential impact on long-term outcomes.

Data availability statement

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

Ethics statement

The studies involving humans were approved by Institutional Ethics Committee of Charité - Universitätsmedizin Berlin (EA2/055/23). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because of the retrospective design of the study.

Author contributions

EY: Conceptualization, Investigation, Data curation, Formal analysis, Writing – original draft. LM-R: Investigation, Formal analysis, Writing – review and editing. RS: Formal analysis, Writing – review and editing. LW: Formal analysis, Writing – original draft, Writing – review and editing. IJ-L: Writing – review and editing. PK: Writing – review and editing. FD: Writing – review and editing. FS: Writing – review and editing. FK: Formal analysis, Writing – review and editing. MY: Writing – review and editing. FB: Writing – review and editing. KS: Writing – review and editing. OM: Writing – review and editing. FL: Conceptualization, Investigation, Formal analysis, Supervision, Writing – original draft, Writing – review and editing.

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Conflict of interest

The author(s) declared that this work 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) declared that generative AI was used in the creation of this manuscript. Generative artificial intelligence was used solely for language editing and grammar correction.

References

- Singh TP, Cherikh WS, Hsich E, Chambers DC, Harhay MO, Hayes D, Jr, et al. The international thoracic organ transplant registry of the international society for heart and lung transplantation: twenty-fourth pediatric heart transplantation report - 2021; focus on recipient characteristics. *J Heart Lung Transpl* (2021) 40(10):1050–9. doi:10.1016/j.healun.2021.07.022
- Rossano JW, Singh TP, Cherikh WS, Chambers DC, Harhay MO, Hayes D, Jr, et al. The international thoracic organ transplant registry of the international society for heart and lung transplantation: twenty-second pediatric heart transplantation report - 2019; focus theme: donor and recipient size match. *J Heart Lung Transpl* (2019) 38(10):1028–41. doi:10.1016/j.healun.2019.08.002
- Dipchand AI, Laks JA. Pediatric heart transplantation: long-term outcomes. *Indian J Thorac Cardiovasc Surg* (2020) 36(Suppl. 2):175–89. doi:10.1007/s12055-019-00820-3
- Kobashigawa JA, Tobis JM, Starling RC, Tuzcu EM, Smith AL, Valentine HA, et al. Multicenter intravascular ultrasound validation study among heart transplant recipients: outcomes after five years. *J Am Coll Cardiol* (2005) 45(9):1532–7. doi:10.1016/j.jacc.2005.02.035
- Tuzcu EM, Kapadia SR, Sachar R, Ziada KM, Crowe TD, Feng J, et al. Intravascular ultrasound evidence of angiographically silent progression in coronary atherosclerosis predicts long-term morbidity and mortality after cardiac transplantation. *J Am Coll Cardiol* (2005) 45(9):1538–42. doi:10.1016/j.jacc.2004.12.076
- Nikolova AP, Kobashigawa JA. Cardiac allograft vasculopathy: the enduring enemy of cardiac transplantation. *Transplantation* (2019) 103(7):1338–48. doi:10.1097/TP.0000000000002704
- Tona F, Marra MP, Fedrigo M, Famoso G, Bellu R, Thiene G, et al. Recent developments on coronary microvasculopathy after heart transplantation: a new target in the therapy of cardiac allograft vasculopathy. *Curr Vasc Pharmacol* (2012) 10(2):206–15. doi:10.2174/157016112799304987
- Tona F, Osto E, Famoso G, Previato M, Fedrigo M, Vecchiati A, et al. Coronary microvascular dysfunction correlates with the new onset of cardiac allograft vasculopathy in heart transplant patients with normal coronary angiography. *Am J Transpl* (2015) 15(5):1400–6. doi:10.1111/ajt.13108
- Ham HM, Khush K, Luikart H, Okada K, Lim HS, Kobayashi Y, et al. Invasive assessment of coronary physiology predicts late mortality after heart transplantation. *Circulation* (2016) 133(20):1945–50. doi:10.1161/CIRCULATIONAHA.115.018741
- Chih S, Chong AY, Erthal F, deKemp RA, Davies RA, Stadnick E, et al. PET assessment of epicardial intimal disease and microvascular dysfunction in cardiac allograft vasculopathy. *J Am Coll Cardiol* (2018) 71(13):1444–56. doi:10.1016/j.jacc.2018.01.062
- Pober JS, Chih S, Kobashigawa J, Madsen JC, Tellides G. Cardiac allograft vasculopathy: current review and future research directions. *Cardiovasc Res* (2021) 117(13):2624–38. doi:10.1093/cvr/cvab259
- Hiemann NE, Wellnhofer E, Knosalla C, Lehmkuhl HB, Stein J, Hetzer R, et al. Prognostic impact of microvasculopathy on survival after heart transplantation: evidence from 9713 endomyocardial biopsies. *Circulation* (2007) 116(11):1274–82. doi:10.1161/CIRCULATIONAHA.106.647149
- Hiemann NE, Knosalla C, Wellnhofer E, Lehmkuhl HB, Hetzer R, Meyer R. Quilty indicates increased risk for microvasculopathy and poor survival after heart transplantation. *J Heart Lung Transpl* (2008) 27(3):289–96. doi:10.1016/j.healun.2007.11.573
- Perez MT, Rizwan R, Gauvreau K, Daly KP, Deng ES, Blume ED, et al. Prognostic value of exercise stress echocardiography in pediatric cardiac transplant recipients. *J Am Soc Echocardiogr* (2022) 35(11):1133–8.e2. doi:10.1016/j.echo.2022.07.006
- Mehra MR, Crespo-Leiro MG, Dipchand A, Ensminger SM, Hiemann NE, Kobashigawa JA, et al. International society for heart and lung transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy-2010. *J Heart Lung Transpl* (2010) 29(7):717–27. doi:10.1016/j.healun.2010.05.017
- Velleca A, Shullo MA, Dhital K, Azeka E, Colvin M, DePasquale E, et al. The international society for heart and lung transplantation (ISHLT) guidelines for the care of heart transplant recipients. *J Heart Lung Transpl* (2023) 42(5):e1–e141. doi:10.1016/j.healun.2022.10.015
- Kindel SJ, Law YM, Chin C, Burch M, Kirklin JK, Naftel DC, et al. Improved detection of cardiac allograft vasculopathy: a multi-institutional analysis of functional parameters in pediatric heart transplant recipients. *J Am Coll Cardiol* (2015) 66(5):547–57. doi:10.1016/j.jacc.2015.05.063
- Billingham ME, Cary NR, Hammond ME, Kemnitz J, Marboe C, McCallister HA, et al. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: Heart rejection study group. The international society for heart transplantation. *J Heart Transpl* (1990) 9(6):587–93.
- Stewart S, Winters GL, Fishbein MC, Tazelaar HD, Kobashigawa J, Abrams J, et al. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. *J Heart Lung Transpl* (2005) 24(11):1710–20. doi:10.1016/j.healun.2005.03.019
- Tan CD, Baldwin WM, Rodriguez ER. Update on cardiac transplantation pathology. *Arch Pathol Lab Med* (2007) 131(8):1169–91. doi:10.5858/2007-131-1169-UOCTP
- Lunze FI, Colan SD, Gauvreau K, Chen MH, Perez-Atayde AR, Blume ED, et al. Cardiac allograft function during the first year after transplantation in rejection-free children and young adults. *Circ Cardiovasc Imaging* (2012) 5(6):756–64. doi:10.1161/CIRCIMAGING.112.976613
- Mills MF, Long J, Quin F, Collins RT, Rosenthal DN, Almond CS, et al. A predictive model for intracardiac pressures in patients free from rejection or allograft vasculopathy after pediatric heart transplantation. *Transplantation* (2020) 104(6):e174–e181. doi:10.1097/TP.0000000000003166
- Reed EF, Demetris AJ, Hammond E, Itescu S, Kobashigawa JA, Reinsmoen NL, et al. Acute antibody-mediated rejection of cardiac transplants. *J Heart Lung Transpl* (2006) 25(2):153–9. doi:10.1016/j.healun.2005.09.003
- Berry GJ, Burke MM, Andersen C, Bruneval P, Fedrigo M, Fishbein MC, et al. The 2013 international society for heart and lung transplantation working formulation for the standardization of nomenclature in the pathologic diagnosis of antibody-mediated rejection in heart transplantation. *J Heart Lung Transpl* (2013) 32(12):1147–62. doi:10.1016/j.healun.2013.08.011
- Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American society of echocardiography and the European association of cardiovascular imaging. *J Am Soc Echocardiogr* (2015) 28(1):1–39.e14. doi:10.1016/j.echo.2014.10.003
- Lopez L, Colan SD, Frommelt PC, Ensing GJ, Kendall K, Younoszai AK, et al. Recommendations for quantification methods during the performance of a pediatric echocardiogram: a report from the pediatric measurements writing group of the

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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.2026.16339/full#supplementary-material>

- American society of echocardiography pediatric and congenital heart disease council. *J Am Soc Echocardiogr* (2010) 23(5):465–95. doi:10.1016/j.echo.2010.03.019
27. Laufs U, Anker SD, Falk V, Pieske B, Baldus S, Perings CA, et al. Kommentar zu den Leitlinien der Europäischen Gesellschaft für Kardiologie (ESC) zur Diagnostik und Behandlung der akuten und chronischen Herzinsuffizienz. *Kardiologe* (2017) 11(3):183–92. doi:10.1007/s12181-017-0143-3
28. Oeing CU, Tschöpe C, Pieske B. Neuerungen der ESC-Leitlinien zur akuten und chronischen Herzinsuffizienz 2016. *Herz* (2016) 41(8):655–63. doi:10.1007/s00059-016-4496-3
29. Tallaj JA, Kirklin JK, Brown RN, Rayburn BK, Bourge RC, Benza RL, et al. Post-heart transplant diastolic dysfunction is a risk factor for mortality. *J Am Coll Cardiol* (2007) 50(11):1064–9. doi:10.1016/j.jacc.2007.06.007
30. Kobayashi Y, Sudini NL, Rhee JW, Aymami M, Moneghetti KJ, Bouajila S, et al. Incremental value of deformation imaging and hemodynamics following heart transplantation: insights from graft function profiling. *JACC Heart Fail* (2017) 5(12):930–9. doi:10.1016/j.jchf.2017.10.011
31. Bollano E, Andersson B, Hjalmarsson C, Dellgren G, Daka B, Karason K. Early post-transplant elevated pulmonary artery pressure predicts adverse outcome in cardiac recipients. *Int J Cardiol Heart Vasc* (2020) 26:100438. doi:10.1016/j.ijcha.2019.100438
32. Patel J, Kittleson M, Deshpande A, Patel N, Singer-Englar T, Hamilton M, et al. Outcome of the development of early restrictive physiology after heart transplantation. *J Heart Lung Transpl* (2021) 40(4):S209. doi:10.1016/j.healun.2021.01.603
33. Foroutan F, Malik A, Nelson LMS, Steve CPF, Guyatt G, Gustafsson F, et al. Association between routine measures of graft function and mortality in heart transplant recipients. *Heart Br Card Soc* (2022) 108(4):307–11. doi:10.1136/heartjnl-2020-318721
34. Tsuji M, Patel JK, Kittleson MM, Chang DH, Kransdorf EP, Nikolova AP, et al. The outcome of restrictive cardiac allograft physiology in severe coronary allograft vasculopathy. *J Heart Lung Transpl* (2025) 44(1):57–63. doi:10.1016/j.healun.2024.09.029
35. Aiyagari R, Nika M, Gurney JG, Donohue JE, Zamberlan MC, King K, et al. Association of pediatric heart transplant coronary vasculopathy with abnormal hemodynamic measures. *Congenit Heart Dis* (2011) 6(2):128–33. doi:10.1111/j.1747-0803.2010.00470.x
36. Clemmensen TS, Eiskjaer H, Løgstrup BB, Valen KPB, Mellemkjaer S, Poulsen SH. Prognostic value of exercise myocardial deformation and haemodynamics in long-term heart-transplanted patients. *ESC Heart Fail* (2019) 6(4):629–39. doi:10.1002/ehf2.12438
37. Rosenthal LM, Krauss A, Miera O, Danne F, Lunze F, Cho MY, et al. Changes in waiting time, need for mechanical circulatory support and outcomes in paediatric heart transplant recipients. *ESC Heart Fail* (2024) 11:3626–35. doi:10.1002/ehf2.14917
38. Smiseth OA, Rider O, Cvijic M, Valković L, Remme EW, Voigt JU. Myocardial strain imaging: theory, current practice, and the future. *JACC Cardiovasc Imaging* (2025) 18(3):340–81. doi:10.1016/j.jcmg.2024.07.011
39. Panatpur A, O'Sullivan DM, Vacher CS, Nguyen MB, Doan TT. Left atrial strain in pediatric cardiology: evidence to date and future directions. *J Am Soc Echocardiogr* (2025) 39(25):S0894–7317. doi:10.1016/j.echo.2025.12.007
40. Lunze FI, Narciso R, Gauvreau K, Blume ED, Colan SD, Singh TP. Is doppler echocardiography useful for estimating left ventricular filling pressures in pediatric heart transplant recipients? *Pediatr Transpl* (2019) 23(7):e13543. doi:10.1111/petr.13543
41. Sridharan A, Dehn MM, Cooper C, Madineedi VS, Ordway LJ, DeNofrio D, et al. Accuracy of echocardiographic estimations of right heart pressures in adult heart transplant recipients. *Clin Cardiol* (2022) 45(7):752–8. doi:10.1002/clc.23835
42. Alfares FA, Nanjagowda C, Swaminathan S, Hunter J, Alkon J, Sandhu S, et al. Echocardiography to predict left ventricular filling pressure for long-term paediatric heart transplant patients. *Cardiol Young* (2024) 34(8):1799–804. doi:10.1017/S1047951124000908
43. Yeh J, Aiyagari R, Gajarski RJ, Zamberlan MC, Lu JC. Left atrial deformation predicts pulmonary capillary wedge pressure in pediatric heart transplant recipients. *Echocardiogr Mt Kisco N* (2015) 32(3):535–40. doi:10.1111/echo.12679
44. Loar RW, Pignatelli RH, Morris SA, Colquitt JL, Feagin DK, Denfield SW, et al. Left atrial strain correlates with elevated filling pressures in pediatric heart transplantation recipients. *J Am Soc Echocardiogr* (2020) 33(4):504–11.e1. doi:10.1016/j.echo.2019.11.004



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Incidence, predictors and outcomes following primary graft dysfunction after cardiac transplantation

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Primary graft dysfunction (PGD) is the leading cause of early morbidity and mortality after heart transplantation (HTx). We retrospectively analyzed 830 consecutive HTx performed between 1984 and 2021. After excluding patients <18 years and those with missing data, 667 adult recipients remained. ISHLT PGD criteria were applied and perioperative variables and outcomes were reviewed. PGD occurred in 70 patients (10.5%), including 41 (6.1%) with left ventricular PGD and 29 (4.3%) with right ventricular PGD. Most LV-PGD cases were severe (88%). Patients with PGD were younger and more frequently had pretransplant dialysis, ventricular assist device support, or prior cardiac surgery. No donor-related factors were associated with PGD. Recipient-related factors and longer cardiopulmonary bypass time were associated with increased risk. PGD was associated with prolonged mechanical ventilation and ICU stay, and increased need for mechanical circulatory support, dialysis, reoperation, and treatment for sepsis. Mortality or re-transplantation was significantly higher in PGD patients at 30 days (45% vs. 3%) and 1 year (51% vs. 8%; HR 6.89, 95% CI 4.01–11.83, $p < 0.0001$).

KEYWORDS

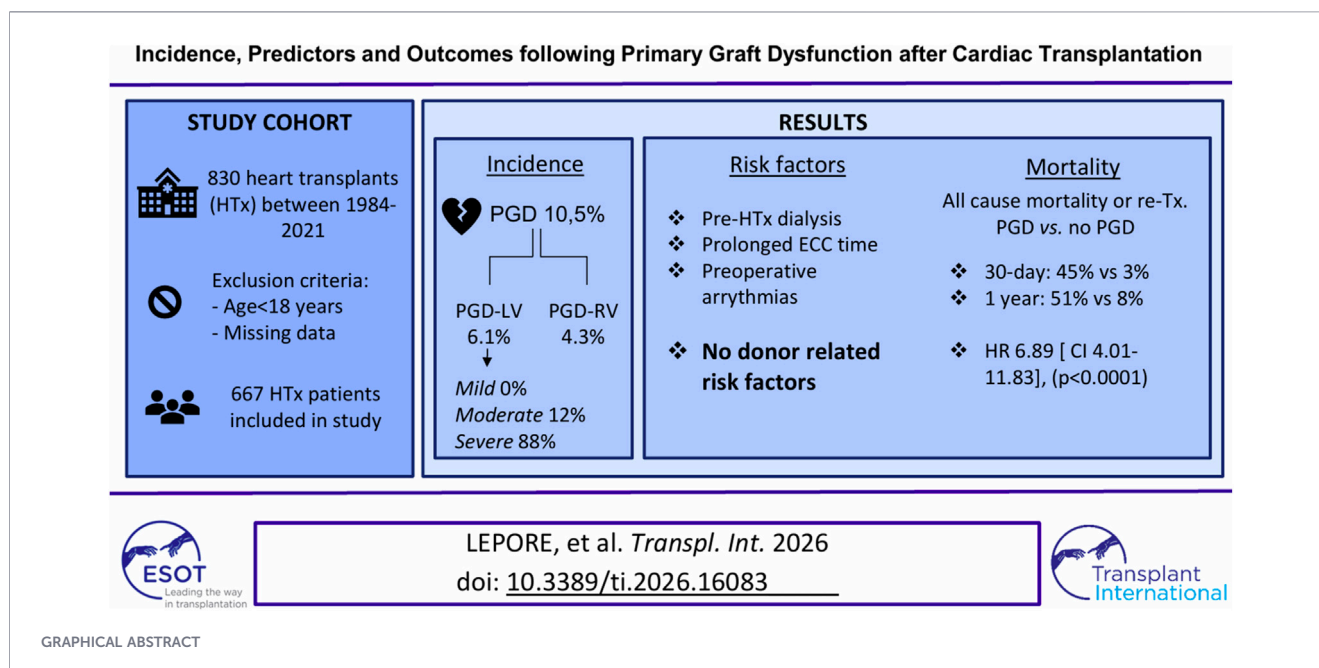
graft rejection, heart failure, heart transplant rejection, heart transplantation, primary graft dysfunction

Introduction

Heart transplantation (HTx) is the gold standard treatment for end-stage heart failure, with close to 9000 patients undergoing the procedure worldwide every year. In the modern era 1-year survival is about 90% and a median-survival of 12.5 years, but even longer survival has been observed in the Scandinavian countries [1–4].

Primary graft dysfunction (PGD) is the leading cause of morbidity and mortality within the first 30 days after HTx [5] and presents as left-, right-, or biventricular allograft failure [3, 6]. Various risk factors for graft dysfunction have been reported, such as graft ischemia time, high donor and recipient age and mechanical circulatory support before transplantation [7–11].

Over time, different definitions of graft dysfunction have been used, leading to challenges in comparing data across time periods and publications. This is exemplified by the reported incidences ranging from 2.5% to 28.2%. In 2014, ISHLT agreed on consensus criteria for graft dysfunction separating primary from secondary graft dysfunction [12]. The



latter, a consequence of, for example, acute rejection, severe bleeding or a surgical complication, while primary graft dysfunction has no obvious discernable cause. Since the ISHLT definition was introduced, transplant centers have utilized the criteria to better identify incidence, risk factors, and mortality, providing a more precise assessment compared to evaluations before 2014. In 2020, a meta-analysis by Buchan et al. reported a pooled incidence with moderate certainty ranging from 1.6% to 7.7%, depending on the subtype of PGD [7]. Further research is essential to achieve a comprehensive understanding of this condition and therefore we investigated our experience of PGD. The aim of this study was to investigate the incidence of PGD thoroughly checked from source data according to the ISHLT guidelines at our center, to identify predictors for PGD, and to study the impact of PGD on the short-term outcome.

Patients and methods

Patient cohort

The study was approved by the Swedish National Research Ethics Committee, following the Helsinki Declaration (Diary

number 728-12, amendment 2020-04281). Being a retrospective study, patient consent was not deemed necessary and waived by the ethical committee. Inclusion criteria were all patients >18 years having had a HTx procedure between June 1984 and December 2021 at Sahlgrenska University Hospital, Sweden. Patients with incomplete medical records preventing assessment of early graft function were excluded. For further details regarding study cohort, see [Supplementary Figure SA1](#).

PGD definitions

Primary graft dysfunction (PGD) is defined in the ISHLT consensus document as acute onset of heart failure affecting the right, left, or both ventricles within the first 24 h after HTx. Additionally, to be classified as PGD, the graft dysfunction may not be attributed to other causes, such as uncontrolled bleeding, surgical complications, pulmonary hypertension or acute rejection. In these circumstances the graft dysfunction is labeled secondary. PGD is categorized into two entities: PGD-Right ventricle (PGD-RV) and PGD-Left ventricle (PGD-LV). The subclassification of PGD-LV includes mild, moderate, and severe, providing a detailed spectrum of severity. For a more comprehensive understanding, see [Supplementary Figure SA1](#).

Data collection and variables

Data was collected retrospectively for all patients included in the study. We are maintaining an updated database of the HTx patients including main patient characteristics as well as follow-up data at our center. However, specific information on PGD has not continuously been collected. Therefore, all anesthesia records and intensive care unit (ICU) charts were retrieved and analyzed (including use of inotropic agents). ICU charts records included hourly measurements of central venous pressure (CVP), continuous cardiac output (CCO), mean arterial pressure (MAP). Pulmonary

Abbreviations: BMI, Body mass index; CCO, Continuous cardiac output; CI, Cardiac index; CVP, Central venous pressure; DCM, Dilated cardiomyopathy; ECC, Extracorporeal circulation; ECMO, Extracorporeal membrane oxygenation; HTx, Heart transplantation; IABP, Intra-aortic balloon pump; ICB, Intracranial bleeding; ICU, Intensive care unit; IHD, Ischemic heart disease; ISHLT, International Society of Heart and Lung Transplantation; LVAD, Left ventricular assist device; MAP, Mean arterial pressure; MCS, Mechanical circulatory support; MPAP, Mean pulmonary arterial pressure; NYHA, New York Heart Association; PCWP, Pulmonary capillary wedge pressure; PGD, Primary graft dysfunction; Re-Tx, Re-transplantation; RV, Right ventricle; RVAD, Right ventricular assist device; TPG, Transpulmonary gradient; VAD, Ventricular assist device.

International criteria for PGD, ISHLT definition

1. PGD-LV

Mild PGD-LV: One of the following criteria must be met:

LVEF < 40% by echocardiography, *or* Hemodynamics with RAP > 15 mmHg, PCWP > 20 mmHg, CI < 2.0 L/min/m² (lasting more than 1 hour requiring low dose inotropes)

Moderate PGD-LV: Must meet one criterion from I and another from II:

I. One criteria from the following: Left ventricular ejection fraction ≤ 40%, *or* Hemodynamic compromise with RAP > 15 mmHg, PCWP > 20 mmHg, CI < 2.0 L/min/m², hypotension with MAP < 70 mmHg (lasting more than 1 hour).

II. One criteria from the following:

- i. High-dose inotropes—Inotrope score > 10a *or*
- ii. Newly placed IABP (regardless of inotropes)

Severe PGD-LV:

Dependence on left or biventricular mechanical support including ECMO, LVAD, BiVAD, or percutaneous LVAD. Excludes requirement for IABP.

2. PGD-RV

Diagnosis requires either both i and ii, or iii alone:

- i. Hemodynamics with RAP > 15 mmHg, PCWP < 15 mmHg, CI < 2.0 L/min/m²
- ii. TPG < 15 mmHg and/or pulmonary artery systolic pressure < 50 mmHg, *or*
- iii. Need for RVAD

FIGURE 1

Definition for primary graft dysfunction adopted from ISHLT consensus report. PGD, primary graft dysfunction. LV, left ventricle; RV, right ventricle; RAP, right atrial pressure; PCWP, pulmonary capillary wedge pressure; MAP, mean arterial pressure; CI, cardiac index; ECMO, extracorporeal membrane oxygenation; IABP, intra-aortic balloon pump; LVAD, left ventricular assist device; BiVAD, biventricular assist device; RVAD, right ventricular assist device; TPG, transpulmonary gradient.

capillary wedge pressure (PCWP) and mean pulmonary arterial pressure (MPAP) via a Swan-Ganz were recorded at regular intervals. From CCO the cardiac index (CI) was calculated using the preoperative body surface area of the patient. Trans pulmonary gradient (TPG) was calculated under the assumption that PCWP is equal to the left atrial pressure (LAP) and was deducted from MPAP to receive the TPG. All hemodynamic parameters must have met the criteria in Figure 1 for at least >1 h for the diagnosis of PGD to be set. To determine the inotropic score, the highest dosage using the concentration in µg/kg/min per inotrope during the first 24 h was selected [12].

By analyzing all the perioperative and postoperative data during the first 24 h in the ICU we were able to identify a graft dysfunction (GD) and non-GD cohort. The data was meticulously reviewed to be able to determine whether PGD had occurred. The operative notes as well as the anesthesia reports were analyzed to exclude secondary graft dysfunction. The PGD group was then divided into four sub-groups using the ISHLT definition (Supplementary Figure SA1).

Statistics

Continuous variables are presented by mean, standard deviation, median, minimum and maximum, and categorical variables by frequency and percentage.

For tests between PGD and no PGD group Fisher's exact test was used for dichotomous variables, Mantel-Haenszel Chi-square trend test for ordered categorical variables, Chi-square test for non-ordered categorical variables and Mann-Whitney U-test for continuous variables. In the analyses on HTx level the

observations were assumed to be independent given the small number of hearts with previous re-HTx (n = 21). All completed transplants were divided into three time-era periods (1984–1999, 2000–2010 and 2011–2022). Associations between pre-HTx and intraoperative clinically relevant a-priori selected variables and incidence of PGD were investigated using logistic regression, adjusted for age and sex. A multivariable model was obtained by running a stepwise selection (forward and backward) and keeping only statistically significant variables beside adjustment for age and sex. From these analyses odds-ratios with 95% confidence intervals (CI), p-values, and area under receiver operating characteristic curve (AUC or c-statistics) were presented. In these analyses only the first HTx was studied. Results from stepwise models should be interpreted as exploratory. No evidence of significant collinearity among the included variables was observed. Missing data were limited and managed using a complete-case approach in the multivariable models, with negligible expected impact on the results.

Associations between pre-HTx and intraoperative variables and the primary outcome all-cause death or re-HTx during first year were performed using Cox regression, adjusted for age and sex. A multivariable model was obtained by running a stepwise selection (forward and backward) and keeping only statistically significant variables beside adjustment for age and sex. From these analyses hazard ratios (HR) and adjusted HR (aHR) with 95% CI and p-values were presented. In the selected multivariable model, the assumption of proportional hazards was tested by introducing interaction terms between variables and log(time) which were non-significant. Hence, proportional hazards could be assumed.

Descriptively, survival data was presented by Kaplan-Meier plots. In these analyses only first HTx was studied.

All tests were two-tailed and conducted at 0.05 significance level. All analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics

Among 830 subjects that underwent HTx between June 1984 and December 2021, 103 (12.4%) were excluded due to age <18 years and 60 (7.2%) due to incomplete medical records. A total of 667 (80.4%) HTx, in 646 unique patients, met the inclusion criteria and were included in the study ([Supplementary Figure SA1](#)). Of these, 504 (75.6%) were men and 163 (24.4%) women.

Incidence of PGD

In total, 16 patients had secondary graft dysfunction due to, e.g., uncontrolled bleeding, stenosis of the vena cava anastomoses or hyperacute rejection. Our primary endpoint PGD was evident in 70 HTx patients and the overall incidence of PGD was 10.5%. This group was now compared to the non-PGD cohort that consisted of 597 HTx in 580 unique patients.

Other secondary endpoints, such as PGD-LV ($n = 41, 58.6\%$) was more common than PGD-RV ($n = 29$) of the PGD cases. Among patients with PGD-LV, 5 had (12%) moderate and 36 (88%) the severe form. Patients who developed PGD were on average younger than those in the non-PGD group (45.5 vs. 49.7, $p = 0.03$). They were also shorter and had a lower body weight (173 cm vs. 176 cm; $p = 0.0063$ and 75 kg vs. 80 kg; $p = 0.0067$, respectively). Patients with and without PGD had different diagnoses leading to HTx ($p = 0.02$). The PGD group more frequently had dialysis preoperatively (7.7% vs. 1.7%; $p = 0.012$), a history of cardiac surgery (60.3% vs. 37.3%, $p = 0.0004$) and pre-HTx ventricular assist device (VAD) (32.8% vs. 18.8%, $p = 0.0098$) compared to the non-PGD group. For further details see [Table 1](#).

Donor characteristics

Other secondary outcomes, donor age for the whole cohort was 41.6 ± 14.9 years. In the donor population, men were overrepresented with 431 individuals (64.6%) compared to 236 women (35.4%). Intracerebral hemorrhage emerged as the predominant cause of death (49.0%), followed by trauma (26.7%). There were no differences in donor characteristics between recipients with or without PGD. Donor-related risk factors are presented in [Table 2](#).

Intraoperative findings

A trend towards longer graft ischemic time in patients with PGD was observed (195 min vs. 181 min, $p = 0.06$) ([Table 3](#)). When re-transplantations were excluded from the analysis, this difference was significant (196 min vs. 181 min, $p = 0.035$). Patients with PGD had longer extra corporeal circulation (ECC) times (median 193.0 min

vs. 145.0 min, p -value <0.0001) indicating more complex procedures.

Postoperative findings

Postoperative secondary outcome data is presented in [Table 4](#). The number of days in the ICU was longer in PGD patients with an average difference of more than 2 weeks (22.1 ± 23.1 vs. 7.8 ± 12.9 , $p < 0.0001$). Postoperative mechanical circulatory support (MCS) was seen in 75.4% of patients with PGD and in 0.9% in the non-PGD group ($p < 0.0001$). PGD subjects required re-operation to a greater extent (70.1% vs. 27.2%, $p < 0.0001$). Post-operative dialysis was more frequent in the PGD cohort (50.0% vs. 19.0%, $p < 0.0001$) and the time with mechanical ventilation was longer (326 ± 458 h vs. 63 ± 167 h, $p < 0.0001$). The proportion of patients who experienced post-operative sepsis was also higher in the PGD group (28.1% vs. 8.9%, $p < 0.0001$).

Association between PGD and mortality or re-transplantation

The 30-day mortality or re-transplant rate in the PGD group was 45.5% compared with 3.3% in the non-PGD group ($p < 0.0001$). Death during hospitalization was 47% in the PGD group and 4.1% in the non-PGD group ($p < 0.0001$). 1-year mortality or re-HTx was 51.5% in the PGD group and 7.6% in the non-PGD group ([Supplementary Table SA1](#); [Figure 1](#)). The median survival for the PGD group was less than 1 year compared to more than 15 years for the non-PGD group ([Figure 2](#)).

Association between pre-HTx or intraoperative variables and PGD

Predictors of PGD in the age- and sex-adjusted logistic regression model were: age (adjusted (a)OR 0.80 (CI 0.66–0.96), $p = 0.017$; weight (aOR 0.90 (CI 0.82–0.98), $p = 0.021$); inverse height risk association (aOR 0.73 (CI 0.62–0.87), $p = 0.0005$); diagnosis (aOR 0.39 (CI 0.19–0.77), $p = 0.0069$); pre-HTx dialysis (aOR 7.03 (CI 1.79–27.5), $p = 0.0051$); pre-HTx sternotomy (aOR 2.51 (CI 1.46–4.29), $p = 0.0008$); ECC (aOR 1.75 (CI 1.45–2.11), $p < 0.0001$); arrhythmias pre-HTx (aOR 1.97 (CI 1.16–3.37), $p = 0.013$); and pre-HTx VAD (aOR 1.85 (CI 1.03–3.33), $p = 0.039$).

Independent predictors for PGD by multivariable logistic regression were: pre-HTx dialysis (aOR 6.73 (CI 1.46–31.6), $p = 0.015$); arrhythmias pre-HTx (aOR 2.08 (CI 1.16–3.75), $p = 0.014$); inverse height risk association (aOR 0.78 (CI 0.64–0.95), $p = 0.012$); longer ECC time (aOR 1.75 (CI 1.44–2.14, $p < 0.0001$), per 60 min increase) ([Supplementary Table SA2](#)).

Association between pre-HTx or intraoperative variables and death of any cause

A stepwise Cox regression analysis of 1-year outcomes of death from any cause and re-HTx ([Supplementary Table SA3](#)) identified 11 predictors in the univariable analysis, and three were

TABLE 1 Preoperative patient characteristics.

Variable	Total N = 667	No PGD N = 597	PGD N = 70	No PGD vs. PGD p-value	PGD-LV N = 41	PGD-RV N = 29
Sex				1.00		
Male	504 (75.6%)	451 (75.5%)	53 (75.7%)		33 (80.5%)	20 (69.0%)
Female	163 (24.4%)	146 (24.5%)	17 (24.3%)		8 (19.5%)	9 (31.0%)
Age	49.3 ± 12.9 52.6 (18.0–70.5) n = 667	49.7 ± 12.6 52.8 (18.0–70.5) n = 597	45.5 ± 14.5 49.9 (18.2–67.4) n = 70	0.030	45.1 ± 14.4 49.8 (18.4–67.4) n = 41	46.1 ± 14.8 50.1 (18.2–65.5) n = 29
Age category				0.0089		
<30y	75 (11.2%)	59 (9.9%)	16 (22.9%)		10 (24.4%)	6 (20.7%)
30–<40y	79 (11.8%)	73 (12.2%)	6 (8.6%)		4 (9.8%)	2 (6.9%)
40–<50y	138 (20.7%)	125 (20.9%)	13 (18.6%)		7 (17.1%)	6 (20.7%)
50–<60y	235 (35.2%)	207 (34.7%)	28 (40.0%)		16 (39.0%)	12 (41.4%)
60 + y	140 (21.0%)	133 (22.3%)	7 (10.0%)		4 (9.8%)	3 (10.3%)
Weight (kg)	79.1 ± 16.2 79.0 (26.0–129.0) n = 662	79.7 ± 16.2 80.0 (26.0–129.0) n = 593	74.5 ± 15.6 73.0 (42.0–111.0) n = 69	0.0067	74.5 ± 14.6 71.0 (50.0–110.0) n = 40	74.6 ± 17.2 75.0 (42.0–111.0) n = 29
Length (cm)	175.6 ± 9.2 176 (139–205) n = 662	176.0 ± 9.1 176 (139–205) n = 593	172.8 ± 8.9 173 (152–190) n = 69	0.0063	173.1 ± 8.9 173 (154–189) n = 40	172.3 ± 9.0 172 (152–190) n = 29
Body mass index (kg/m ²)	25.5 ± 4.3 25.2 (13.5–40.7) n = 661	25.6 ± 4.3 25.2 (13.5–40.7) n = 592	24.9 ± 4.4 24.5 (15.5–34.1) n = 69	0.22	24.8 ± 4.1 24.5 (16.9–34.1) n = 40	25.0 ± 4.9 24.8 (15.5–33.8) n = 29
Diagnosis				0.020		
Ischemic cardiomyopathy	158 (24.5%)	138 (23.9%)	20 (29.4%)		10 (25.0%)	10 (35.7%)
Dilated cardiomyopathy	334 (51.7%)	311 (53.8%)	23 (33.8%)		14 (35.0%)	9 (32.1%)
Myocarditis	12 (1.9%)	10 (1.7%)	2 (2.9%)		1 (2.5%)	1 (3.6%)
Re-transplantation	21 (3.3%)	17 (2.9%)	4 (5.9%)		3 (7.5%)	1 (3.6%)
Valve disease	12 (1.9%)	11 (1.9%)	1 (1.5%)		1 (2.5%)	0 (0.0%)
Congenital heart disease	43 (6.7%)	33 (5.7%)	10 (14.7%)		5 (12.5%)	5 (17.9%)
Arrhythmogenic right valve dysplasia	17 (2.6%)	16 (2.8%)	1 (1.5%)		1 (2.5%)	0 (0.0%)
Restrictive cardiomyopathy	14 (2.2%)	10 (1.7%)	4 (5.9%)		3 (7.5%)	1 (3.6%)
Hypertrophic cardiomyopathy	27 (4.2%)	24 (4.2%)	3 (4.4%)		2 (5.0%)	1 (3.6%)
Amyloidosis	7 (1.1%)	7 (1.2%)	0 (0.0%)			
Restrictive pericarditis	1 (0.2%)	1 (0.2%)	0 (0.0%)			
Missing	21	19	2		1	1
Smoking				0.34		
No	363 (55.8%)	320 (54.9%)	43 (64.2%)		25 (64.1%)	18 (64.3%)
Smoked within 6 months	52 (8.0%)	48 (8.2%)	4 (6.0%)		0 (0.0%)	4 (14.3%)

(Continued)

TABLE 1 Continued

Variable	Total N = 667	No PGD N = 597	PGD N = 70	No PGD vs. PGD p-value	PGD-LV N = 41	PGD-RV N = 29
Previous smoker (>6 months)	235 (36.2%)	215 (36.9%)	20 (29.9%)		14 (35.9%)	6 (21.4%)
Missing	17	14	3		2	1
Dialysis				0.012		
No	630 (97.7%)	570 (98.3%)	60 (92.3%)		34 (91.9%)	26 (92.9%)
Yes	15 (2.3%)	10 (1.7%)	5 (7.7%)		3 (8.1%)	2 (7.1%)
Missing	22	17	5		4	1
Previous sternotomy				0.0004		
No	397 (60.3%)	370 (62.7%)	27 (39.7%)		16 (40.0%)	11 (39.3%)
Yes	261 (39.7%)	220 (37.3%)	41 (60.3%)		24 (60.0%)	17 (60.7%)
Missing	9	7	2		1	1
Previous heart transplantation				0.26		
No	646 (96.9%)	580 (97.2%)	66 (94.3%)		38 (92.7%)	28 (96.6%)
Yes	21 (3.1%)	17 (2.8%)	4 (5.7%)		3 (7.3%)	1 (3.4%)
Amiodarone				0.20		
No	511 (79.0%)	464 (79.7%)	47 (72.3%)		27 (71.1%)	20 (74.1%)
Yes	136 (21.0%)	118 (20.3%)	18 (27.7%)		11 (28.9%)	7 (25.9%)
Missing	20	15	5		3	2
Inotrope				0.17		
No	478 (74.8%)	435 (75.7%)	43 (67.2%)		25 (69.4%)	18 (64.3%)
Yes	161 (25.2%)	140 (24.3%)	21 (32.8%)		11 (30.6%)	10 (35.7%)
Missing	28	22	6		5	1
Missing	14	9	5		3	2
NYHA				0.37		
I-II	32 (5.0%)	31 (5.4%)	1 (1.6%)		1 (2.7%)	0 (0.0%)
III	434 (68.5%)	390 (68.3%)	44 (69.8%)		25 (67.6%)	19 (73.1%)
IV	168 (26.5%)	150 (26.3%)	18 (28.6%)		11 (29.7%)	7 (26.9%)
Missing	33	26	7		4	3
Arrhythmias				0.039		
No	425 (65.7%)	390 (67.0%)	35 (53.8%)		18 (48.6%)	17 (60.7%)
Yes	222 (34.3%)	192 (33.0%)	30 (46.2%)		19 (51.4%)	11 (39.3%)
Missing	20	15	5		4	1
VAD				0.0098		
No	521 (79.8%)	476 (81.2%)	45 (67.2%)		25 (64.1%)	20 (71.4%)
Yes	132 (20.2%)	110 (18.8%)	22 (32.8%)		14 (35.9%)	8 (28.6%)
Missing	14	11	3		2	1
Calendar year				0.07		

(Continued)

TABLE 1 Continued

Variable	Total N = 667	No PGD N = 597	PGD N = 70	No PGD vs. PGD p-value	PGD-LV N = 41	PGD-RV N = 29
1984–1999	201 (30.1%)	178 (29.8%)	23 (32.9%)		15 (36.6%)	8 (27.6%)
2000–2010	176 (26.4%)	149 (25.0%)	27 (38.6%)		14 (34.1%)	13 (44.8%)
2011–2022	290 (43.5%)	270 (45.2%)	20 (28.6%)		12 (29.3%)	8 (27.6%)

Data are presented as mean $\pm$ standard deviation, median (range) and number of observations, or number (percentage).

For test between two groups with respect to dichotomous variables Fisher's exact test was used, for non-ordered categorical variables Chi-square test, for ordered categorical variables Mantel-Haenszel Chi-square trend test, and for continuous variables Mann-Whitney U-test.

PGD, primary graft dysfunction; LV, left ventricle; RV, right ventricle; VAD, Ventricular assist device; NYHA, New York Heart Association.

TABLE 2 Donor characteristics.

Variable	Total N = 667	No PGD N = 597	PGD N = 70	No PGD vs. PGD p-value	PGD-LV N = 41	PGD-RV N = 29
Donor sex				0.60		
Male	431 (64.6%)	388 (65.0%)	43 (61.4%)		26 (63.4%)	17 (58.6%)
Female	236 (35.4%)	209 (35.0%)	27 (38.6%)		15 (36.6%)	12 (41.4%)
Donor age (years)	41.6 $\pm$ 14.9 43.0 (12.0–73.0) n = 666	41.5 $\pm$ 15.1 43.0 (12.0–73.0) n = 596	42.9 $\pm$ 12.5 46.0 (15.0–65.0) n = 70	0.50	41.7 $\pm$ 13.0 44.0 (15.0–65.0) n = 41	44.7 $\pm$ 11.9 49.0 (21.0–64.0) n = 29
Donor blood group				0.99		
0	287 (43.2%)	256 (43.1%)	31 (44.3%)		20 (48.8%)	11 (37.9%)
A	296 (44.6%)	265 (44.6%)	31 (44.3%)		17 (41.5%)	14 (48.3%)
B	72 (10.8%)	65 (10.9%)	7 (10.0%)		4 (9.8%)	3 (10.3%)
AB	9 (1.4%)	8 (1.3%)	1 (1.4%)		0 (0.0%)	1 (3.4%)
Missing	3	3	0			
Donor height (cm)	176.1 $\pm$ 10.7 177 (80–205) n = 662	176.1 $\pm$ 10.2 176 (89–205) n = 592	175.8 $\pm$ 14.5 177 (80–195) n = 70	0.62	177.3 $\pm$ 8.3 177 (160–195) n = 41	173.6 $\pm$ 20.2 175 (80–195) n = 29
Donor weight (kg)	78.8 $\pm$ 16.8 77.0 (45.0–192.0) n = 664	78.6 $\pm$ 16.5 77.0 (45.0–192.0) n = 594	81.0 $\pm$ 19.0 76.0 (52.0–175.0) n = 70	0.51	80.8 $\pm$ 16.8 78.0 (58.0–135.0) n = 41	81.2 $\pm$ 22.0 75.0 (52.0–175.0) n = 29
Reason for death				0.36		
Unknown	25 (4.3%)	25 (4.8%)	0 (0.0%)			
ICB	282 (49.0%)	249 (48.1%)	33 (56.9%)		19 (61.3%)	14 (51.9%)
Trauma	154 (26.7%)	138 (26.6%)	16 (27.6%)		9 (29.0%)	7 (25.9%)
Thromboembolic	17 (3.0%)	16 (3.1%)	1 (1.7%)		1 (3.2%)	0 (0.0%)
Other	98 (17.0%)	90 (17.4%)	8 (13.8%)		2 (6.5%)	6 (22.2%)
Missing	91	79	12		10	2

Data are presented as mean $\pm$ standard deviation, median (range) and number of observations, or number (percentage).

For test between two groups with respect to dichotomous variables Fisher's exact test was used, for non-ordered categorical variables Chi-square test, and for continuous variables Mann-Whitney U-test.

PGD, primary graft dysfunction; ICB, intracranial cerebral bleeding; LV, left ventricle; RV, right ventricle.

independently significant after adjustment for age and sex in the multivariable analysis. In the multivariable analysis, PGD had an almost 7-fold higher risk of death vs. non-PGD (aHR of 6.89 [CI 4.01–11.83], $p < 0.0001$, [Supplementary Table SA3](#)). Individuals

transplanted 2011–2022 had a lower risk of death or re-HTx compared to 1984–1999, (aHR 0.27 [CI 0.14–0.52], $p = 0.0001$). For every 60 min of increased ECC time, the risk of death or re-HTx was increased (aHR 1.38 [CI 1.17–1.63], $p = 0.0002$).

TABLE 3 Intraoperative data.

Variable	Total N = 667	No PGD N = 597	PGD N = 70	No PGD vs. PGD p-value	PGD-LV N = 41	PGD-RV N = 29
Ischemic time (min)	182.7 ± 59.1 185.5 (44.0–431.0) n = 642	181.3 ± 59.5 185.0 (44.0–431.0) n = 576	194.7 ± 54.9 196.5 (51.0–375.0) n = 66	0.06	186.8 ± 50.5 191.0 (51.0–284.0) n = 37	204.8 ± 59.5 209.0 (73.0–375.0) n = 29
ECC	159.9 ± 65.6 149.0 (56.0–583.0) n = 653	153.4 ± 56.2 145.0 (56.0–583.0) n = 584	214.6 ± 103.7 193.0 (82.0–495.0) n = 69	<0.0001	225.3 ± 110.6 198.0 (92.0–495.0) n = 40	199.7 ± 93.1 169.0 (82.0–446.0) n = 29
Cross clamp duration at implant	96.7 ± 54.9 80.0 (17.0–450.0) n = 479	96.2 ± 54.9 79.0 (17.0–450.0) n = 428	101.4 ± 55.3 85.0 (23.0–257.0) n = 51	0.62	101.5 ± 52.7 83.0 (43.0–202.0) n = 31	101.3 ± 60.7 95.5 (23.0–257.0) n = 20

Data are presented as mean ± standard deviation, median (range) and number of observations.

For test between two groups Mann-Whitney U-test was used for continuous variables.

PGD, primary graft dysfunction; LV, left ventricle; RV, right ventricle; ECC, extra corporeal circulation.

Discussion

Primary graft dysfunction occurred in 10.5% of all adult patients that underwent a HTx in our single center analysis. PGD was associated with a severely increased risk of early mortality with 50% of the patients not surviving the first year.

The incidence of PGD in the analysis was lower compared to other publications. Buchan conducted a meta-analysis in 2021 that included 36 studies. The pooled incidence of PGD was 20.5% [7]. Singh et al published a national study from the UK in 2019, 450 heart transplants were included and the incidence of moderate or severe PGD was 36% [13]. Dronovalli reported an incidence of 32.4%, however not based on the ISHLT criteria, based on the 290 transplantations in a single center study [14]. Based on ISHLT criteria and a data set from Duke University Hospital between 2009 and 2014, Nicoara et al reported a PGD incidence of 31% in 317 patients [15]. However, Rhee et al included 570 patients an analysis and reported an PGD incidence of 6.1%, comparable to our study also based on ISHLT criteria [16]. D'Alessandro et al conducted a study including 1221 donor hearts in-between 2015–2022, and based on ISHLT criteria the overall incidence for PGD was 18.5% [17]. Similar to our data set, the studies above included a majority of male recipients, with the mean age under 60 years, and similar preoperative conditions.

Despite the lower incidence of PGD in our population, both 30-day (45.5%) and 1-year mortality or re-Tx (51.5%) in the PGD population were higher compared to the other above-mentioned studies with 30-day mortality being 14.5%–39.3% [13–16] and 1-year mortality of 27.5%–41.5% [14, 16]. In our population the majority of the PGD cases were either severe PGD-LV or PGD-RV and these more severe forms carry a higher risk of mortality. This should be compared to the before mentioned studies where a large part of the cohort consisted of the mild or moderate PGD-LV. In Rhee's study population, only 0.5% of subjects were diagnosed with PGD-RV, and of the 6.1% with PGD-LV, 40% had the moderate and 57.1% had the severe form [16]. In the meta-analysis, the pooled incidence of PGD-LV was mild 3.5%, moderate 6.6% and severe PGD-LV 7.7%. The incidence of PGD-RV was only 1.6% [7]. In D'Alessandro's study the incidence of severe PGD was 8.2%. The 30-day and 1 year mortality in the severe

PGD group was 19% respectively 31.2%. Their multivariable analysis showed that the recipients who develop severe PGD face a 7.8-fold higher risk of death within 1 year compared to those who do not. These results are similar to our study with a HR of 6.89 in the PGD-group (Supplementary Table SA3) [17]. The overall lower mortality rate can therefore be explained by two primarily factors. Firstly, a larger portion of the PGD cohorts consisting of the mild or moderate form and secondly, our patient inclusion spanned a broader temporal range during which therapeutic modalities have undergone significant advancements and refinements.

Our study also aimed to identify donor, pre- and intraoperative variables associated with an increased risk of developing PGD after heart transplant. Most interestingly, no donor factor was associated with PGD, instead recipient factors linked to a higher degree of disease (dialysis, arrhythmias) or more complex surgery (long ECC-time) were the ones found significant in multivariable analyses. Although donor data was not detailed, this indicated that recipient factors may be of higher importance for development of PGD. The significance of recipient length may be attributed to challenges in finding an appropriate size match of an appropriate donor heart, resulting in prolonged waiting times for transplantation and potentially under sizing of donors in relationship to the recipient. Singh and Buchan et al both reported that under sized donor hearts, as measured by predicted heart mass, were associated with the development of PGD postoperatively. In a similar study by Singh et al, they identified additional risk factors, such as elevated recipient body mass index, preoperative creatinine, recipient hospitalized at the time of transplantation, ischemic cardiomyopathy, undersized donor (by difference in predicted heart mass of greater than 30%), and requirement for ECMO prior to transplantation. The pre-operative recipient related risk-factors in Buchans meta-analysis was usage of amiodarone pre-Tx, an increase in creatinine levels, pre-Tx VAD or ECMO, where the results certainty regarding pre-tx VAD or ECMO was deemed moderate due to high inconsistency in-between the studies. We found that pre-Tx VAD was associated with PGD in an age- and sex-adjusted analyses. However, in our analysis neither ECMO nor pre-Tx

TABLE 4 Postoperative data.

Variable	Total N = 646	No PGD N = 580	PGD N = 66	No PGD vs. PGD p-value	PGD-LV N = 38	PGD-RV N = 28
Mechanical ventilation time (h)	88.9 ± 229.7 10 (0–1906) n = 629	61.6 ± 166.3 9 (0–1531) n = 567	338.5 ± 465.1 126 (2–1906) n = 62	<0.0001	306.0 ± 499.3 79 (2–1906) n = 35	380.6 ± 422.3 255 (5–1691) n = 27
Postoperative MCS				<0.0001		
No	576 (91.3%)	561 (99.1%)	15 (23.1%)		6 (16.2%)	9 (32.1%)
Yes	55 (8.7%)	5 (0.9%)	50 (76.9%)		31 (83.8%)	19 (67.9%)
Missing	15	14	1		1	0
ICU time (days)	8.9 ± 14.6 4 (0–219) n = 607	7.7 ± 12.9 4 (0–219) n = 561	23.4 ± 23.6 13 (1–81) n = 46	<0.0001	23.0 ± 26.9 10 (1–81) n = 23	23.9 ± 20.4 20 (3–77) n = 23
In-hospital stay (days)	32.1 ± 17.7 29 (0–153) n = 640	32.0 ± 15.6 29 (0–153) n = 574	33.2 ± 30.9 26 (0–141) n = 66	0.08	26.5 ± 32.0 16 (0–141) n = 38	42.4 ± 27.3 33 (1–100) n = 28
Re-operation at first admission				<0.0001		
No	426 (68.6%)	408 (73.1%)	18 (28.6%)		9 (25.0%)	9 (33.3%)
Yes	195 (31.4%)	150 (26.9%)	45 (71.4%)		27 (75.0%)	18 (66.7%)
Missing	25	22	3		2	1
Inotrope score	39.4 ± 35.1 31.1 (0.0–186.0) n = 615	38.3 ± 34.5 29.0 (0.0–186.0) n = 556	49.8 ± 38.6 42.5 (3.2–161.3) n = 59	0.012	49.7 ± 37.2 42.5 (3.2–143.3) n = 35	50.0 ± 41.3 44.9 (3.5–161.3) n = 24
Postoperative dialysis				<0.0001		
No postoperative dialysis	490 (78.4%)	458 (81.8%)	32 (49.2%)		20 (54.1%)	12 (42.9%)
Postoperative dialysis	135 (21.6%)	102 (18.2%)	33 (50.8%)		17 (45.9%)	16 (57.1%)
	21	20	1		1	0
Postoperative sepsis				<0.0001		
No	562 (89.6%)	518 (91.5%)	44 (72.1%)		26 (78.8%)	18 (64.3%)
Yes	65 (10.4%)	48 (8.5%)	17 (27.9%)		7 (21.2%)	10 (35.7%)
Missing	19	14	5		5	0

Data are presented as mean ± standard deviation, median (range) and number of observations, or number (percentage).

For test between two groups with respect to dichotomous variables Fisher's exact test was used, for non-ordered categorical.

variables Chi-square test, and for continuous variables Mann-Whitney U-test.

PGD, primary graft dysfunction; LV, left ventricle; RV, right ventricle; MCS, mechanical circulatory support; ICU, intensive care unit.

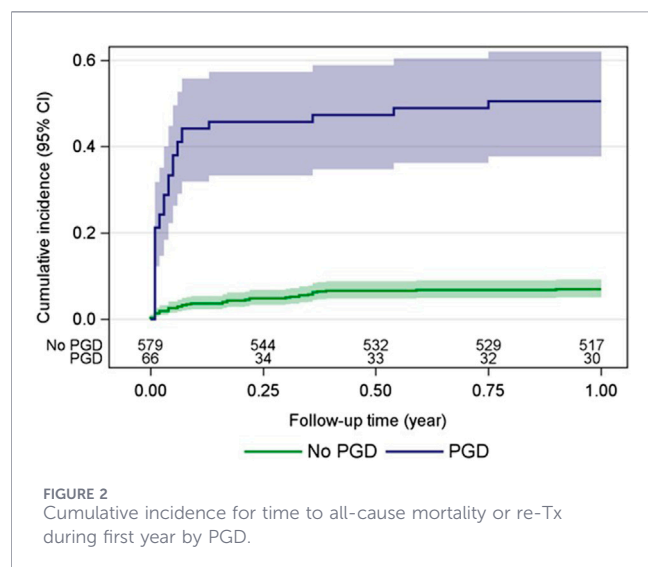
VAD were related to increased risk of PGD in the multivariable analyses, in the presence of other strong association variables, most likely related to a type-2-error, indicating a small cohort. Nonetheless, decreased kidney function was in both our, Buchans and Singh et al's studies significantly related to increased risk of PGD [7, 13].

Part of the criteria for determining PGD is the inotropic score. To differentiate between mild and moderate PGD, a threshold of 10 is required. The fact that we did not diagnose anyone in our population with mild PGD-LV may be since this threshold does not adequately reflect the clinical reality observed in post-transplant care. In practice, early and aggressive use of inotropic support within the first 24 h post-transplantation appears to be common, resulting in most patients exhibiting

inotropic scores above 10, regardless of their PGD status. This trend is evident in the data presented in Table 4, where even non-PGD patients demonstrate a mean inotropic score of 29, well above the current threshold for moderate PG. The matter was also discussed by Kaye et al, suggesting removing norepinephrine all together from the score to prevent up grading patients from mild to moderate PGD, something that needs to be discussed further [18].

Limitations and strengths

The limitations include the retrospective nature, some missing data and limited information from echocardiography that was simply not used routinely in the early eras of HTx. Furthermore, in the current



study moderate PGD were diagnosed primarily based on hemodynamic criteria as echocardiography within 24 h of transplantation was usually not available. Thus, the incidence of moderate PGD could be underestimated leading to a PGD population consisting of more severe forms of PGD which in turn may have overestimated the mortality [14]. Furthermore, the lack of relevant donor data prevents us from investigating mismatch issues. In addition, the study spans many decades without detailing such things as preservation techniques and perioperative management. Finally the study has been worked up over several years but does not take into account the new and modified PGD definition.

However, the strength of this study is the consecutively transplanted and followed population, with all charts manually checked for PGD retrospectively, in a system where all patients can be tracked according to social security numbers. There are therefore no patients lost to follow-up.

In conclusion, primary graft dysfunction, using the ISHLT 2014 definitions, was a frequent and severe complication following HTx, characterized by poor prognosis in terms of both 30-day and 1-year survival rates. Four independent predictors for PGD were pre-HTx dialysis, pre-transplant arrhythmias, inverse height association and longer ECC time. However, there were no donor variables identified as predictors for PGD. However, caution in interpretations is encouraged since cohort is relatively small.

Data availability statement

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

Ethics statement

The study was approved by the Swedish National Research Ethics Committee, following the Helsinki Declaration (Diary number 728-12, amendment 2020-04281). Being a retrospective

study, patient consent was not deemed necessary and waived by the ethical committee.

Author contributions

IL collected all study data, performed data curation, and drafted the manuscript. AP conducted the statistical analyses and contributed to data interpretation. JO, AW, and MT provided critical feedback, contributed with ideas, and revised the manuscript for important intellectual content. GD supervised the project, contributed to study conception and design, and critically reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

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Supplementary material

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References

- Dellgren G, Westerlind A, Liden H, Gabel J, Bartfay SE, Bollano E, et al. Continuous improvement in outcome after heart transplantation - long-term follow-up after three decades of experience. *Int J Cardiol* (2017) 231:188–94. doi:10.1016/j.ijcard.2016.12.186
- Dellgren G, Geiran O, Lemstrom K, Gustafsson F, Eiskjaer H, Koul B, et al. Three decades of heart transplantation in scandinavia: long-term follow-up. *Eur J Heart Fail* (2013) 15(3):308–15. doi:10.1093/eurjhf/hfs160
- Khush KK, Cherikh WS, Chambers DC, Harhay MO, Hayes D, Jr., Hsich E, et al. The international thoracic organ transplant registry of the international society for heart and lung transplantation: thirty-sixth adult heart transplantation report - 2019; focus theme: donor and recipient size match. *J Heart Lung Transpl* (2019) 38(10):1056–66. doi:10.1016/j.healun.2019.08.004
- Khush KK, Hsich E, Potena L, Cherikh WS, Chambers DC, Harhay MO, et al. The international thoracic organ transplant registry of the international society for heart and lung transplantation: thirty-eighth adult heart transplantation report - 2021; focus on recipient characteristics. *J Heart Lung Transpl* (2021) 40(10):1035–49. doi:10.1016/j.healun.2021.07.015
- Iyer A, Kumarasinghe G, Hicks M, Watson A, Gao L, Doyle A, et al. Primary graft failure after heart transplantation. *J Transpl* (2011) 2011:175768. doi:10.1155/2011/175768
- Lund LH, Khush KK, Cherikh WS, Goldfarb S, Kucheryavaya AY, Levvey BJ, et al. The registry of the international society for heart and lung transplantation: thirty-fourth adult heart transplantation Report-2017; focus theme: allograft ischemic time. *J Heart Lung Transpl* (2017) 36(10):1037–46. doi:10.1016/j.healun.2017.07.019
- Buchan TA, Moayed Y, Truby LK, Guyatt G, Posada JD, Ross HJ, et al. Incidence and impact of primary graft dysfunction in adult heart transplant recipients: a systematic review and meta-analysis. *J Heart Lung Transpl* (2021) 40(7):642–51. doi:10.1016/j.healun.2021.03.015
- Hong KN, Iribarne A, Worku B, Takayama H, Gelijns AC, Naka Y, et al. Who is the high-risk recipient? Predicting mortality after heart transplant using pretransplant donor and recipient risk factors. *Ann Thorac Surg* (2011) 92(2):520–7. doi:10.1016/j.athoracsurg.2011.02.086
- Russo MJ, Iribarne A, Hong KN, Ramlawi B, Chen JM, Takayama H, et al. Factors associated with primary graft failure after heart transplantation. *Transplantation* (2010) 90(4):444–50. doi:10.1097/TP.0b013e3181e6f1eb
- Segovia J, Cosio MD, Barcelo JM, Bueno MG, Pavia PG, Burgos R, et al. RADIAL: a novel primary graft failure risk score in heart transplantation. *J Heart Lung Transpl* (2011) 30(6):644–51. doi:10.1016/j.healun.2011.01.721
- Young JB, Hauptman PJ, Naftel DC, Ewald G, Aaronson K, Dec GW, et al. Determinants of early graft failure following cardiac transplantation, a 10-year, multi-institutional, multivariable analysis. *J Heart Lung Transpl* (2001) 20(2):212. doi:10.1016/s1053-2498(00)00460-5
- Kobashigawa J, Zuckermann A, Macdonald P, Leprince P, Esmailian F, Luu M, et al. Report from a consensus conference on primary graft dysfunction after cardiac transplantation. *J Heart Lung Transpl* (2014) 33(4):327–40. doi:10.1016/j.healun.2014.02.027
- Avtaar Singh SS, Banner NR, Rushton S, Simon AR, Berry C, Al-Attar N. ISHLT primary graft dysfunction incidence, risk factors, and outcome: a UK national study. *Transplantation* (2019) 103(2):336–43. doi:10.1097/TP.0000000000002220
- Dronavalli VB, Rogers CA, Banner NR. Primary cardiac allograft dysfunction-validation of a clinical definition. *Transplantation* (2015) 99(9):1919–25. doi:10.1097/TP.0000000000000620
- Nicoara A, Ruffin D, Cooter M, Patel CB, Thompson A, Schroder JN, et al. Primary graft dysfunction after heart transplantation: incidence, trends, and associated risk factors. *Am J Transpl* (2018) 18(6):1461–70. doi:10.1111/ajt.14588
- Rhee Y, Kim HJ, Kim JJ, Kim MS, Lee SE, Yun TJ, et al. Primary graft dysfunction after isolated heart Transplantation- incidence, risk factors, and clinical implications based on a single-center experience. *Circ J* (2021) 85(9):1451–9. doi:10.1253/circj.CJ-20-0960
- D'Alessandro D, Schroder J, Meyer DM, Vidic A, Shudo Y, Silvestry S, et al. Impact of controlled hypothermic preservation on outcomes following heart transplantation. *J Heart Lung Transpl* (2024) 43(7):1153–61. doi:10.1016/j.healun.2024.03.010
- Kaye DM, Kure CE, Wallinder A, McGiffin DC. Limitations of the inotrope score use as a measure of primary graft dysfunction. *J Heart Lung Transpl* (2024). 44(2):289–292. doi:10.1016/j.healun.2024.10.002



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Postoperative detection of *Klebsiella pneumoniae* in clinical cultures and recurrent primary sclerosing cholangitis after liver transplantation: a single-center retrospective cohort study

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Recurrent primary sclerosing cholangitis (rPSC) remains a significant challenge. Although *Klebsiella pneumoniae* (Kp) has been implicated in PSC pathogenesis, its association with rPSC remains unclear. We retrospectively analyzed 62 patients who underwent LT for PSC at our institution between 1996 and July 2024 and survived for more than 1 year. We assessed the association between Kp detection in postoperative clinical cultures and rPSC. rPSC was observed in 22 of 62 patients (35.5%). Kp was detected more frequently in the rPSC group than in the non-rPSC group (31.8% vs. 7.5%). In multivariate Cox regression analysis, Kp detection in clinical cultures (Hazard ratio: 3.83, 95% confidence interval: 1.44–10.21, $p = 0.007$) and identification of three or more bacterial species in clinical cultures (Hazard ratio: 0.31, 95% confidence interval: 0.12–0.78, $p = 0.01$) were independently associated with rPSC. Recurrence-free survival was significantly shorter in patients with Kp detection (10-year recurrence-free survival: 28% with Kp vs. 56.7% without Kp, $p = 0.03$). These findings suggest a potential association between postoperative Kp detection and rPSC.

KEYWORDS

clinical cultures, *Klebsiella pneumoniae*, liver transplantation, primary sclerosing cholangitis, recurrence of primary sclerosing cholangitis

Introduction

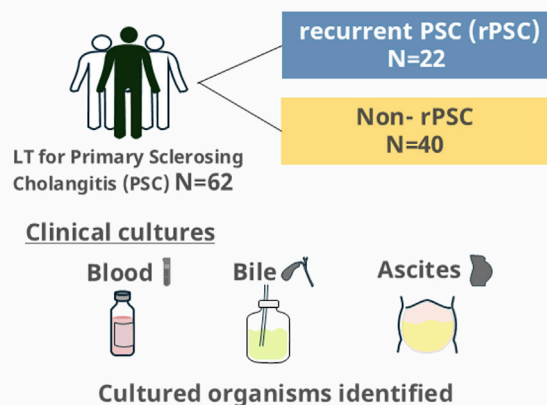
Primary sclerosing cholangitis (PSC) is a progressive cholestatic disease characterized by chronic inflammation and fibrosis of the intrahepatic and extrahepatic bile ducts, ultimately leading to cirrhosis and liver failure. Currently, there is no effective medical therapy, and liver transplantation (LT) remains the only curative treatment option [1].

Recurrence of the primary disease, termed recurrent PSC (rPSC), may occur after LT and is associated with impaired graft function and adverse long-term outcomes [1]. Various risk factors for rPSC have been reported, including active inflammatory bowel disease [2, 3], reduction of immunosuppressive therapy [4], older donor age [4, 5], a high preoperative Model for End-Stage Liver Disease (MELD) score [5], postoperative biliary complications [6], living donation from a first-degree relative [7], and cytomegalovirus (CMV) disease

Postoperative detection of *Klebsiella pneumoniae* in Clinical Cultures and Recurrent Primary Sclerosing Cholangitis After Liver Transplantation

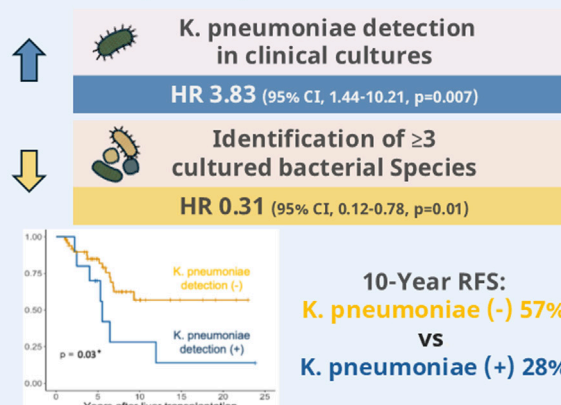
STUDY DESIGN

Single-center, Retrospective cohort



FINDINGS

Variables independently associated with rPSC



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GRAPHICAL ABSTRACT

within 3 months post transplantation [7]. However, the reproducibility of these findings and the underlying mechanisms have yet to be clarified.

In recent years, increasing attention has been paid to the role of gut microbiota, particularly *Klebsiella pneumoniae* (Kp), in the pathogenesis of PSC [8]. Nakamoto et al. [8] reported that in a PSC mouse model, Kp disrupts the intestinal barrier, triggers immune responses via Th17 cells, and contribute to hepatic fibrosis. Hole et al. [9] analyzed colonic mucosal biopsy specimens from rPSC patients and demonstrated that these patients exhibited significantly greater dysbiosis compared with non-recurrent patients. They also reported that, although the difference was not significant, the abundance of *Klebsiella* genus was higher in rPSC patients than in non-recurrent patients. As Hole et al. [9] investigated *Klebsiella* at the genus level, the relationship between Kp and rPSC remains to be elucidated.

The aim of the present study was to investigate the association between Kp detection in postoperative clinical cultures and rPSC after LT.

Patients and methods

Patients and data collection

This study included 76 patients who underwent their first LT for PSC at a tertiary referral center between January 1996 and July 2024. For the analysis of rPSC, 12 patients who died within 1 year post-transplant and two recurrent cases for whom sufficient clinical information could not be obtained were excluded. Ultimately, 62 patients were enrolled in the final analysis. The study protocol was approved by the institutional ethics committee (No. R1473-8) and performed in compliance with the Helsinki Declaration.

Demographic characteristics, perioperative findings, and long-term outcomes were retrospectively collected from medical records. In addition to risk factors for rPSC, we collected microbiological and treatment-related variables, including the number of submitted culture tests, the types of organisms detected, the number of positive detections, and antimicrobial use. Data on first-time liver transplantation cases were included in the analysis.

Definition of rPSC, assessment of Kp

rPSC was defined according to the criteria proposed by Graziadei et al [10]. Specifically, patients with a preoperative diagnosis of PSC were considered to have recurrence if, at 90 days or more after LT, they exhibited either multiple intrahepatic bile duct strictures on cholangiography or magnetic resonance cholangiopancreatography (MRCP), or histological findings consistent with fibrous cholangitis or fibro-obliterative lesions. Other potential causes, such as ischemic cholangitis,

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; DDLT, deceased donor liver transplantation; HLA, human leukocyte antigen; HR, hazard ratio; IQR, interquartile range; Kp, *Klebsiella pneumoniae*; LDLT, living donor liver transplantation; LT, liver transplantation; MELD, Model for End-Stage Liver Disease; MNZ, metronidazole; MRCP, magnetic resonance cholangiopancreatography; OS, overall survival; PGS, primary graft survival; PSC, primary sclerosing cholangitis; rPSC, recurrent primary sclerosing cholangitis; RFS, recurrence-free survival; TEIC, teicoplanin; UC, ulcerative colitis; VCM, vancomycin.

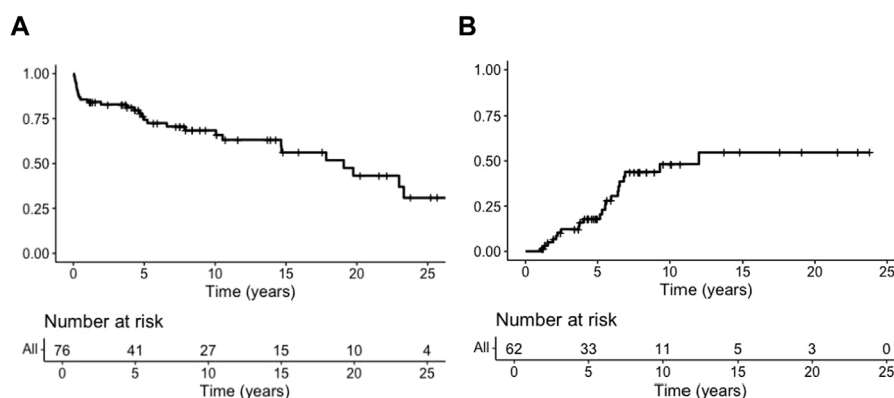


FIGURE 1
Patient and graft outcomes after liver transplantation for primary sclerosing cholangitis (PSC) (n = 76). **(A)** Overall patient survival. **(B)** Cumulative incidence of recurrent primary sclerosing cholangitis (rPSC).

chronic ductopenic rejection, and anastomotic stricture within 90 days post-transplantation, were excluded.

For Kp detection in rPSC cases, Kp positivity was defined as those with Kp identified in blood, bile, or ascitic fluid cultures obtained between LT and rPSC diagnosis, whereas in non-rPSC cases, as those with Kp identified in same clinical cultures between LT and the end of follow-up. The same criteria were applied to the evaluation of other bacterial species and the number of clinical culture tests performed. Only clinical cultures with species-level identification were included; those identified only to the genus level were excluded.

Operative procedure and management

All procedures and postoperative management followed the standard protocol of our institution in all cases. Roux-en-Y hepaticojejunostomy was the standard biliary reconstruction method for PSC cases. Biliary stents were placed intraoperatively and externally drained through the blind end of the duodenum. Patients were discharged with the stent in place, which was removed under fluoroscopic guidance after approximately 3 months if no complications occurred. Preoperative prophylactic antibiotics typically included ampicillin and second-generation cephalosporins. If preoperative clinical cultures were positive, antibiotic selection was adjusted based on sensitivity testing. Antibiotics were continued for 2 days after LT and discontinued if no signs of infection were observed. When infection was suspected, based on fever, elevated liver enzymes, or inflammatory markers, blood, bile, ascites, and urine cultures were obtained, and empirical antibiotics administered. In addition, clinical cultures were submitted at the surgeons' discretion. The same protocol was applied in the case of readmission due to fever or similar symptoms. Bile samples were collected aseptically from a stent placed intraoperatively until its removal. Thereafter, further bile samples were collected via endoscopic retrograde cholangiopancreatography or percutaneous transhepatic cholangial drainage. Ascitic fluid was collected via intraperitoneal drains or paracentesis. Immunosuppressive regimens consisted of dual or triple therapy combining a calcineurin inhibitor with steroids, mycophenolate mofetil, and everolimus, which were administered

from 1 month after transplantation. Tapering of immunosuppressive agents during the maintenance phase was performed at the surgeon's discretion. Rituximab was administered as induction therapy for ABO-incompatible living donor liver transplantation (LDLT) or in patients with preoperatively strong positive donor-specific antibodies. Histological diagnoses of acute and chronic rejection were made according to the Banff criteria.

Statistical analysis

Continuous variables were expressed as medians with interquartile ranges (IQR) and compared using the Mann-Whitney U test. Categorical variables were compared using the chi-square test or Fisher's exact test. Kaplan-Meier method and log-rank test were used for survival analyses. Factors associated with rPSC were evaluated using a Cox proportional hazards model, with death or re-transplantation treated as censoring events. Cut off values for continuous variables were determined by receiver operating characteristic curve analysis and the Youden index or based on previously published studies and clinical relevance. Variables considered clinically and statistically relevant were included in the multivariable analysis, with model selection via the backward stepwise method. In addition, bootstrap resampling with 1,000 iterations was conducted to assess the robustness and stability of the multivariable model estimates. All statistical analyses were performed using R software (version 4.3.0).

Results

Liver transplantation outcomes for PSC at our institution

Among 76 patients who underwent initial LT for PSC at our institution during the study period, the median age was 36.0 years (IQR, 25.8–49.0 years), 66 were adults (86.8%), and 36 were male (47.4%). LDLT was performed in 64 cases (84.2%), deceased donor liver transplantation in eight cases (10.5%), and domino

TABLE 1 Patient characteristics.

Variables	rPSC (n = 22)	Non-rPSC (n = 40)	p
Recipient factors			
Age (years old)	37.0 (26.5–47.5)	41.0 (27.5–50)	0.70
Adult/Pediatric	20/2	36/4	>0.99
Sex, male/Female	8/14	23/17	0.19
MELD score	18.5 (12.3–21.8)	15.0 (9.0–20.3)	0.26
Mayo PSC risk score	3.0 (2.1–3.4)	2.8 (1.9–3.6)	0.89
Child–Pugh class A/B/C	3/8/11	5/13/22	0.93
History of UC (yes/no)	8/14	19/21	0.56
HLA-DR15 (yes/no)	7/15	21/19	0.19
Donor factors			
Age (years old)	48.0 (37.0–55.0)	41.0 (33.0–51.0)	0.12
Sex, M/F	12/10	28/12	0.35
Sex mismatch (yes/no)	12/10	23/17	>0.99
ABO-incompatible (yes/no)	3/19	3/37	0.66
First degree relationship (yes/no)	14/8	24/16	>0.99
HLA mismatch number (≥4/<4)	16/5	31/6	0.72
HLA-DR15 (yes/no)	12/9	13/24	0.18
Operative factors			
LDLT/DDLT/Domino	18/3/1	32/5/3	0.90
Operation time (min)	685 (561–828)	715 (654–891)	0.29
Estimated blood loss (mL)	2,400 (1,430–4,980)	2,900 (2010–5,530)	0.67
Cold ischemic time (min)	90.0 (58.0–227)	87.0 (43.5–222)	0.61
Warm ischemic time (min)	40.0 (33.5–43.8)	36.0 (32.3–39.0)	0.30
Hepaticojejunostomy/duct-to-duct	1/25	1/38	>0.99
Postoperative factors			
Major complication (yes/no)	6/16	10/30	>0.99
Biliary tract complication (yes/no)	4/18	7/33	>0.99
CMV infection (yes/no)	8/14	20/20	0.44
Acute rejection (yes/no)	10/12	18/22	>0.99
Active UC after LT (yes/no)	10/12	6/34	0.02*
Immunosuppression 1 year after LT (single/dual/triple)	5/10/7	3/16/21	0.14
Cultures and antibiotics			
No. of total clinical cultures (blood, biliary juice, ascites)	26.5 (11.3–46.3)	27.5 (19–35.5)	0.66
≥3 bacterial species identified in clinical cultures (yes/no)	12/10	26/14	0.59
Kp (+) in clinical cultures after LT (yes/no)	7/15	3/37	0.03*
Oral antibiotic treatment with MNZ and VCM (yes/no)	5/17	5/35	0.31
Intravenous antibiotic therapy with MNZ, VCM, and TEIC (yes/no)	9/13	22/18	0.56

Continuous variables are described as median (interquartile range [IQR]). RPSC, Recurrent primary sclerosing cholangitis; MELD score, Model for end-stage liver disease score; UC, Ulcerative colitis; HLA, Human leukocyte antigen; LDLT, Living donor liver transplantation; DDLT, Deceased donor liver transplantation; CMV, Cytomegalovirus; LT, Liver transplantation; Kp, *Klebsiella pneumoniae*; MNZ, Metronidazole; VCM, Vancomycin; TEIC, Teicoplanin. *p values <0.05.

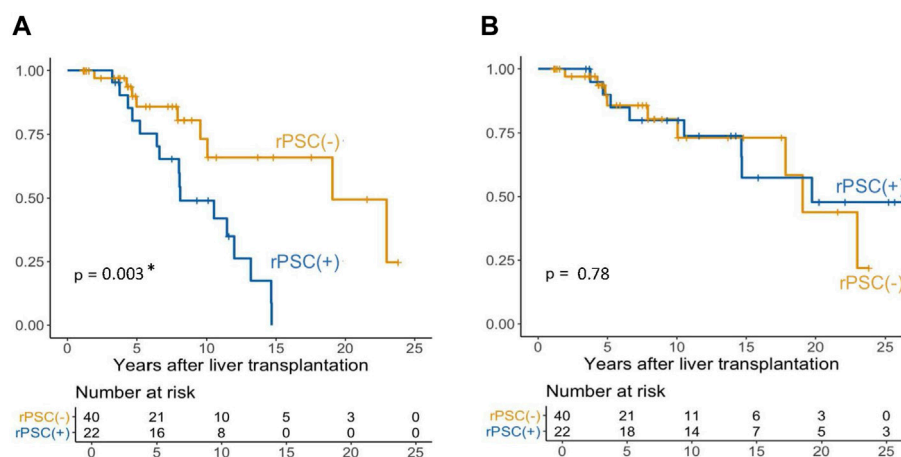


FIGURE 2

Patient and graft outcomes after liver transplantation for primary sclerosing cholangitis (PSC) (n = 62). (A) Graft survival stratified by the presence or absence of recurrent PSC (rPSC). (B) Overall patient survival stratified by the presence or absence of rPSC. *p values <0.05. RPSC: Recurrent primary sclerosing cholangitis.

transplantation in four cases (5.3%). ABO-incompatible LT was performed in eight cases (10.5%). The median follow-up period was 75.1 months (IQR, 37.9–168.0 months; mean, 105.1 months). rPSC occurred in 24 patients (31.6%), with a median time to recurrence of 55.0 months (IQR, 26.4–76.9 months). Kaplan–Meier curves for overall survival (OS) and recurrence rates among the 76 patients are shown in Figure 1. The 5-, 10-, and 15-year OS rates were 74.3%, 68.4%, and 56.1%, respectively (Figure 1A). The 5-, 10-, and 15-year recurrence rates were 20.9%, 50.1%, and 56.3%, respectively (Figure 1B). Primary graft survival (PGS) rates were 69.6% at 5 years, 50.1% at 10 years, and 25.0% at 15 years.

Patient demographics

The background characteristics of the 62 patients included in the recurrence analysis are summarized in Table 1. rPSC occurred in 22 patients (35.5%), and 40 patients (64.5%) were in the non-recurrent group. The median age in the rPSC group was 37.0 years (IQR, 26.5–47.5), and 41.0 years (IQR, 27.5–50.0) in the non-rPSC group, with no significant difference. There were no significant differences between both groups in the proportion of adult cases, sex, preoperative MELD score, Mayo PSC risk score, Child–Pugh classification, history of ulcerative colitis (UC), or human leukocyte antigen (HLA)-DR15 positivity. Regarding donor factors, the median donor age was 48.0 years (IQR, 37.0–55.0) in the rPSC group and 41.0 years (IQR, 33.0–51.0) in the non-rPSC group ($p = 0.12$), with no significant difference. There were no significant differences between both groups in sex mismatch, ABO-incompatible transplantation, first-degree-related donors, or number of HLA mismatches. The presence of donor HLA-DR15 also did not significantly differ between the groups (rPSC vs. non-rPSC = 12 cases [57.1%] vs. 13 cases [35.1%], $p = 0.18$). Surgical factors, including LDLT, operative time, blood loss, cold ischemia time, warm ischemia time, and biliary reconstruction method, were not significantly different. Postoperative factors, including major complications, biliary complications, CMV infection, acute rejection, and immunosuppressive regimen at

1 year post-LT (alone/dual/triple), also showed no significant differences between the groups. On the other hand, active postoperative UC was significantly more frequent in the rPSC group (rPSC vs. non-rPSC = 10 cases [45.5%] vs. six cases [15.0%], $p = 0.02$). As for bacterial cultures, the median number of clinical cultures submitted postoperatively (including blood, bile, and ascitic fluid) was 26.5 (IQR: 11.3–46.3) in the rPSC group and 27.5 (IQR: 19.0–35.5) in the non-rPSC group, with no significant difference ($p = 0.66$). However, the detection rate of Kp was significantly higher in the rPSC group (seven cases [31.8%] vs. three cases [7.5%], $p = 0.03$). No significant differences were observed between the groups in the identification of three or more bacterial species or the use of antibiotics.

RPSC impairs primary graft survival but not OS

Kaplan–Meier curves for PGS and OS stratified by recurrence status among the 62 patients are shown in Figure 2. The 5- and 10-year PGS rates in the rPSC group were 80.4% and 49.0%, respectively, with a median graft survival of 97.2 months. In the non-rPSC group, the 5- and 10-year PGS rates were 85.9% and 73.3%, respectively, with a median graft survival of 229.2 months. PGS was significantly lower in the rPSC group ($p = 0.003$, Figure 2A). OS stratified by recurrence status showed no significant difference between both groups: in the rPSC group, 5-, 10-, and 15-year OS rates were 90.0%, 80.0%, and 57.4%, respectively, with a median survival of 236.4 months; in the non-rPSC group, the corresponding rates were 85.8%, 80.4%, and 73.1%, with a median survival of 229.2 months ($p = 0.78$, Figure 2B).

Kp detection and lower diversity of cultured organisms associated with rPSC

Table 2 summarizes the results of univariate and multivariate Cox proportional hazards models assessing variables associated

TABLE 2 Univariate and multivariate Cox proportional hazards models for time to recurrence of primary sclerosing cholangitis after liver transplantation.

Variables	Univariate analysis		Multivariate analysis	
	HR 95% CI	p	HR 95%CI	p
Recipient factors				
Age <20	0.90 0.23–4.28	>0.99		
Sex, Male	0.54 0.22–1.28	0.16		
Mayo PSC risk score >2	1.02 0.38–2.79	0.96		
HLA-DR15	0.70 0.28–1.72	0.44		
Donor factors				
Age ≥45	2.22 0.91–5.42	0.08		
Sex mismatch	1.02 0.44–2.36	0.97		
ABO-incompatible	1.84 0.53–6.31	0.34		
First degree relationship	0.99 0.41–2.38	0.98		
HLA loci mismatch number ≥4	0.77 0.28–2.12	0.61		
HLA-DR15	2.17 0.90–5.22	0.08		
Operative factors				
LDLT	0.67 0.27–1.70	0.40		
Operation time [min] ≥ 600	0.43 0.16–1.13	0.09		
Cold ischemic time [min] ≥ 60	1.20 0.42–3.41	0.73		
Postoperative factors				
Biliary tract complication	0.71 0.24–2.10	0.53		
CMV infection	0.62 0.26–1.49	0.29		
Immunosuppressant 1 year after LT (alone)	1.36 0.50–3.69	0.55		
Immunosuppression 1 year after LT (triple)	0.93 0.38–2.30	0.87		
Active UC after LT	2.53 1.08–5.88	0.03*	2.37 0.99–5.64	0.052
Cultures and antibiotics				
≥3 bacterial species identified in clinical cultures	0.37 0.15–0.88	0.02*	0.31 0.12–0.79	0.01*
Kp (+) in clinical cultures	2.64 1.06–6.54	0.03*	3.83 1.44–10.21	0.007*
Oral antibiotic treatment with MNZ or VCM	1.20 0.44–3.26	0.72		

HR, Hazard ratio; 95%CI, 95% confidence intervals; HLA, Human leukocyte antigen; LDLT, Living donor liver transplantation; DDLT, Deceased donor liver transplantation; CMV, Cytomegalovirus; LT, Liver transplantation; UC, Ulcerative colitis; Kp, *Klebsiella pneumoniae*. *p values <0.05.

with rPSC. In the univariate analysis, postoperative active UC ($p = 0.03$), Kp detection ($p = 0.03$) and the identification of three or more bacterial species in clinical ($p = 0.02$) were significantly associated with rPSC. In the multivariate analysis, Kp detection (HR: 3.83, 95% CI: 1.44–10.21, $p = 0.007$) and three or more bacterial species in clinical cultures (HR: 0.31, 95% CI: 0.12–0.79, $p = 0.01$) were independently associated with rPSC. Even when the multivariate analysis was restricted to culture data obtained within 1 year after LT, similar results were observed. Kp detection (HR: 3.47, 95% CI: 1.26–9.54, $p = 0.02$), the identification of three or more bacterial species in clinical cultures (HR: 0.35, 95% CI: 0.14–0.86, $p = 0.02$), and postoperative active UC (HR 2.52, 95% CI 1.05–6.07, $p = 0.04$) remained independently associated with rPSC. Bootstrap resampling analysis (1,000 iterations) showed similar results, with Kp detection (median HR: 4.10, 95% CI: 1.57–14.71), identification

of three or more cultured bacterial species (median HR: 0.29, 95% CI: 0.09–0.73), and postoperative active UC (median HR: 2.43, 95% CI: 0.89–7.53).

Table 3 presents an exploratory analysis of bacterial species detected in at least three patients. Only Kp showed a significant association with rPSC ($p = 0.03$). Supplementary Table 1 lists all other bacterial species detected in the study cohort, as well as their respective detection counts.

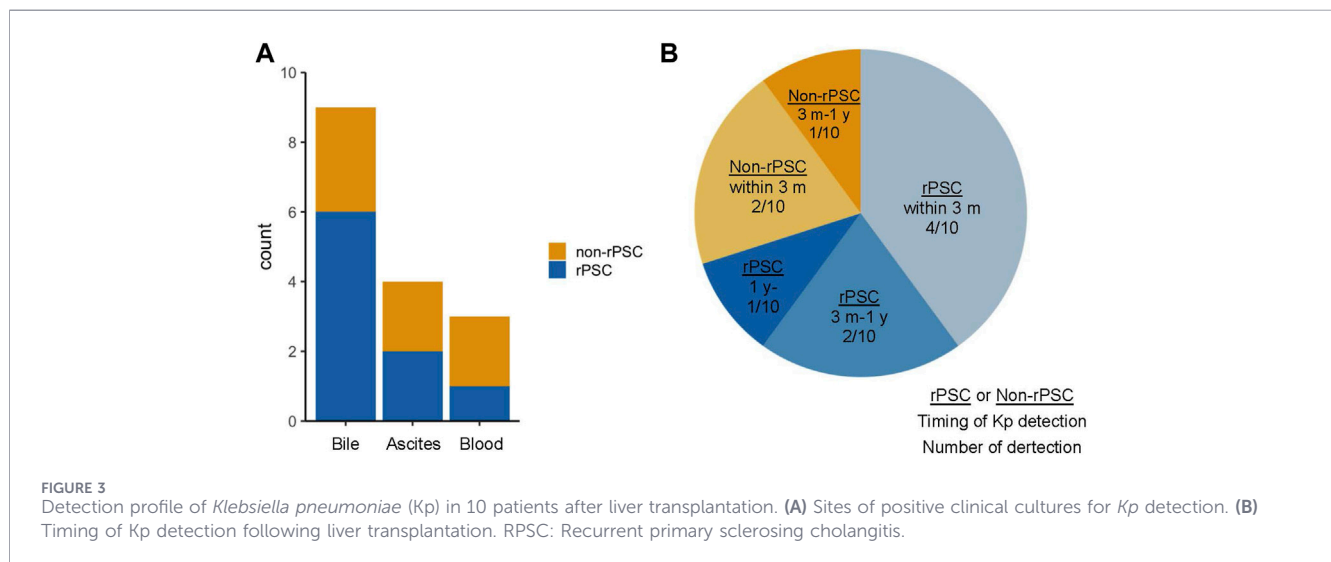
Characteristics and timing of Kp detection and recurrence-free survival

Figure 3 shows the types and timing of Kp detection specimens. Among the 10 patients in whom Kp was detected,

TABLE 3 Univariate Cox proportional hazards models exploratorily evaluating the association between individual bacterial species detected in postoperative clinical cultures and recurrence of primary sclerosing cholangitis after liver transplantation.

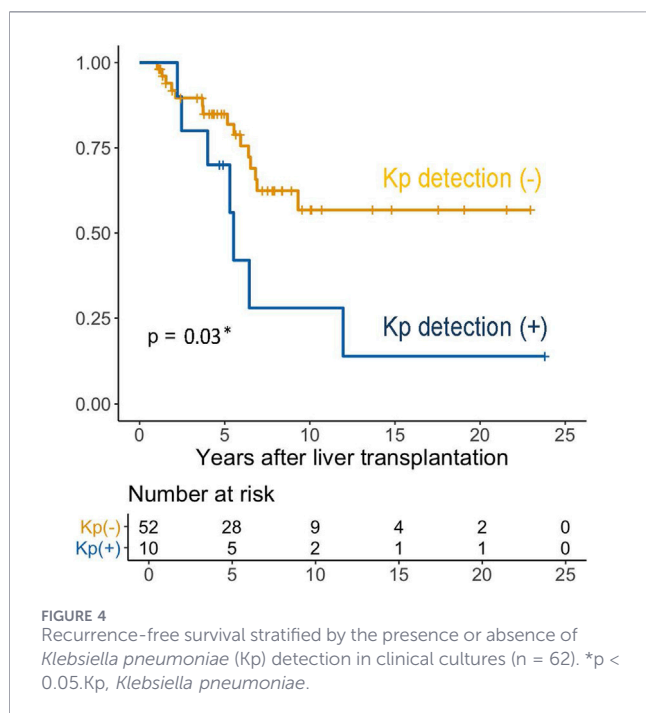
Variables	rPSC (n = 22)	Non-rPSC (n = 40)	Univariate analysis	
			HR 95%CI	p
<i>Klebsiella</i>				
<i>Klebsiella pneumoniae</i> (yes/no)	7/15	3/37	2.64 1.06–6.54	0.03*
<i>Klebsiella oxytoca</i>	2/20	1/39	1.81 0.42–7.82	0.43
<i>Citrobacter freundii</i>	2/20	2/38	1.08 0.25–4.62	0.92
<i>Escherichia coli</i>	3/19	8/2	0.56 0.16–1.89	0.35
<i>Enterobacter cloacae</i>	8/14	10/30	1.02 0.42–2.46	0.97
<i>Enterococcus</i>				
<i>Enterococcus avium</i>	2/20	3/37	2.14 0.48–9.53	0.31
<i>Enterococcus faecium</i>	8/14	13/27	0.95 0.40–2.26	0.90
<i>Enterococcus faecalis</i>	4/18	8/32	0.91 0.30–2.71	0.86
<i>Enterococcus gallinarum</i>	4/18	4/36	0.82 0.27–2.46	0.72
<i>Pseudomonas aeruginosa</i>	5/17	11/29	0.61 0.22–1.66	0.33
<i>Staphylococcus</i>				
<i>Staphylococcus aureus</i>	5/17	7/33	1.03 0.38–2.84	0.95
<i>Staphylococcus epidermidis</i>	6/16	11/29	1.10 0.43–2.82	0.84
<i>Staphylococcus haemolyticus</i>	0/22	3/37	-	>0.99
<i>Stenotrophomonas maltophilia</i>	0/22	8/32	-	>0.99

PSC, primary sclerosing cholangitis; HR, hazard ratio; 95%CI, 95% confidence intervals. *p values <0.05.



Kp was found in bile in nine cases, blood in three cases, and ascitic fluid in four cases. In nine of the 10 cases, Kp was detected within 1 year post-LT, and in six cases within 3 months post-LT. Among the 10 patients with Kp detection, Kp was identified from clinical cultures obtained during suspected cholangitis episodes accompanied by symptoms such as fever or abdominal pain and/

or abnormal blood test findings in four of seven rPSC patients and two of three non-rPSC patients. Figure 4 shows recurrence-free survival (RFS) stratified by Kp detection. The 5- and 10-year RFS rates were 70.0% and 28.0% in the Kp (+) group, and 84.9% and 56.7% in the Kp (-) group, respectively. RFS was significantly worse in the Kp (+) group ($p = 0.03$).



Discussion

This retrospective clinical study investigated factors associated with rPSC after LT. To our knowledge, this is the first study to investigate a possible association between rPSC and Kp, which has recently been implicated in the pathogenesis of PSC. The main findings of this study are as follows: 1) rPSC was significantly associated with poorer graft survival, 2) Kp detection and lower diversity of cultured organisms were independently associated with rPSC, and 3) RFS was shorter in patients with Kp detection.

Our findings support previous studies that reported that rPSC is a major determinant of poor PGS [2–7]. Furthermore, several studies have shown that OS may not be compromised even in patients with recurrence if retransplantation is successful [5, 10, 11], and our findings were consistent with these studies. Nevertheless, some patients experience repeated episodes of rPSC and undergo multiple retransplantations, indicating that rPSC remains a significant unresolved challenge.

In the present study, the recurrence rate of PSC after LT among 76 patients was 31.6%. Although surveillance imaging, including MRCP and computed tomography, and liver biopsy were not performed according to a fully standardized protocol, patients underwent repeated examinations during follow-up. During the first 5 years after discharge, the median numbers of imaging and liver biopsy examinations per patient were 2.00 (IQR, 1.00–4.00) and 4.00 (IQR, 2.00–6.75), respectively. Furthermore, the frequencies of imaging and liver biopsy examinations did not significantly differ between the rPSC and non-rPSC groups. Compared with our previous institutional report [12], this suggests an improving trend; however, it remains higher than that reported in other studies [13, 14]. This modest improvement may be partly attributable to recent advances in UC treatment,

which have improved remission rates [15, 16] and contributed to better postoperative disease control. UC has long been recognized as an important factor in PSC pathogenesis [17], and in the present study, postoperative active UC was associated with rPSC in univariate analysis, a finding that is consistent with our previous report. However, it is also notable that several recurrent cases were observed without an apparent association with UC. PSC patients in Japan have a relatively low prevalence of UC [18], highlighting the need to focus on additional factors beyond UC. Genetic factors, such as HLA, have been implicated in previous studies [12, 19]; however, these factors were not identified as independent associated factors in the present analysis. Taken together, the etiology of PSC and rPSC is likely multifactorial [1, 14]. Further investigation of additional contributing factors is warranted.

In the present study, we focused on Kp. Kp is an intestinal bacterium, that has recently been implicated in PSC pathogenesis. Kp detection in clinical cultures was independently associated with rPSC. Nakamoto et al. [8] demonstrated that Kp translocates from the colon to the mesenteric lymph nodes, induces Th17 cells, and contributes to PSC pathogenesis. Based on this evidence, we assessed the detection of bacteria, including Kp, in cultures from blood, bile, and ascitic fluid obtained between LT and the diagnosis of rPSC. Since these sites are normally sterile, bacterial detection may reflect pathological conditions such as infection or bacterial translocation from the gut. Notably, no clear association was observed for other bacterial species in this exploratory analysis, suggesting that Kp may have a distinct association with rPSC. Furthermore, lower diversity of cultured organisms was also independently associated with rPSC. Although culture-based measures do not fully capture the entire microbiome, our findings are consistent with previous reports demonstrating decreased bile microbial diversity in PSC patients [20] and dysbiosis being associated with detection of *Klebsiella* species in patients with rPSC [9]. Collectively, these observations suggest a potential contribution of gut microbiota, including Kp, in the pathogenesis of PSC and rPSC.

The clinical contribution of Kp should be interpreted with caution, as it was not detected in all patients who later developed rPSC. In this study, Kp was detected in only seven of the rPSC cases (31.8%), suggesting that its involvement is limited to a subset of patients. Nevertheless, Kp detection was associated with poorer outcomes. Similar findings were also observed when the analysis was restricted to culture data obtained within 1 year after LT. Furthermore, most Kp detections were from bile cultures obtained within 3 months postoperatively, indicating its potential early marker associated with recurrence risk. From a therapeutic perspective, we also explored the potential preventive effect of perioperative antibiotics. Although Nakamoto et al. [8] reported that oral administration of metronidazole or vancomycin improved hepatic fibrosis in a PSC mouse model, no significant association was observed in our cohort, likely due to the small sample size and limited exposure to oral antibiotics. More recently, bacteriophage therapy targeting Kp was proposed as a potential therapeutic strategy [21]. Further studies are warranted to investigate its clinical applicability in preventing rPSC.

Several limitations should be noted. First, this was a single-center retrospective study; therefore, selection and information biases cannot be excluded. Second, the criteria and timing for submitting culture samples were not standardized. Although similar findings were observed in analyses restricted to culture data obtained within 1 year after LT, potential differences between the rPSC and non-rPSC groups may still have influenced the findings. In addition, some bile and ascitic fluid cultures were obtained from postoperative drainage tubes, and Kp detection in these samples may partly reflect bacterial colonization rather than clinically significant infection. Third, this study does not indicate whether Kp detection is a cause or consequence of rPSC; thus, no definitive causal relationship can be established. Future studies should involve multicenter prospective cohorts with standardized specimen collection protocols and regulated antibiotic use. In addition, stool-based microbiota analysis and molecular or immunological characterization of Kp will be essential to elucidate its direct etiological relevance in rPSC.

In conclusion, this study is the first clinical investigation to suggest a possible association between Kp and rPSC. It represents an important step toward improving our understanding of rPSC and exploring future preventive strategies.

Data availability statement

Data involved in the present study is available from the corresponding author upon request. As the data is sourced from the hospital's database, we are unable to publicly disclose it.

Ethics statement

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

anonymized clinical data, and an opt-out approach was applied. For minors, consent was managed through an opt-out procedure approved by the ethics committee.

Author contributions

Data collection, analysis, and interpretation: JI; Writing – original draft: JI, KS, and TI; Writing – review and editing: KS, TI, SO, DU, TY, HA, RA, SO, KN, YU, TIs, and EH. All authors contributed to the article and approved the submitted version.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2026.16698/full#supplementary-material>

References

1. Manns MP, Bergquist A, Karlsen TH, Levy C, Muir AJ, Ponsioen C, et al. Primary sclerosing cholangitis. *Nat Rev Dis Primers* (2025) 11(1):17. doi:10.1038/s41572-025-00600-x
2. Montano-Loza AJ, Bhanji RA, Wasilenko S, Mason AL. Systematic review: recurrent autoimmune liver diseases after liver transplantation. *Aliment Pharmacol Ther* (2017) 45(4):485–500. doi:10.1111/apt.13894
3. Hildebrand T, Pannicke N, Dechene A, Gotthardt DN, Kirchner G, Reiter FP, et al. Biliary strictures and recurrence after liver transplantation for primary sclerosing cholangitis: a retrospective multicenter analysis. *Liver Transpl* (2016) 22(1):42–52. doi:10.1002/lt.24350
4. Akamatsu N, Hasegawa K, Egawa H, Ohdan H, Yoshizawa A, Kokudo N, et al. Donor age (≥45 years) and reduced immunosuppression are associated with the recurrent primary sclerosing cholangitis after liver transplantation - a multicenter retrospective study. *Transpl Int* (2021) 34(5):916–29. doi:10.1111/tri.13852
5. Gordon FD, Goldberg DS, Goodrich NP, Lok AS, Verna EC, Selzner N, et al. Recurrent primary sclerosing cholangitis in the adult-to-adult living donor liver transplantation cohort study: comparison of risk factors between living and deceased donor recipients. *Liver Transpl* (2016) 22(9):1214–22. doi:10.1002/lt.24496
6. Egawa H, Taira K, Teramukai S, Haga H, Ueda Y, Yonezawa A, et al. Risk factors for recurrence of primary sclerosing cholangitis after living donor liver transplantation: a single center experience. *Dig Dis Sci* (2009) 54(6):1347–54. doi:10.1007/s10620-009-0773-9
7. Egawa H, Ueda Y, Ichida T, Teramukai S, Nakanuma Y, Onishi S, et al. Risk factors for recurrence of primary sclerosing cholangitis after living donor liver transplantation in Japanese registry. *Am J Transpl* (2011) 11(3):518–27. doi:10.1111/j.1600-6143.2010.03402.x
8. Nakamoto N, Sasaki N, Aoki R, Miyamoto K, Suda W, Teratani T, et al. Gut pathobionts underlie intestinal barrier dysfunction and liver T helper 17 cell immune

- response in primary sclerosing cholangitis. *Nat Microbiol* (2019) 4(3):492–503. doi:10.1038/s41564-018-0333-1
9. Hole MJ, Jørgensen KK, Holm K, Braadland PR, Meyer-Myklestad MH, Medhus AW, et al. A shared mucosal gut microbiota signature in primary sclerosing cholangitis before and after liver transplantation. *Hepatology* (2023) 77(3):715–28. doi:10.1002/hep.32773
10. Graziadei IW, Wiesner RH, Batts KP, Marotta PJ, LaRusso NF, Porayko MK, et al. Recurrence of primary sclerosing cholangitis following liver transplantation. *Hepatology* (1999) 29(4):1050–6. doi:10.1002/hep.510290427
11. Catanzaro E, Gringeri E, Cazzagon N, Floreani A, Cillo U, Burra P, et al. Risk factors for recurrence of primary sclerosing cholangitis after liver transplantation: single-center data. *J Pers Med* (2024) 14(3):222. doi:10.3390/jpm14030222
12. Ueda Y, Kaido T, Okajima H, Hata K, Anazawa T, Yoshizawa A, et al. Long-term prognosis and recurrence of primary sclerosing cholangitis after liver transplantation: a single-center experience. *Transpl Direct* (2017) 3(12):e334. doi:10.1097/TXD.0000000000000751
13. Steenstraten IC, Sebib Korkmaz K, Trivedi PJ, Inderson A, van Hoek B, Rodriguez Gironde MDM, et al. Systematic review with meta-analysis: risk factors for recurrent primary sclerosing cholangitis after liver transplantation. *Aliment Pharmacol Ther* (2019) 49(6):636–43. doi:10.1111/apt.15148
14. Karlsen TH, Folseraas T, Thorburn D, Vesterhus M. Primary sclerosing cholangitis - a comprehensive review. *J Hepatol* (2017) 67(6):1298–323. doi:10.1016/j.jhep.2017.07.022
15. Aslam N, Lo SW, Sikafi R, Barnes T, Segal J, Smith PJ, et al. A review of the therapeutic management of ulcerative colitis. *Therap Adv Gastroenterol* (2022) 15:17562848221138160. doi:10.1177/17562848221138160
16. Ungaro R, Colombel JF, Lissos T, Peyrin-Biroulet L. A treat-to-target update in ulcerative colitis: a systematic review. *Am J Gastroenterol* (2019) 114(6):874–83. doi:10.14309/ajg.0000000000000183
17. Buchholz BM, Lykoudis PM, Ravikumar R, Pollok JM, Fusai GK. Role of colectomy in preventing recurrent primary sclerosing cholangitis in liver transplant recipients. *World J Gastroenterol* (2018) 24(28):3171–80. doi:10.3748/wjg.v24.i28.3171
18. Tanaka A, Tazuma S, Okazaki K, Tsubouchi H, Inui K, Takikawa H. Nationwide survey for primary sclerosing cholangitis and IgG4-related sclerosing cholangitis in Japan. *J Hepatobiliary Pancreat Sci* (2014) 21(1):43–50. doi:10.1002/jhbp.50
19. Okamoto T, Okajima H, Kadohisa M, Yamamoto M, Uebayashi EY, Okumura S, et al. Association of primary sclerosing cholangitis with HLA-C*07:02 in a Japanese population. *Liver Int* (2025) 45(11):e70376. doi:10.1111/liv.70376
20. Vaughn BP, Kaiser T, Staley C, Hamilton MJ, Reich J, Graiziger C, et al. A pilot study of fecal bile acid and microbiota profiles in inflammatory bowel disease and primary sclerosing cholangitis. *Clin Exp Gastroenterol* (2019) 12:9–19. doi:10.2147/CEG.S186097
21. Ichikawa M, Nakamoto N, Kreda-Russo S, Weinstock E, Weiner IN, Khabra E, et al. Bacteriophage therapy against pathological *Klebsiella pneumoniae* ameliorates the course of primary sclerosing cholangitis. *Nat Commun* (2023) 14(1):3261. doi:10.1038/s41467-023-39029-9



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Daratumumab as rescue therapy in refractory recurrent FSGS after kidney transplantation: a case series

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KEYWORDS

anti-CD38-antibodies, daratumumab, focal segmental glomerulosclerosis, kidney transplantation, recurrent disease

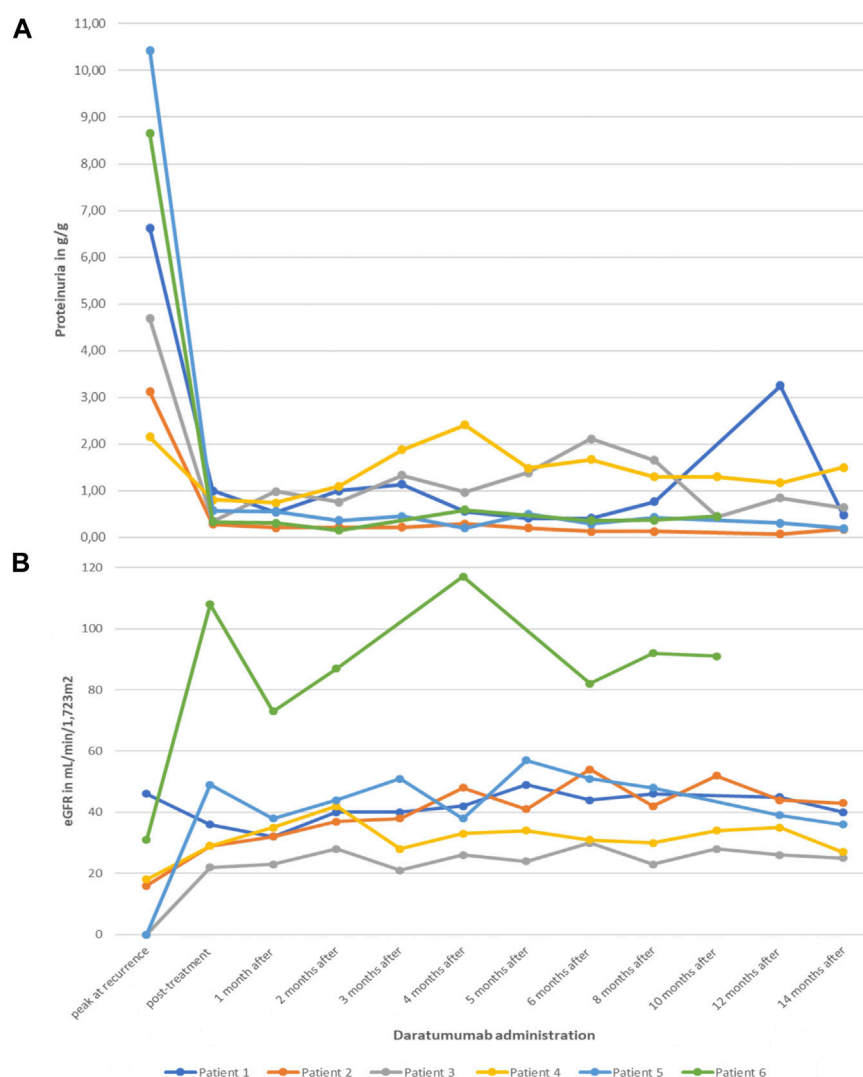
Dear Editors,

Recurrent focal segmental glomerulosclerosis (FSGS) remains one of the most challenging complications after kidney transplantation, frequently leading to early graft dysfunction and premature graft loss [1–3]. Despite advances in immunosuppressive therapy, treatment options remain limited, particularly in patients refractory to standard therapies including plasmapheresis and B-cell-depleting strategies. In this context, plasma cell-targeted therapies such as daratumumab have emerged as a promising strategy in refractory cases [4–7]. However, current evidence is limited to small case series and heterogeneous cohorts, and the effectiveness of daratumumab across different clinical scenarios remains unclear.

We report a single-center case series of six kidney transplant recipients with biopsy-proven recurrent FSGS who were refractory to conventional treatment and received daratumumab as rescue therapy. All patients had kidney failure due to primary FSGS with biopsy-confirmed recurrence after transplantation. Electron microscopy demonstrated a podocytopathy with diffuse podocyte foot process effacement in all cases. Genetic testing using a targeted NGS panel was performed in four patients and did not reveal a genetic cause of FSGS.

All patients received induction therapy with basiliximab followed by standard triple maintenance immunosuppression consisting of tacrolimus, mycophenolate mofetil, and corticosteroids. All patients were dialysis-dependent and anuric prior to transplantation, no pre-emptive transplantations were performed; one patient had previously undergone kidney transplantation. Following biopsy-confirmed recurrence of FSGS, five patients were switched from tacrolimus to cyclosporine. All patients received anti-proteinuric therapy with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers at maximally tolerated doses. One patient additionally received an SGLT2-inhibitor.

Prior to daratumumab, all patients were treated with plasmapheresis and anti-CD20 therapy (rituximab in all cases with escalation to obinutuzumab in one patient), reflecting current standard approaches, but failed to achieve sustained remission. Treatment timing varied, reflecting real-world practice. The interval between initiation of anti-CD20 therapy and the first daratumumab administration was a median of 110 days (IQR 72–133).

**FIGURE 1**

Longitudinal course of proteinuria and graft function during and after multimodal therapy for recurrent post-transplant FSGS. Each line represents an individual patient with recurrent post-transplant FSGS ($n = 6$). **(A)** Urine protein-to-creatinine ratio (UPCR; g/g) values are shown at peak proteinuria at recurrence, after completion of treatment, and during follow-up. Peak values were obtained prior to daratumumab initiation, although some patients were already receiving plasmapheresis or rituximab, with escalation to obinutuzumab in one patient. Post-treatment values were assessed at a median of 16 days (IQR 7–18) after the last daratumumab administration. Four patients achieved complete remission (<0.5 g/g creatinine), and two achieved partial remission (<2 g/g creatinine with $\geq 50\%$ reduction in proteinuria). Patient 1 experienced a relapse 12 months after the last daratumumab dose and received a single additional administration. **(B)** Estimated glomerular filtration rate (eGFR; mL/min/1.73 m²) values are shown at recurrence, after completion of treatment, and during follow-up. eGFR was calculated using the CKD-EPI equation. Treatment consisted of plasmapheresis, rituximab/obinutuzumab, and daratumumab. Follow-up time points at 1, 2, and 3 months and thereafter refer to the interval since the last daratumumab administration.

Daratumumab was administered subcutaneously at a fixed dose of 1800 mg, with patients receiving between three and six administrations. Median follow-up after the last daratumumab administration was 12.3 months (IQR 11–14.7).

Following daratumumab treatment, four patients achieved complete remission and two achieved partial remission, accompanied by stabilization or improvement of graft function. These responses were observed despite prior failure of multimodal therapy and may reflect meaningful treatment effects in a highly refractory population, likely driven by a synergistic effect of B-cell and plasma cell depletion. One patient experienced a relapse 12 months after treatment but regained complete remission after a

single additional dose of daratumumab, suggesting that retreatment may be effective in selected cases. Infectious complications, including cytomegalovirus infection, hepatitis E virus infection, herpes zoster, and bacterial infections, occurred in three patients. All infections were mild to moderate, managed as outpatients, and resolved without sequelae, indicating an acceptable safety profile despite intensive immunosuppression.

Importantly, our cohort reflects a broad spectrum of clinical presentations and disease trajectories. Recurrence occurred within days of transplantation in most patients, consistent with previous reports, but included one patient with delayed clinical progression requiring treatment escalation more than 4 years post-transplant.

Notably, this patient had an early histological recurrence but remained clinically stable 4 years before developing overt nephrotic syndrome with graft dysfunction. This highlights the dynamic nature of recurrent FSGS and suggests that disease activity may evolve before manifesting clinically. The longitudinal course of proteinuria and graft function following daratumumab therapy is illustrated in [Figure 1](#).

In contrast to previously reported case series, our observations emphasize that daratumumab may have potential across broader clinical scenarios than previously appreciated. While prior reports have focused on early recurrence or selected refractory cases, our cohort captures both early and delayed treatment settings and diverse disease trajectories. This provides additional insight into the potential role of plasma cell-targeted therapy in clinical practice.

Recent multicenter analyses have reported encouraging remission rates with combined B-cell and plasma cell-targeted strategies [7]. Our findings are consistent with these observations but extend them by providing detailed longitudinal clinical data in a well-characterized cohort, allowing a more granular and clinically relevant assessment of treatment response. In particular, the inclusion of patients with delayed disease progression and prolonged disease courses prior to treatment initiation suggests that the therapeutic window for daratumumab may be broader than previously assumed.

Circulating anti-nephrin autoantibodies have recently been proposed as potential biomarkers and possible mediators of recurrent podocytopathies [8, 9]. Anti-nephrin autoantibodies were serologically assessed in all patients at recurrence during active nephrotic disease and were negative in all cases. These findings argue against a dominant role of anti-nephrin-mediated mechanisms in these cases and further underscore the biological heterogeneity of recurrent FSGS. They also suggest that additional, as yet unidentified circulating factors may contribute to disease pathogenesis in a subset of patients. However, additional podocyte-associated markers were not systematically assessed and therefore cannot be evaluated in this cohort.

The mechanism of action of daratumumab in this setting likely extends beyond plasma cell depletion. CD38 is expressed on multiple immune cell subsets, including plasma cells, activated B cells, T cells, natural killer cells, and dendritic cells. Targeting CD38 may exert broader immunomodulatory effects influencing circulating permeability factors [10]. While the precise mechanisms remain incompletely understood, the consistent clinical responses observed in our cohort support further investigation of CD38-directed therapies in recurrent FSGS.

Our study has several limitations, including its retrospective design, small sample size, short follow-up, and lack of systematic B-cell and plasma cell count data, precluding conclusions regarding long-term outcomes. In addition, treatment strategies were not standardized but reflected real-world clinical practice. The absence of a control group limits attribution of treatment effects to daratumumab alone. Nevertheless, the consistent responses observed across a heterogeneous patient population provide clinically relevant insights into treatment effectiveness in a salvage setting.

In conclusion, daratumumab may represent a promising therapy in patients with recurrent FSGS after kidney transplantation who are refractory to standard treatment. Our findings suggest potential

benefit across diverse clinical scenarios, including delayed treatment escalation and prolonged disease courses, and support further evaluation in prospective, multicenter studies.

Sincerely,

Parthenopi Avaniadi.

Data availability statement

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

Ethics statement

The studies involving humans were approved by Ethics Committee of Charité University Medicine Berlin. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from a by-product of routine care or industry. 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. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

PA, FH, and KB conducted the study. PA, ES, SL, GE, FB, LG, and FH contributed to patient care and outcome assessment. FH, AM, TH, and NT provided laboratory testing for anti-nephrin-antibodies. PA, AA, AG, and FH performed the data analysis. PA and AA wrote the manuscript in consultation with FH, KB, and K-UE. All authors have read and agreed to the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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References

1. Ponticelli C, Glasscock RJ. Posttransplant recurrence of primary glomerulonephritis. *Clin J Am Soc Nephrol* (2010) 5(12):2363–72. doi:10.2215/CJN.06720810
2. Uffing A, Pérez-Sáez MJ, Mazzali M, Manfro RC, Bauer AC, de Sottomaor Drumond F, et al. Recurrence of FSGS after kidney transplantation in adults. *Clin J Am Soc Nephrol* (2020) 15(5):703–15. doi:10.2215/CJN.08970719
3. Hickson LJ, Gera M, Amer H, Iqbal CW, Moore TB, Milliner DS, et al. Kidney transplantation for primary FSGS: outcomes and response to therapy in the modern era. *Am J Kidney Dis* (2009) 53(6):964–76. doi:10.1097/TP.0b013e31819f12be
4. Delbet JD, Hogan J, Parmentier C, Ulinski T, Dossier C. Successful global anti-B-cell strategy with daratumumab in a patient with post-transplant nephrotic syndrome recurrence unresponsive to immunoadsorption and obinutuzumab. *Pediatr Transplant* (2023) 27(5):e14544. doi:10.1111/ptr.14544
5. Angeletti A, Bin S, Magnasco A, Bruschi M, Cravedi P, Ghiggeri GM. Efficacy of combined rituximab and daratumumab treatment in posttransplant recurrent focal segmental glomerulosclerosis. *Am J Transplant* (2024) 24(4):688–692. doi:10.1016/j.ajt.2023.12.010
6. Randone P, Sanna E, Dolla C, Gallo E, Mingozzi S, Tarragoni R, et al. Rescue with obinutuzumab and daratumumab as combined B cell/plasma cell targeting approach in severe posttransplant focal segmental glomerulosclerosis recurrence. *Am J Transpl* (2024) 24(10):1896–900. doi:10.1016/j.ajt.2024.06.010
7. Cravedi P, Maggiore U, Mortari G, Alberici F, La Manna G, Goes N, et al. Combined anti-CD20/anti-CD38 therapy in posttransplant focal segmental glomerulosclerosis recurrence: a retrospective, international, multicenter study. *Transpl Direct* (2026) 12(2):e1908. doi:10.1097/TXD.0000000000001908
8. Batal I, Watts AJB, Gibier JB, Hamroun A, Top I, Provot F, et al. Pre-transplant Anti-nephrin antibodies are specific predictors of recurrent diffuse podocytopathy in the kidney allograft. *Kidney Int* (2024) 106(4):749–52. doi:10.1016/j.kint.2024.07.022
9. Hengel FE, Dehde S, Lassé M, Zahner G, Seifert L, Schnarre A, et al. Autoantibodies targeting Nephrin in podocytopathies. *N Engl J Med* (2024) 391(5):422–33. doi:10.1056/NEJMoa2314471
10. Doberer K, Kläger J, Gualdoni GA, Mayer KA, Eskandary F, Farkash EA, et al. CD38 antibody daratumumab for the treatment of chronic active antibody-mediated kidney allograft rejection. *Transplantation* (2021) 105(2):451–7. doi:10.1097/TP.0000000000003247



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Incidence and factors associated with Herpes Zoster infection in kidney transplant recipients, a recent epidemiological study

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epidemiology, herpes zoster, incidence, kidney transplant recipients, vaccine

Dear Editors,

We want to address a letter about incidence and factors associated with herpes zoster infection in Kidney transplant recipients through a recent epidemiological study.

Herpes zoster (HZ), resulting from varicella-zoster virus reactivation, occurs more frequently in immunocompromised patients, including kidney transplant recipients (KTRs). However, contemporary epidemiological data focusing on age-specific incidence and risk factors in homogeneous cohorts of KTRs remain limited [1–6].

We conducted a monocentric observational cohort study including all adult patients who underwent kidney transplantation at our center between 2004 and 2015, had a functioning graft at day 30, and received a standard immunosuppressive regimen based on a calcineurin inhibitor combined with mycophenolate or everolimus. Patients with multi-organ transplantation or HIV infection were excluded. Follow-up extended until January 2017, death, or graft failure. Cytomegalovirus prophylaxis consisted of valganciclovir for 3 or 6 months according to donor and recipient serostatus, or a preemptive strategy.

Herpes zoster cases were identified through a systematic keyword search of shared electronic medical records and validated by expert review based on clinical description; virological confirmation was required only for disseminated or organ-invasive disease. Incidence rates were calculated overall and by age group, with 95% confidence intervals obtained by bootstrap resampling. Cumulative incidence was estimated using the Aalen–Johansen method, accounting for death and graft failure as competing events. Risk factors for HZ were evaluated using cause-specific Cox proportional hazards models, with valganciclovir exposure modeled as a time-varying covariate. Variables associated with HZ at $p < 0.20$ in univariable analyses were included in multivariable models, followed by backward elimination retaining variables with $p < 0.05$, after collinearity checking.

A total of 1,101 KTRs were included, with a median follow-up of 5.6 [3.3–9.2] years, representing 6092 patient-years. Eighty-nine patients experienced at least one episode of HZ, yielding an incidence rate of 14.5 per 1,000 patient-years (95% CI:

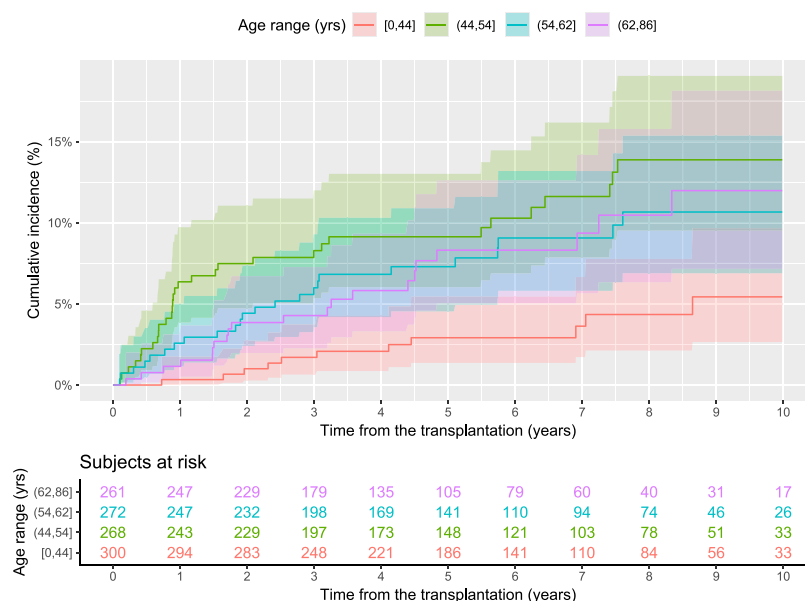


FIGURE 1

Incidence rate of Herpes Zoster of our cohort vs. Pinchinat et al. (DOI: [10.1186/1471-2334-13-170](https://doi.org/10.1186/1471-2334-13-170)) across ages. Bar and error plot comparing incidence rates of cases per one thousand patient-years for kidney transplant recipients and general population by age group, showing higher rates for transplant recipients in most age ranges, with rate ratios listed below the chart.

11.5–17.6). The median time to HZ onset was 5.3 years post-transplantation. Among the 99 zoster herpes, 20 were severe (22.5%; ophthalmic or neurological involvement, post-herpetic neuralgia, disseminated VZV, tissue invasive/organ disease), 53 non severe (59.5%); and 16 undetermined (18%).

Recipients who developed HZ were older at transplantation ($p = 0.042$), had a higher number of HLA class II mismatches ($p = 3.2 \times 10^{-4}$), and were more likely to receive maintenance corticosteroids ($p = 0.004$). When stratified by age quartiles, cumulative incidence analyses showed a significantly higher risk of HZ in recipients aged >44 years at transplantation compared with younger patients ($p = 0.0045$).

In the multivariable model, older age (HR 1.30 per decade, 95% CI 1.10–1.50; $p = 0.008$), induction with rabbit anti-thymocyte globulin (HR 1.70, 95% CI 1.10–2.60; $p = 0.02$), and higher HLA class II mismatch (HR 1.40, 95% CI 1.20–1.70; $p = 6 \times 10^{-4}$) remained independently associated with HZ occurrence, whereas valganciclovir exposure was not.

In addition, kidney transplant recipients experienced a substantially increased risk of herpes zoster across all age groups, with incidence rates in younger recipients comparable to those observed in elderly individuals in the general population (Figure 1). In all age ranges the incidence rate of HZ was much higher than in the general population (geometric mean of incidence rate ratios: 3.6). It is worth noting that even below 25 years of age at the time of transplantation the incidence rate of HZ was 6.33/1,000 patient-years [0–16.3], approximately at the same level as in the general population between 55 and 65-year (5.77 [4.97–6.97] [7]. These findings provide robust epidemiological support for systematic use of the

recombinant zoster vaccine in all adult KTRs irrespective of age. As the vaccine has a good immunogenicity in immunocompromised patients [8], they support broader implementation and reimbursement strategies in transplant populations, for futures European Guidelines.

Data availability statement

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

Ethics statement

The studies involving humans were approved by decision 2009-413, no 1357154, 2 July 2009. 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

BT, PP, MN, FJ, IG, LC, PM, KM, and HK conceived the study. BT and PP collected the data. BT performed the statistic analyses. BT, PP, MN, FJ, IG, LC, PM, KM, and HK interpreted the results. BT and HK wrote the manuscript. BT, PP, MN, FJ, IG, LC, PM, KM,

and HK revised the manuscript. All authors contributed to the article and approved the submitted version.

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References

1. Hurley JK, Greenslade T, Lewy PR, Ahmadian Y, Firlit C. Varicella-zoster infections in pediatric renal transplant recipients. *Arch Surg Chic Ill* (1960) 115(6):751–2. doi:10.1001/archsurg.1980.01380060049012
2. Hogewoning AA, Goettsch W, van Loveren H, de Fijter JW, Vermeer BJ, Bouwes Bavinck JN. Skin infections in renal transplant recipients. *Clin Transpl* (2001) 15(1):32–8. doi:10.1034/j.1399-0012.2001.150106.x
3. Gourishankar S, McDermid JC, Jhangri GS, Preiksaitis JK. Herpes zoster infection following solid organ transplantation: incidence, risk factors and outcomes in the current immunosuppressive era. *Am J Transpl* (2004) 4(1):108–15. doi:10.1046/j.1600-6143.2003.00287.x
4. Martin-Gandul C, Stampf S, Héquet D, Mueller NJ, Cusini A, van Delden C, et al. Preventive strategies against cytomegalovirus and incidence of α -Herpesvirus infections in solid organ transplant recipients: a nationwide cohort study. *Am J Transpl* (2017) 17(7):1813–22. doi:10.1111/ajt.14192
5. Fernández-Ruiz M, Origüen J, Lora D, López-Medrano F, González E, Polanco N, et al. Herpes zoster in kidney transplant recipients: protective effect of anti-cytomegalovirus prophylaxis and natural killer cell count. A single-center cohort study. *Transpl Int* (2018) 31(2):187–97. doi:10.1111/tri.13076
6. Sarı N, Erol Ç, Yanık Yalçın T, Kurt Azap Ö, Arslan H, Karakaya E, et al. Herpes zoster infections in solid-organ transplant recipients. *Exp Clin Transpl* (2023) 21(9):764–71. doi:10.6002/ect.2023.0185
7. Gonzalez CS, Sarazin M, Turbelin C, Lasserre A, Pelat C, Bonmarin I, et al. Herpes zoster: burden of disease in France. *Vaccine* (2010) 28(50):7933–8. doi:10.1016/j.vaccine.2010.09.074
8. Vink P, Ramon Torrell JM, Sanchez Fructuoso A, Kim SJ, Kim SI, Zaltzman J, et al. Immunogenicity and safety of the adjuvanted recombinant zoster vaccine in chronically immunosuppressed adults following renal transplant: a phase 3, randomized clinical trial. *Clin Infect Dis* (2020) 70(2):181–90. doi:10.1093/cid/ciz177



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Decisional preferences and distress among kidney transplant recipients with impaired graft function

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KEYWORDS

graft loss, kidney transplantation, patient-centered care, patient-reported outcome measure (PROM), shared decision making (SDM)

Dear Editors,

After transplantation, 57% of kidney transplant recipients (KTR) experience graft loss, while 43% die with a functioning graft [1], [Supplementary References S1, S2](#). When graft loss occurs, KTR face preference-sensitive decisions similar to those in advanced chronic kidney disease (CKD) and kidney failure (KF) [2], [Supplementary Reference S3](#). In CKD and KF, implementing shared decision-making (SDM) improves satisfaction with the selected kidney replacement therapy [3], [Supplementary References S4, S6](#). For KTR approaching graft loss, however, evidence-based SDM interventions are lacking. In conventional physician-centered care, reported rates of conversations about treatment options after graft loss are as low as 13%, and the associated SDM process has not been studied [4]. The KDIGO (Kidney Disease: Improving Global Outcomes) guideline “Challenges in the management of the kidney allograft: from decline to failure” underscores the importance of SDM and advance care planning in this phase while also highlighting the limited evidence base [2].

The “Prospectively Investigating the Impact of AI on Shared Decision-Making in Post-Kidney Transplant Care” (PRIMA-AI; NCT06056518) study investigates whether implementing an AI-based model predicting 1-year risk of graft loss increases the frequency of conversations about kidney replacement therapy in case of graft loss and supports shared decision-making (SDM) as graft function declines. In this research letter, we report patients’ self-reported decisional preferences, their decisional experiences, and patient-reported outcome measures (PROMs) including distress [5], [Supplementary References S7–S9](#). This sequential, longitudinal mixed-methods study was nested within the randomized, single-center PRIMA-AI study comparing the AI-supported approach with usual care. We enrolled 76 German-proficient KTRs with eGFR <30 mL/min/1.73 m² from two outpatient transplant clinics. Recruitment was terminated early (planned n = 122) due to recruitment difficulties. We summarize quantitative survey data for the full cohort from the baseline visit. The ethics committee of Charité - Universitätsmedizin Berlin approved the

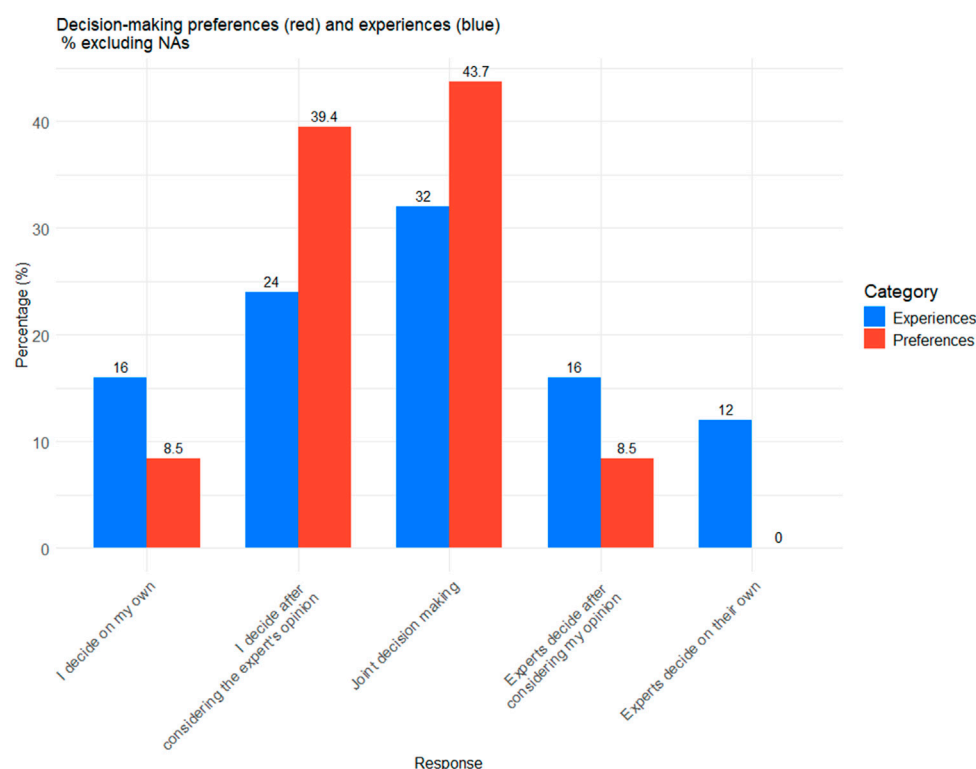


FIGURE 1

Decision-making preferences (red) of 71/76 participants assessed with the Control Preferences Scale (CPS), and decision-making experiences (blue) of 25/76 participants assessed with the CPS post questionnaire show that patients experienced passive decision-making roles more often than the overall cohort preferred those.

study and detailed protocols have been published previously [6], [Supplementary Reference S11](#). Full methods are provided in the [Supplementary Material](#).

From January 19, 2024 to September 18, 2024, 76 KTRs (age: 58.6 ± 17 years; transplant age: 14.4 ± 7.5 years) were enrolled, 32% (24/76) of which were female. Most participants reported German nationality (93%, 71/76), as well as German origin and mother tongue (89%, 68/76). Educational attainment was high: 65.8% (50/76) reported completing high school or higher; 50% (38/76) reported a job qualification; and 39.4% (30/76) reported a university degree or comparable qualification (Detailed demographics: [Supplementary Table S1](#), CONSORT flowchart: [Supplementary Figure S1](#)).

Preferred decision-making roles were assessed using the validated Control Preferences Scale (CPS) with the question: “How would you like to decide on kidney replacement therapy after graft loss?”. Of 76 enrolled patients, 71 (93%) provided valid baseline CPS data. At baseline, 48% (34/71) preferred an active role, 44% (31/71) a collaborative role, and 8.5% (6/71) a passive (paternalistic) role ([Supplementary Table S2](#)). A minority of 33% (25/76) already provided information about their decision-making experiences regarding graft loss at the baseline visit. They reported a higher proportion of passive decision-making experiences compared to the overall decision-making preferences (28% vs. 8.5%, [Figure 1](#)).

From baseline PROMs, we report self-rated general health and the Distress Thermometer (DT), a brief, intuitive, and cross-culturally validated measure of psychological distress (0 = no

distress to 10 = extreme distress). General health was rated as bad by 7.9% (6/76), mediocre by 42.1% (32/76), good by 39.5% (30/76), and very good by 1.3% (1/76), with 9.2% (7/76) missing. Median distress in the past week was 6 (interquartile range 4–7) ([Supplementary Figure S2](#)). The following reasons for distress were reported in >30% of the surveys: exhaustion (61.9%), sleep (51.6%), pain (48.2%), concerns (46.2%), mobility (43.8%), dry/itching skin (35.5%), diarrhea (35.0%), digestive problems (32.3%), sadness (31.2%), and fears (30.3%).

Most KTRs with impaired graft function preferred an active or collaborative role when considering kidney replacement therapy after potential graft loss. Decisional preferences resemble patterns seen in other serious illnesses such as cancer and heart disease [7], [Supplementary References S12, S13](#). In the baseline surveys, a higher proportion of patients reported experiencing a passive role than was preferred in the overall cohort. Together with previously reported low conversation rates in standard care, this supports prioritizing interventions that reliably enable SDM as graft function deteriorates. One promising approach are patient decision aids, which provide clear, comprehensive information on risks and benefits and can be delivered as booklets or web-based tools [3, 8]. They improve patient knowledge, risk perception, and decisional conflict related to feeling uninformed [3, 8]. Since KTR at risk for graft loss face comparable decisions as patients with CKD and KF, adapting validated decision aids from those settings seems sensible [3]. Importantly, consultation length is generally unchanged when

decision aids are used beforehand and increases by only 1.5 min when used during a visit [8].

We also observed substantial distress in this cohort, comparable to levels reported in patients receiving chemotherapy. In oncology, DT thresholds of 4–5 are commonly used to indicate clinically significant distress, and a recent meta-analysis suggests that around half of lung cancer patients meet such criteria [9], [Supplementary Reference S9](#). Importantly, the reasons for distress were physical symptoms above all, with almost half of the patients reporting pain, which as a manageable symptom should deserve more attention in our practice. While these findings should be confirmed in multi-center observational studies including patients with more diverse backgrounds and all levels of graft function, our findings still warrant broader routine assessment of distress in post-transplant care. A systematic evaluation for physical symptoms and clearer pathways to psychological and social support is needed when distress is high. There is a need for evidence-based, easy-to-adopt guidelines and strategies on how to increase the uptake of such offers in KTR. For example, individually tailored referrals to supportive care programmes, including social prescriptions, may deliver accessible information and support to address unmet psychosocial and practical needs [Supplementary Reference S14](#). This may improve both patients' and their support persons' health and wellbeing and enhance multidisciplinary collaboration among various healthcare providers.

Limitations include the single-center and non-observational design both of which resulted in a relatively small sample size. Given the inclusion criteria such as German-fluency and the topic of the trial (AI-based risk prediction), we assume that there was considerable participation bias toward participants of German origin and higher socioeconomic status than the general transplant population.

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 the ethics committee of Charité - Universitätsmedizin Berlin. 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.

References

- Mayrdorfer M, Liefeldt L, Wu K, Rudolph B, Zhang Q, Friedersdorff F, et al. Exploring the complexity of death-censored kidney allograft failure. *J Am Soc Nephrol* (2021) 32(6): 1513–26. doi:10.1681/ASN.2020081215
- Josephson MA, Becker Y, Budde K, Kasiske BL, Kiberd BA, Loupy A, et al. For conference participants. Challenges in the management of the kidney allograft: from decline to failure: conclusions from a kidney disease: improving global outcomes (KDIGO) controversies conference. *Kidney Int* (2023) 104(6):1076–91. doi:10.1016/j.kint.2023.05.010
- Amir N, McCarthy HJ, Tong A. A working partnership: a review of shared decision-making in nephrology. *Nephrology (Carlton)*. (2021) 26(11):851–7. doi:10.1111/nep.13902
- Bissonnette J, Woodend K, Davies B, Stacey D, Knoll GA. Evaluation of a collaborative chronic care approach to improve outcomes in kidney transplant recipients. *Clin Transpl* (2013) 27(2):232–8. doi:10.1111/ctr.12068

Author contributions

Conceptualization: BO and AH. Methodology: BO, ZS, SE, RR, KB, and AH. Software: RR. Formal Analysis: JS, BO, RR. Investigation: JS, ÖÖ, ZS, SE, and BO. Data Curation: JS, ÖÖ, ZS, SE, and BO. Writing (Original Draft): BO, AH, RR, AB, MH, and TO. Writing (Review and Editing): all authors. Visualization: BO. Supervision: AH, KB, PD, and SM. Project Administration: BO, ZS, RR, MH, and TO. Funding Acquisition: AH, KB, PD, and SM. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2026.16268/full#supplementary-material>

5. Degner LF, Sloan JA, Venkatesh P. The control preferences scale. *Can J Nurs Res* 1997 Fall (1997) 29(3):21–43.
6. Osmanodja B, Sassi Z, Eickmann S, Hansen CM, Roller R, Burchardt A, et al. Investigating the impact of AI on shared decision-making in post-kidney transplant care (PRIMA-AI): protocol for a randomized controlled trial. *JMIR Res Protoc* (2024) 13: e54857. doi:10.2196/54857
7. Singh JA, Sloan JA, Atherton PJ, Smith T, Hack TF, Huschka MM, et al. Preferred roles in treatment decision making among patients with cancer: a pooled analysis of studies using the control preferences scale. *The Am Journal Managed Care* (2010) 16(9): 688–96.
8. Stacey D, Lewis KB, Smith M, Carley M, Volk R, Douglas EE, et al. Decision aids for people facing health treatment or screening decisions. *Cochrane Database Syst Rev* (2024) 1(1). doi:10.1002/14651858.CD001431.pub6
9. Zhang L, Liu X, Tong F, Zou R, Peng W, Yang H, et al. Lung cancer distress: screening thermometer meta-analysis. *BMJ Support Palliat Care* (2024) 13(e3):e1084–e1092. doi:10.1136/bmjspcare-2021-003290



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Joint exposure to plasma heavy metals and the risk of kidney graft failure

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arsenic, cadmium, heavy metal, kidney graft failure, lead

Dear Editors,

Long-term kidney allograft survival remains a major clinical challenge. Nearly half of deceased-donor transplants fail within a decade, and identifying modifiable risk factors is therefore a priority [1]. Environmental heavy metals such as cadmium (Cd), lead (Pb), and arsenic (As) are established nephrotoxins that promote oxidative stress, tubular injury, and progression of chronic kidney disease [2]. Kidney transplant recipients (KTRs) are particularly susceptible because of reduced renal clearance, comorbidities, and immunosuppressive therapy. Previous work by our group demonstrated that each of these metals independently predicts graft failure [3, 4]. However, real-world exposure involves mixtures rather than single agents, and no prior study has evaluated their combined impact on long-term graft outcomes. We addressed this gap using advanced mixture modeling in a large prospective cohort.

We analyzed 668 outpatient KTRs from the TransplantLines Food and Nutrition Biobank and Cohort Study. Plasma Cd, Pb, and As were quantified by inductively coupled plasma mass spectrometry. A composite mixture index was derived using quantile-based g-computation (Q-gcomp), which jointly estimates the effect of increasing all metals by one quantile while weighting each metal's contribution [5]. The primary outcome was death-censored graft failure. Bayesian Kernel Machine Regression (BKMR) was applied to capture non-linear and interactive effects, multivariable linear regression identified clinical determinants of the mixture index, and prespecified subgroup analyses were stratified by baseline estimated glomerular filtration rate (eGFR) and proteinuria.

The median age was 54.7 years (IQR 44.8–62.9), 58% of participants were male, and median eGFR was 38.7 mL/min/1.73 m², assessed at a median of 5.3 years post-transplant. Plasma metal concentrations rose significantly across mixture-index tertiles, while inter-metal Spearman correlations between metals were generally weak (all $r < 0.4$), with distinct concentration distributions across KTRs (Figure 1A), supporting their treatment as distinct yet co-occurring exposures. Higher tertiles were accompanied by lower eGFR (47.4–31.3 mL/min/1.73 m², $P < 0.001$), greater proteinuria, lower transferrin saturation (TSAT), hemoglobin, and iron, and higher high-sensitivity C-reactive protein (hs-CRP) and uric acid, suggesting clustering of metal burden with kidney injury, anemia, and inflammation.

During a median follow-up of 5.3 (IQR 4.5–6.0) years, 80 graft failure events occurred. In Q-gcomp analysis, a simultaneous one-tertile increase in all three metals was associated

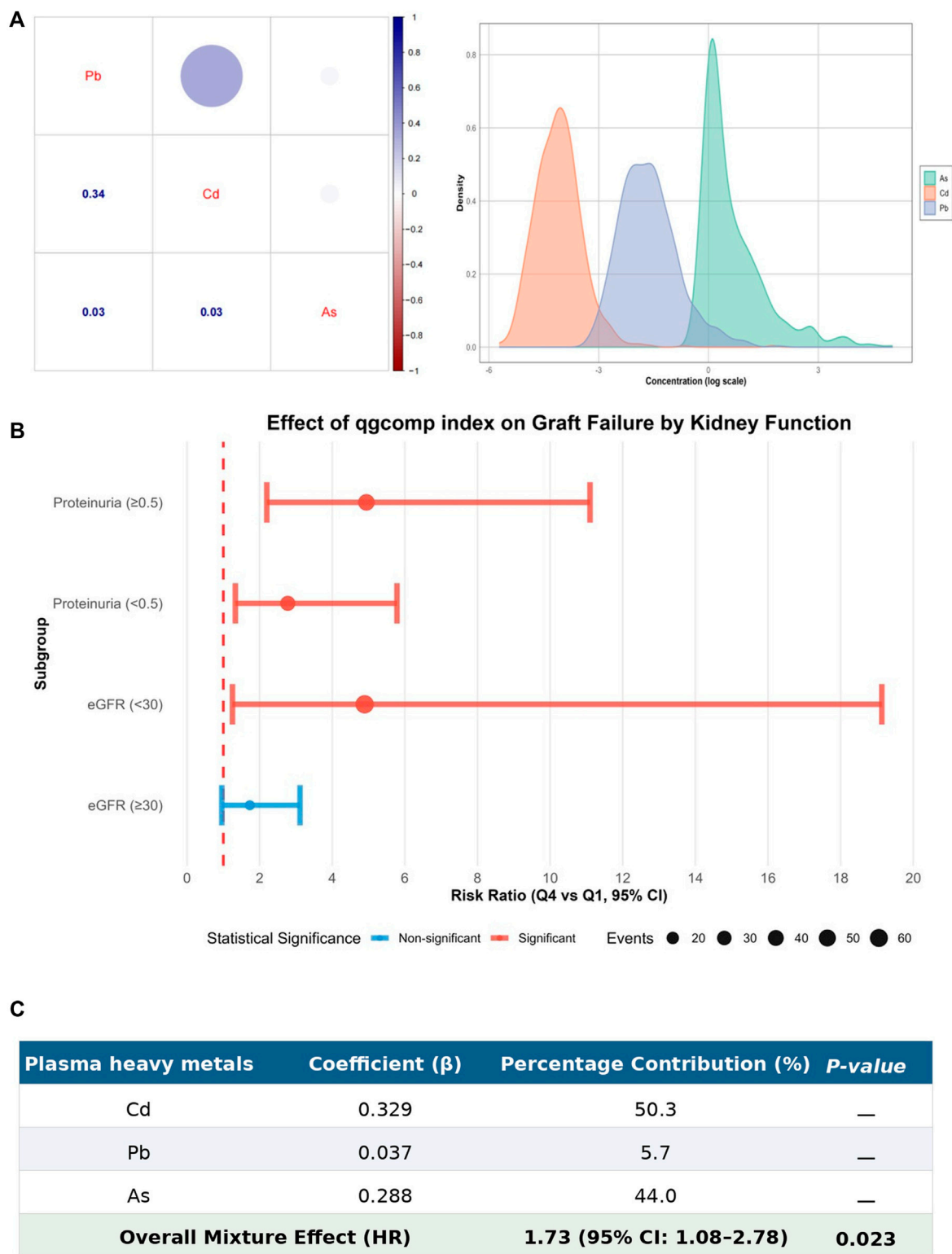


FIGURE 1
Combined heavy metal exposure and graft failure risk in kidney transplant recipients. **(A)** Correlation matrix and log-scale concentration distributions of plasma Cd, Pb, and As. **(B)** Subgroup analysis of the mixture effect on graft failure stratified by baseline kidney function (risk ratio, Q4 vs. Q1). **(C)** Q-gcomp mixture weights and overall hazard ratio for graft failure.

with a 73% higher risk of graft failure (HR 1.73, 95% CI 1.08 to 2.78, $P = 0.023$, [Figure 1C](#)). Cd contributed 50.3% of the overall mixture effect, As 44.0%, and Pb only 5.7%, identifying Cd and As being the dominant nephrotoxic drivers in this population. For comparison, individual metal associations with graft failure are presented in [Supplementary Results \(Supplementary Figure S1\)](#), which showed consistent patterns with Cd and As independently associated with increased risk. BKMR analysis confirmed a dose-dependent increase in risk above the 50th exposure percentile and revealed a synergistic interaction in which the nephrotoxic effect of Cd was amplified at higher As concentrations ([Supplementary Figure S2](#)). Multivariable regression further identified higher eGFR ($\beta = -0.260$, $P < 0.001$), longer transplant duration, and higher TSAT as inverse correlates of mixture burden, whereas higher fish intake was a positive correlate, pointing to clinically actionable determinants of systemic exposure.

A key observation was the strong variation in metal-associated graft failure risk according to baseline kidney function. Among KTRs with severe renal impairment (eGFR below 30 mL/min/1.73 m² or proteinuria of at least 0.5 g/24 h), those in the highest quartile of metal exposure had nearly a fivefold increased risk of graft failure compared with the lowest quartile (RR 4.89, 95% CI 1.25 to 19.12, and RR 4.87, 95% CI 2.10 to 11.30, respectively, [Figure 1B](#)). In contrast, the risk difference was much smaller and only borderline significant among recipients with preserved eGFR (RR 1.74, 95% CI 0.96–3.16) or low-grade proteinuria (RR 2.79, 95% CI 1.35–5.77). This gradient likely reflects impaired metal clearance and increased tissue accumulation in damaged kidneys, compounded by elevated oxidative stress and inflammation that sensitize the kidney to metal toxicity [6, 7]. These findings support incorporating baseline kidney function into risk assessment strategies for KTRs and identify a high-risk subgroup that may benefit most from targeted intervention.

From an environmental perspective, our findings align with concerns that current regulations, which typically evaluate metals individually, may underestimate risks from combined exposures. Epidemiological data from CKD-endemic regions such as Nicaragua and Sri Lanka show that agricultural workers chronically exposed to mixed metals develop kidney injury even at low levels [8]. Our results extend these observations to a vulnerable transplant population in a high-income setting, indicating that the burden of mixed exposure is not confined to occupationally or geographically high-risk groups, and underscoring the need for safety standards that explicitly address metal mixtures.

The inverse association between TSAT and the mixture index suggests a protective effect of adequate iron status, as iron and cadmium compete for shared transporters such as DMT1 and ZIP proteins, affecting both intestinal absorption and tissue retention [9]. This finding is consistent with our previous report of an inverse relationship between TSAT and cadmium levels in KTRs and underscores the bidirectional interplay between iron metabolism and metal toxicity in this population [9]. The positive association with fish intake likely reflects dietary exposure to bioaccumulated environmental contaminants, particularly inorganic As and Cd in long-lived predatory species. Beyond lifestyle modification, chelation therapies such as ethylenediaminetetraacetic acid (EDTA) have proven effective in lowering circulating metals and

slowing renal decline in other chronic conditions, raising the possibility of benefit in selected KTRs with elevated mixture indices [10]. Limitations include reliance on a single baseline plasma measurement, the observational design that precludes causal inference, and limited generalizability beyond similar outpatient cohorts.

In conclusion, this prospective study establishes that combined heavy metal exposure significantly increases graft failure risk in KTRs, with Cd and As demonstrating synergistic nephrotoxic effects. Importantly, we identified that recipients with compromised kidney function are particularly vulnerable to metal-induced graft injury, suggesting the need for risk-stratified clinical management. These findings support integrating routine heavy metal monitoring into post-transplant care, especially for high-risk recipients, which may help mitigate metal-associated graft loss and improve long-term transplant outcomes.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: Access to the data can be requested through the University Medical Center Groningen. Requests to access these datasets should be directed to Michele F. Eisenga, m.f.eisenga@umcg.nl.

Ethics statement

The studies involving humans were approved by TransplantLines Food and Nutrition Biobank and Cohort Study. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from all participants, and the study complied with the Declaration of Helsinki.

Author contributions

YY: Formal Analysis, Visualization, Data curation, Writing – original draft. PR: Investigation, Writing – review and editing. MS: Investigation, Data curation. AG-N: Data curation. JB: Supervision, Writing – review and editing. MB: Supervision, Writing – review and editing. DT: Methodology, Resources, Writing – review and editing. SB: Conceptualization, Supervision, Writing – review and editing. RG: Supervision, Writing – review and editing. ME: Conceptualization, Supervision, Project administration, Writing – review and editing.

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Conflict of interest

The author(s) declared that this work 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) declared that generative AI was not 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.2026.16919/full#supplementary-material>

References

1. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med* (2000) 342(9):605–12. doi:10.1056/NEJM200003023420901
2. Xu X, Nie S, Ding H, Hou FF. Environmental pollution and kidney diseases. *Nat Rev Nephrol* (2018) 14(5):313–24. doi:10.1038/nrneph.2018.11
3. Sotomayor CG, Groothof D, Vodegel JJ, Eisenga MF, Knobbe TJ, Ijmker J, et al. Plasma cadmium is associated with increased risk of long-term kidney graft failure. *Kidney Int* (2021) 99(5):1213.1224–. doi:10.1016/j.kint.2020.08.027
4. Sotomayor CG, Giubergia F, Groothof D, Ferreccio C, Nolte IM, Navis GJ, et al. Plasma lead concentration and risk of late kidney allograft failure. *Am J Kidney Dis* (2022) 80(1):87–97. doi:10.1053/j.ajkd.2021.10.009
5. Keil AP, Buckley JP, O'Brien KM, Ferguson KK, Zhao S, White AJ. A quantile-based g-computation approach to addressing the effects of exposure mixtures. *Environ Health Perspect* (2020) 128(4):47004. doi:10.1289/EHP5838
6. Barbier O, Jacquillet G, Tauc M, Coughnon M, Poujeol P. Effect of heavy metals on, and handling by, the kidney. *Nephron Physiol* (2005) 99(4):p105–110. doi:10.1159/000083981
7. Krishnan N, Moledina DG, Perazella MA. Toxic nephropathies of the tubulointerstitium. *Am J Kidney Dis* (2024) 83(5):659–676. doi:10.1053/j.ajkd.2023.09.017
8. Smpokou ET, Gonzalez-Quiroz M, Martins C, Alvito P, Le Blond J, Glaser J, et al. Environmental exposures in young adults with declining kidney function in a population at risk of Mesoamerican nephropathy. *Occup Environ Med* (2019) 76(12):920–6. doi:10.1136/oemed-2019-105772
9. Rawee P, Kremer D, Nolte IM, Leuvenink HGD, Touw DJ, De Borst MH, et al. Iron deficiency and nephrotoxic heavy metals. *Int J Mol Sci* (2023) 24(6) 5315. doi:10.3390/ijms24065315
10. Glicklich D, Shin CT, Frishman WH. Heavy metal toxicity in chronic renal failure and cardiovascular disease. *Cardiol Rev* (2020) 28(6):312–8. doi:10.1097/CRD.0000000000000304



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