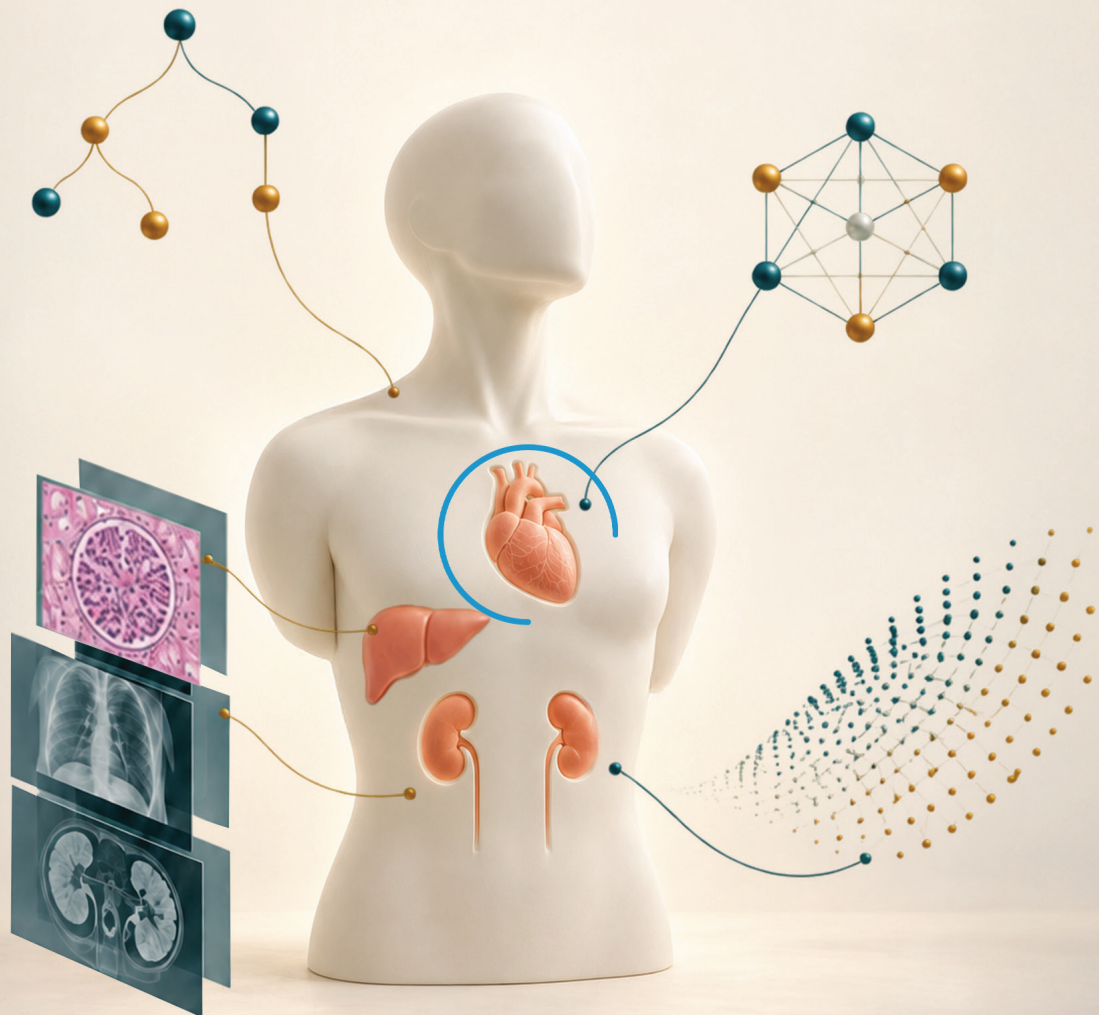


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**Decoding AI for Transplantation:  
Many Models, One Patient**



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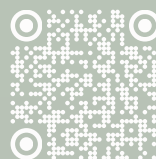
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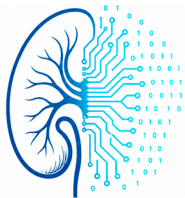
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# Decoding AI for transplantation: many models, one patient: why there is no single “AI for transplantation” – and what to ask instead

Olivier Aubert<sup>1,2,3\*</sup>, Esteban Cortes Garcia<sup>3</sup>, Silvia Pineda<sup>4,5</sup>, Thomas Neyens<sup>4,6,7</sup> and Nina Pilat<sup>8,9,10</sup>

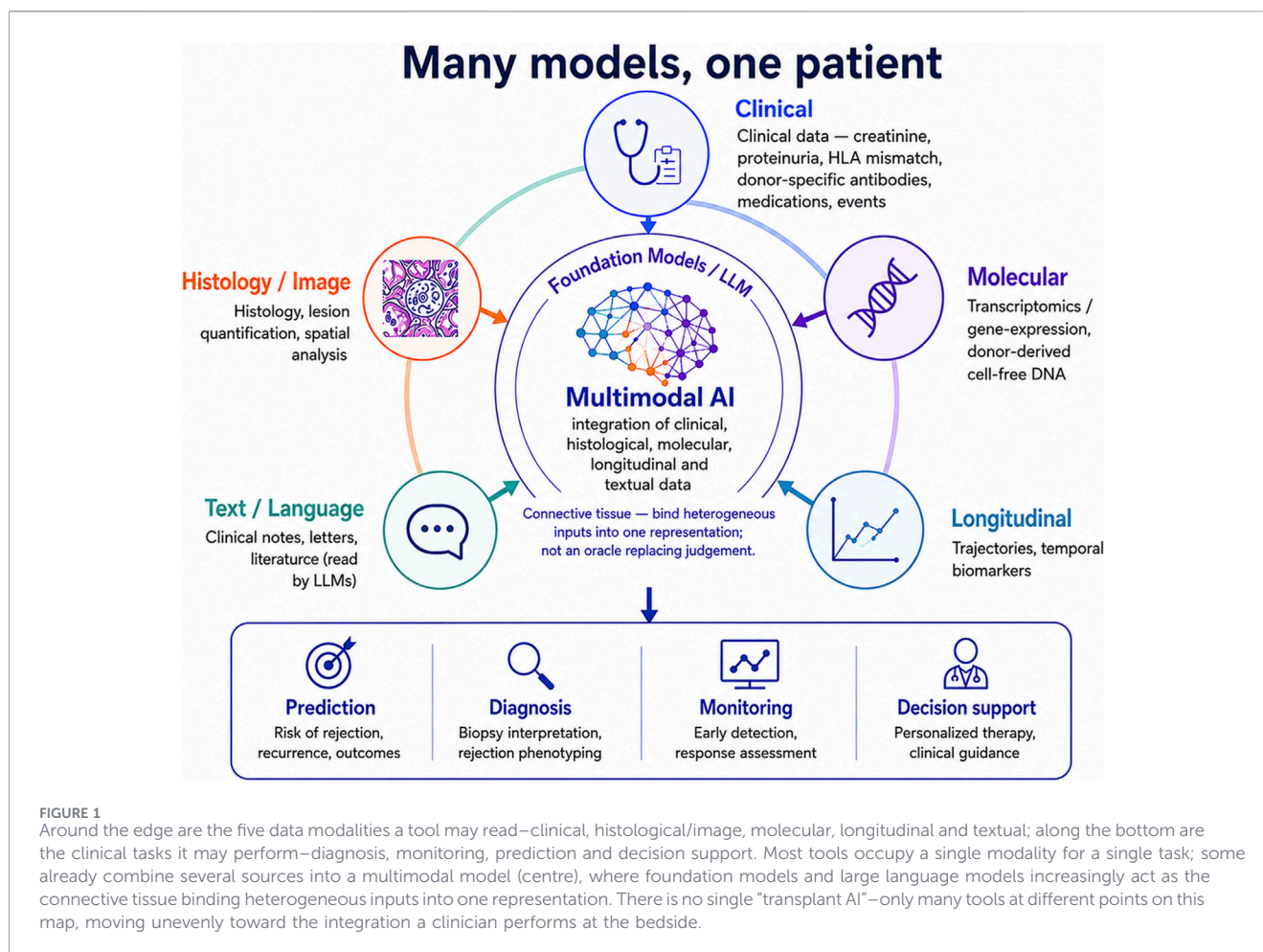
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#### KEYWORDS

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## Opening vignette

Consider a routine follow-up with a kidney transplant recipient: You are sitting with your patient presenting with an elevated serum creatinine. To assess potential allograft rejection or injury you do not reach for a single piece of information. You look at the trend rather than one value, you check the proteinuria, you recall the donor-specific antibodies from last year, you open the most recent biopsy results and you weigh all of it against everything you know about this particular patient. In a few minutes you have combined five different kinds of information into one judgement, almost without noticing. This ordinary act—holding many sources together and deciding what they mean as a whole—turns out to be the single hardest thing for artificial intelligence to do. The current landscape of AI tools in transplantation largely features fragmented, siloed architectures. Most of these tools read a single type of data—the biopsy, the transcriptomic signature, or the cfDNA value alone—and are built to answer one specific diagnostic or prognostic question.



## There is no single “transplant AI”

With the launch of this inaugural piece, we proudly introduce our new educational series, “Decoding AI for Transplantation.” This opening contribution is intended to paint the overarching landscape of the field, establishing a baseline framework before subsequent educational pieces look closely at specific computational methods, novel algorithmic approaches, and pressing ethical concerns.

A pervasive challenge in contemporary medical discourse is the tendency to evaluate “Artificial Intelligence” as a single, monolithic entity, like a uniform “black box” expected to revolutionize clinical care overnight. In reality, there is no singular, all-encompassing AI tool for transplantation; instead, the technology consists of highly narrow algorithms (or “AI tools”) designed for a specific purpose, each reading a particular kind of data [1, 2]. The fundamental clinical skill this series aims to cultivate, therefore, is not the capacity to critique “AI” in the abstract, but rather the practical ability to differentiate between these disparate technologies. This analytical categorization can largely be achieved by interrogating two fundamental questions: first, *what specific modality of data does the tool read* and second, *what precise clinical objective is it engineered to fulfil?*

## What does it read, and what can it do?

Take the two questions in turn, because together they locate almost any tool you will meet (Figure 1).

The first question is simple: what kind of data does the tool read? We call this its modality. Some tools read structured clinical data—creatinine, proteinuria, HLA mismatch, donor-specific antibodies. Others read images, typically the digitized biopsy slide [3]. Some read molecular data – the gene-expression signature of a tissue sample [4, 5], or donor-derived cell-free DNA in the blood [6]. Some read longitudinal data, following how a value moves over months rather than its level on a single day. And some now read free text—notes, letters, the literature—using large language models [7]. Each modality is, in effect, a different language, and most tools speak only one.

The second question is the task—the job the tool performs. A tool may be built to diagnose (what is happening in this graft now?), to screen or monitor (is something developing before it becomes clinically obvious?), to predict (what is the long-term risk?), or to guide a treatment decision. The same modality can serve different jobs, and the same job can be approached through different modalities—which is precisely why the word “AI” tells you so little on its own.



Placing real transplant tools on these two axes makes the point concrete. Digital-pathology models read images to help diagnose rejection on biopsy [3]. Transcriptomic classifiers read molecular data for the same diagnostic question [4, 5], by an entirely different route. Donor-derived cell-free DNA, increasingly embedded in multimodal screening tools, reads the blood to monitor for subclinical rejection before a biopsy is taken. A risk score such as the iBox reads clinical, histological and immunological data together to predict long-term graft survival [8]—the first of our four tools to combine modalities rather than rely on one. Four tools, four positions on the map, and only by naming each tool's data and task can you judge what it offers, and what it cannot.

## The illusion of completeness: unimodal precision vs. clinical synthesis

Categorizing a tool by its data input and clinical function reveals a critical limitation that a single headline metric can easily hide: most algorithms capture only a narrow slice of the total clinical picture. The biopsy-reading model has never seen the cell-free DNA result; a purely clinical risk score knows nothing of the histology. Each model can be excellent within its specific (diagnostic) domain and is, by design, blind to everything outside it.

That is the reverse of what a clinician does. At the bedside you are the integrator: the value of your judgement comes not from any one number but from seeing the big picture and holding the individual results together—the rising creatinine made meaningful by the proteinuria, the antibodies reinterpreted in the light of the biopsy. A tool that returns one confident result from one source is, in this sense, doing something you cannot; it answers a narrower question—often very well, but a narrower one. A narrow answer delivered with confidence is easy to mistake for a complete one.

To be fair, some tools already reach beyond a single modality. But these still remain narrow next to the synthesis a clinician performs without thinking. The honest description of the field is a spectrum: many single-modality tools, a growing few that combine two or three sources, and none that yet sees the whole patient, but then neither does the clinician alone, and multimodal integration is precisely the path toward seeing more than either the eye or a single tool can on its own.

## Putting the patient back together—and why this is so difficult

The direction of travel follows from all this, and it is the ambition behind the centre of Figure 1: to combine these tools, feeding clinical, histological, molecular and longitudinal information into models that produce one integrated picture. This is what multimodal AI means—and the principle is not speculative.

It is worth understanding why this frontier is genuinely hard, because this is where many promising tools stumble. Different modalities live on different scales and timeframes: a slide, a single blood value and a years-long trajectory are not naturally

comparable and forcing them into one model confronts us with a real problem, not a formality. Sources are often missing—no biopsy this visit, the anti-HLA DSA result not yet back—and a model trained on complete data can behave unpredictably without them [9]. Unlike traditional statistics, which offers a wide range of flexible methods for missing data [10], AI methodology is not yet at that stage; the more sources a model blends, the harder it becomes to see why it reached its conclusion, exactly when you most want to know. This is the role increasingly proposed for foundation models and large language models: not as oracles that replace judgement, but as connective tissue able to take in very different kinds of input and help bind them—a useful job, and a difficult one.

The holy grail, stated simply, would be to train computational models to replicate what clinicians already perform instinctively: to evaluate the patient holistically rather than viewing an isolated organ system or a singular assay. In reality we should not try to develop machine learning methods that replicate what clinicians do, but provide better tools to help clinicians make better decisions. This conceptual thread unifies this article series and underlines the message visualized in Figure 1, illustrating a trajectory that moves from the fragmented, highly specialized tools of today toward a fully integrated clinical paradigm tomorrow.

## A framework for critical appraisal

For the reader, this reframing turns an intimidating field into a set of answerable questions. Faced with any “AI in transplantation” paper or product, the first move is not to ask whether “AI works”, but to locate the tool. The first two questions have been introduced: what data does it read, and what job does it do? Three further questions complete the appraisal: how much does it integrate, how was it tested, and does it challenge my decision [7, 11–13]? A tool that reads one modality for one task can be excellent and still be only a part of the picture. A tool that claims to integrate many should be held to a higher standard, not a lower one, because its reach is wider and its failures quieter. And the ordinary questions never disappear—was it validated elsewhere, does it change a decision, does it help the patient. There is no single “transplant AI” to adopt or dismiss; there are many tools, each to be read on its own terms. Embracing this complexity is a more demanding approach, but ultimately a more useful one. In the end the question is not whether AI can replace our judgement but how we can use AI to make the best possible judgement to help patients.

### Box 1 – Decoding AI: five fundamental questions

1. What kind of data does this tool read—clinical, image, molecular, longitudinal, or text?
2. What is its job—diagnosis, screening or monitoring, prediction, or decision support?
3. Does it read one modality or combine several—and if it integrates, how does it cope when a source is missing?
4. Was it tested in patients and centre other than the ones it was built on?
5. Does it change a decision or improve an outcome—or only describe a risk you already sensed?

## Box 2 - Key takeaways and glossary

### Three takeaways

1. There is no single “transplant AI”: the field is many tools, each defined by the data it reads and the clinical job it does.
2. Most tools see only a slice of the patient; some already combine modalities, but none yet matches the integration a clinician performs at the bedside.
3. The direction is integration (multimodal AI) - already underway, genuinely hard, and to be read more critically as it grows, not less.

### Glossary

**Data modality.** The kind of information a tool reads - clinical numbers, biopsy images, molecular signals, time trajectories, or text.

**Task.** The clinical job a tool performs - diagnosis, screening/monitoring, prediction, or decision support.

**Multimodal AI.** A model that combines several data types into one output, rather than relying on a single source.

**Foundation model/large language model (LLM).** A large, general-purpose model (often trained on text) that can take in varied inputs and be adapted to many tasks.

**External validation.** Testing a tool in patients or centres other than those it was developed on, to see whether it travels.

## Author contributions

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

## References

1. Acosta JN, Falcone GJ, Rajpurkar P, Topol EJ. Multimodal biomedical AI. *Nat Med* (2022) 28(9):1773–84. doi: 10.1038/s41591-022-01981-2
2. Rajpurkar P, Chen E, Banerjee O, Topol EJ. AI in health and medicine. *Nat Med* (2022) 28(1):31–8. doi: 10.1038/s41591-021-01614-0
3. Kers J, Bulow RD, Klinkhammer BM, Breimer GE, Fontana F, Abiola AA, et al. Deep learning-based classification of kidney transplant pathology: a retrospective, multicentre, proof-of-concept study. *Lancet Digit Health* (2022) 4(1):e18–e26. doi: 10.1016/S2589-7500(21)00211-9
4. Zielinski D, Goutaudier V, Sablik M, Divard G, Aubert O, Piedrafita A, et al. Molecular diagnosis of kidney allograft rejection based on the Banff Human Organ Transplant gene panel: a multicenter international study. *Am Journal Transplantation* (2025) 25(8):1631–42. doi: 10.1016/j.ajt.2025.04.025
5. Halloran PF, Madill-Thomsen KS, Reeve J. The molecular phenotype of kidney transplants: insights from the MMDx project. *Transplantation* (2024) 108(1):45–71. doi: 10.1097/TP.0000000000004624
6. Aubert O, Ursule-Dufait C, Brousse R, Gueguen J, Racapé M, Raynaud M, et al. Cell-free DNA for the detection of kidney allograft rejection. *Nat Med* (2024) 30(8):2320–7. doi: 10.1038/s41591-024-03087-3
7. Moor M, Banerjee O, Abad ZSH, Krumholz HM, Leskovec J, Topol EJ, et al. Foundation models for generalist medical artificial intelligence. *Nature* (2023) 616(7956):259–65. doi: 10.1038/s41586-023-05881-4
8. Loupy A, Aubert O, Orandi BJ, Naesens M, Bouatou Y, Raynaud M, et al. Prediction system for risk of allograft loss in patients receiving kidney transplants: international derivation and validation study. *Bmj* (2019) 366:l4923. doi: 10.1136/bmj.l4923
9. Finlayson SG, Subbaswamy A, Singh K, Bowers J, Kupke A, Zittrain J, et al. The clinician and dataset shift in artificial intelligence. *The New Engl Journal Medicine* (2021) 385(3):283–6. doi: 10.1056/NEJMc2104626
10. Little RJA, Rubin DB. *Statistical Analysis with Missing Data*. 3rd ed. John Wiley and Sons (2019).
11. Van Calster B, McLernon DJ, van Smeden M, Wynants L, Steyerberg EW. Topic Group ‘Evaluating diagnostic tests and prediction models’ of the STRATOS initiative. Calibration: the achilles heel of predictive analytics. *BMC Med* (2019) 17(1):230. doi: 10.1186/s12916-019-1466-7
12. Collins GS, Moons KGM, Dhiman P, Riley RD, Beam AL, Van Calster B, et al. TRIPOD+AI statement: updated guidance for reporting clinical prediction models that use regression or machine learning methods. *Bmj* (2024) 385:e078378. doi: 10.1136/bmj-2023-078378
13. Moons KGM, Damen JAA, Kaul T, Hooft L, Andaur Navarro C, Dhiman P, et al. PROBAST+AI: an updated quality, risk of bias, and applicability assessment tool for prediction models using regression or artificial intelligence methods. *Bmj* (2025) 388:e082505. doi: 10.1136/bmj-2024-082505

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# Reshaping the landscape of HLA desensitization through immune engineering

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access to transplantation, CAR T cells, desensitization, HLA allosensitization, kidney transplantation

Kidney transplantation in highly HLA-sensitized candidates remains one of the greatest unmet challenges in transplantation. Since transplantation with donor specific antibodies (DSA) is associated with an increased risk of rejection and graft loss [1], strategies to enable HLA compatible transplantation should be encouraged, such as kidney exchange programs and different prioritization programs [2]. However, despite major advances in histocompatibility testing and allocation policies prioritizing sensitized recipients, patients with broad sensitization (cPRA >99%) continue to experience prolonged waiting times, low access to compatible donors, and increased mortality while on the waiting list [3–5]. Consequently, HLA desensitization has emerged as the only viable option for transplantation in many of these patients.

Over the past decades, numerous desensitization strategies have sought to overcome HLA sensitization and expand access to transplantation. However, most have relied on off-label immunosuppressive therapies targeting isolated components of the humoral immune response, circulating antibodies, plasma cells, or memory B cells, rather than the integrated biology of alloimmune memory. Consequently, clinical efficacy has been inconsistent and durable desensitization has remained largely elusive [6, 7].

The introduction of imlifidase represented the first major breakthrough by enabling rapid elimination of circulating donor-specific antibodies (DSA) and successful HLA-incompatible transplantation in selected highly sensitized recipients [8–10]. Although encouraging short- and intermediate-term outcomes have been reported, its long-term impact remains uncertain because rapid antibody rebound, driven by persistent memory B cells and long-lived plasma cells, frequently occurs within days to weeks after transplantation [11, 12]. Thus, while imlifidase established proof-of-concept that deeply entrenched humoral alloimmunity can be therapeutically overcome, it also highlighted the need for strategies capable of durably targeting the cellular reservoirs that sustain HLA antibody production.

Three reports published in the New England Journal of Medicine [13–15] provide compelling proof-of-concept that immune-engineering approaches may substantially expand the therapeutic armamentarium for HLA desensitization. The remarkable efficacy of chimeric antigen receptor (CAR)-T cells and T-cell engager (TCE) bispecific antibodies targeting B-cell and plasma-cell lineages in hematologic malignancies, together with their emerging success in severe refractory autoimmune diseases [16, 17], has now prompted their translation into transplantation. Four highly

TABLE 1 Main goals and biological features of different desensitization strategies.

| Feature                                     | Conventional desensitization (PLEX/IVIG ± anti-CD20) | Imlifidase                                    | CD19 CAR-T                                                                                                                                          | CD19/BCMA CAR-T                                                                                       | BCMA T-cell engager (TCE)                                                     |
|---------------------------------------------|------------------------------------------------------|-----------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| Primary humoral immune compartment targeted | Circulating antibodies + partial B-cell depletion    | Circulating IgG antibodies                    | CD19 <sup>+</sup> B-cell lineage (including naïve, memory B cells and some plasma cells; largely sparing CD19 <sup>+</sup> long-lived plasma cells) | CD19 <sup>+</sup> B-cell lineage + BCMA <sup>+</sup> plasma cells (including long-lived plasma cells) | BCMA <sup>+</sup> plasma cells (sparing BCMA <sup>+</sup> memory B cells)     |
| HLA antibody kinetics                       | Partial and variable reduction                       | Immediate, complete but transient elimination | Progressive decline                                                                                                                                 | Progressive, potentially more sustained decline                                                       | Progressive decline                                                           |
| Access to HLA-compatible transplantation    | Limited                                              | ✓                                             | ✓                                                                                                                                                   | ✓                                                                                                     | ✓                                                                             |
| Observed DSA rebound                        | Frequent                                             | Frequent                                      | None observed*                                                                                                                                      | None observed*                                                                                        | None observed*                                                                |
| Principal biological limitation             | Incomplete control of humoral alloimmunity           | Persistence of humoral immune memory          | Persistence of CD19 <sup>+</sup> long-lived plasma cells                                                                                            | Prolonged hypogammaglobulinemia and immune deficiency                                                 | Persistence of memory B cells                                                 |
| Major unanswered question                   | —                                                    | How can antibody rebound be prevented?        | Is depletion of CD19 <sup>+</sup> B-cell compartments sufficient for durable desensitization?                                                       | Does dual targeting provide superior durability that justifies deeper immune depletion?               | Is plasma-cell depletion alone sufficient without eliminating memory B cells? |
| Conceptual advance                          | Suppress humoral alloimmunity                        | Remove circulating antibodies                 | Reprogram humoral immune memory                                                                                                                     | Reprogram humoral immune memory                                                                       | Reprogram humoral immune memory                                               |

\*No DSA, rebound has been observed during the available follow-up. However, long-term durability remains unknown and will likely depend on both the completeness of depletion of the relevant humoral immune compartments and the nature of subsequent HLA, antigen re-exposure, including repeated HLA, antigen encounters, shared epitopes, and persistent antigen expression from retained failed allografts.

sensitized kidney transplant candidates (cPRA >99.8%) underwent successful desensitization followed by HLA-compatible kidney transplantation after receiving different immune-engineering platforms targeting complementary cellular compartments through CD19, B-cell maturation antigen (BCMA), or both. Rather than simply removing circulating donor-specific antibodies (DSAs), these pioneering approaches aim to eradicate the cellular reservoirs responsible for sustaining humoral alloimmune memory. Although based on only a handful of patients, these reports provide an important biological proof-of-concept and establish a strong rationale for future clinical investigation.

Direct comparison among these studies is challenging because they employed distinct therapeutic platforms (one CD19 CAR-T, two dual CD19/BCMA CAR-T, and one CD3×BCMA TCE), different cell doses, and variable lymphodepletion regimens. Nevertheless, all strategies produced profound reductions in circulating HLA antibodies, enabling timely HLA-compatible kidney transplantation. Perhaps even more strikingly, none of the treated patients experienced DSA rebound following

transplantation despite re-exposure to donor HLA antigens, suggesting that these approaches may interfere with the immunological mechanisms underlying anamnestic humoral responses. This observation is particularly intriguing for strategies primarily targeting only one of the two principal compartments sustaining alloantibody production (memory B cells or long-lived plasma cells) as compensatory responses arising from the untargeted compartment have previously been described with other, predominantly monoclonal antibody-based, desensitization approaches [18, 19]. Indeed, these observations are consistent with a recent desensitization experience using a BCMA-directed TCE combined with obinutuzumab, a next-generation anti-CD20 monoclonal antibody, which similarly induced marked reductions in HLA antibodies, enabled successful transplantation, and maintained durable biological effects after transplantation [20].

Importantly, despite the intensity of these immune interventions, all reports describe an acceptable short-term safety profile. Treatment-related toxicities were limited, with only one patient receiving high-dose CD19 CAR-T therapy



developing mild grade I cytokine release syndrome (CRS), no cases of immune effector cell-associated neurotoxicity syndrome (ICANS), and no major opportunistic infections either during dialysis or after transplantation under conventional induction and maintenance immunosuppression. However, all patients developed profound hypogammaglobulinemia due to depletion of polyclonal immunoglobulins, necessitating prolonged intravenous immunoglobulin replacement in most cases.

Beyond their clinical findings, these reports provide valuable mechanistic insights into the biology of HLA antibody persistence. Serial antibody measurements illustrate the extraordinary burden of circulating HLA antibodies in highly sensitized patients and suggest that several physiological antibody turnover cycles are required before complete clearance becomes evident. This was particularly evident for anti-HLA-DQ antibodies, consistent with their typically higher circulating antibody burden in highly sensitized recipients. Furthermore, in patients who underwent a single immunoadsorption session before transplantation, a marked reduction in HLA antibody levels was achieved without subsequent rebound, further supporting the notion that antibody production had been effectively suppressed rather than merely transiently reduced.

Comprehensive immune phenotyping further supports the proposed mechanisms of action. Peripheral blood B cells were completely depleted in all patients throughout follow-up, except in the two recipients of dual CD19/BCMA CAR-T cells, in whom B-cell reconstitution occurred after approximately 53 days. Bone marrow analyses demonstrated effective depletion of CD19-positive cellular populations across all strategies, whereas profound elimination of bone marrow-resident plasma cells was observed only in patients receiving BCMA-targeted therapies. Likewise, lymph node analyses showed marked depletion of B-cell populations following both CD19-only and dual CD19/BCMA CAR-T therapies, although comparable tissue analyses were unfortunately unavailable in the patient treated with the CD3×BCMA bispecific antibody. In this line, antibodies directed against previously encountered HLA specificities appeared more resistant to depletion following CD19 CAR-T therapy. In contrast, no preferential persistence of antibodies against repeated donor HLA mismatches was observed in the patient receiving BCMA-targeted therapy despite antigen rechallenge at kidney transplantation. Although based on a single case, these observations suggest that the durability of established alloantibody responses may depend on the underlying mechanisms sustaining humoral immune memory, including the relative contribution of memory B cells, long-lived plasma cells, and persistent antigenic stimulation.

Collectively, these pioneering studies provide compelling proof-of-concept that immune-engineering approaches may reshape the landscape of HLA desensitization. However, many fundamental questions remain. Which therapeutic platform, or combination of cellular targets, provides the optimal balance between efficacy and safety? The slower decline of anti-HLA-DQ antibodies, likely reflecting their particularly high antibody burden, together with the persistence of antibodies directed against previously encountered HLA antigens after some

treatment approaches, illustrates that distinct components of humoral alloimmunity may differ in their susceptibility to these new immune engineering therapies (Table 1). Whether depletion of memory B cells alone is sufficient, or sustained plasma-cell depletion is also required to prevent anamnestic alloantibody responses, remains unknown. Addressing these questions will require adequately powered prospective clinical trials comparing these different strategies, which should invariably be accompanied by comprehensive mechanistic studies to define which cellular compartments must be effectively targeted for durable control of humoral alloimmunity. Equally important, the long-term consequences of prolonged hypogammaglobulinemia, the optimal post-transplant immunosuppressive strategy, and the risk of opportunistic viral infections remain to be established before these transformative approaches can be broadly implemented. Ultimately, lessons from these emerging immune-engineering platforms may pave the way toward HLA-specific desensitization, selectively eliminating pathogenic alloimmune memory while preserving protective immunity, likely complemented by non-antigen-specific plasma cell-directed therapies [21–23].

## 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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## References

- Kahanpää M, Lauronen J, Lempinen M, Ahoelto K, Sallinen V, Helanterä I. Long-term HLA-incompatible kidney transplant outcomes. *Transpl Int* (2026) 39:16478. doi: 10.3389/ti.2026.16478
- Mamode N, Bestard O, Claas F, Furian L, Griffin S, Legendre C, et al. European guideline for the management of kidney transplant patients with HLA antibodies: by the European Society for organ transplantation working group. *Transpl Int* (2022) 35:10511. doi: 10.3389/ti.2022.10511
- Manook M, Koeser L, Ahmed Z, Robb M, Johnson R, Shaw O, et al. Post-listing survival for highly sensitised patients on the UK kidney transplant waiting list: a matched cohort analysis. *The Lancet* (2017) 389(10070):727–34. doi: 10.1016/S0140-6736(16)31595-1
- Montgomery RA, Lonze BE, King KE, Kraus ES, Kucirka LM, Locke JE, et al. Desensitization in HLA-incompatible kidney recipients and survival. *N Engl J Med* (2011) 365(4):318–26. doi: 10.1056/NEJMoa1012376
- Sapir-Pichhadze R, Tinckam KJ, Laupacis A, Logan AG, Beyene J, Kim SJ. Immune sensitization and mortality in wait-listed kidney transplant candidates. *J Am Soc Nephrol JASN* (2016) 27(2):570–8. doi: 10.1681/ASN.2014090894
- Schinstock C, Tambur A, Stegall M. Current approaches to desensitization in solid organ transplantation. *Front Immunol* (2021) 12:686271. doi: 10.3389/fimmu.2021.686271
- Bestard O, Couzi L, Crespo M, Kessaris N, Thauan O. Stratifying the humoral risk of candidates to a solid organ transplantation: a proposal of the ENGAGE working group. *Transpl Int* (2021) 34(6):1005–18. doi: 10.1111/tri.13874
- Jordan SC, Lorant T, Choi J, Kjellman C, Winstedt L, Bengtsson M, et al. IgG endopeptidase in highly sensitized patients undergoing transplantation. *N Engl J Med* (2017) 377(5):442–53. doi: 10.1056/NEJMoa1612567
- Furian L, Heemann U, Bengtsson M, Bestard O, Binet I, Böhmig GA, et al. Desensitization with imlifidase for HLA-incompatible deceased donor kidney transplantation: a Delphi international expert consensus. *Transpl Int* (2025) 37:13886. doi: 10.3389/ti.2024.13886
- Couzi L, Malvezzi P, Amrouche L, Anglicheau D, Blanche G, Caillard S, et al. Imlifidase for kidney transplantation of highly sensitized patients with a positive crossmatch: the French consensus guidelines. *Transpl Int* (2023) 36:11244. doi: 10.3389/ti.2023.11244
- Kamar N, Bertrand D, Caillard S, Pievani D, Apithy MJ, Congy-Jolivet N, et al. Imlifidase in highly sensitized kidney transplant recipients with a positive crossmatch against a deceased donor. *Kidney Int Rep* (2024) 9(10):2927–36. doi: 10.1016/j.ekir.2024.07.024
- Lorant T, Lonze BE, Montgomery RA, Desai NM, Legendre C, Lundgren T, et al. Five years Follow-up of imlifidase desensitized kidney transplant recipients. *Transpl Int Off J Eur Soc Organ Transpl* (2025) 38:15425. doi: 10.3389/ti.2025.15425
- Schrezenmeier J, Rincon-Arevalo H, Lachmann N, Stauch D, Globke B, Öllinger R, et al. Kidney transplantation after clearing Anti-HLA antibodies with CD19 CAR T cells. *N Engl J Med* (2026) 394(21):2166–8. doi: 10.1056/NEJM2517277
- Bhoj VG, Kaminski M, Zhao H, Jackson K, Wang W, Liu C, et al. Kidney transplantation in two highly sensitized candidates after CAR T-Cell therapy. *N Engl J Med* (2026) 394(21):2117–25. doi: 10.1056/NEJMoa2513428
- Schatzl M, Mayer KA, Agis H, Haindl S, Kriks D, Fischer G, et al. T-Cell engager-mediated HLA antibody depletion before kidney transplantation. *N Engl J Med* (2026) 395(5):511–3. doi: 10.1056/NEJM2603853
- Müller F, Hagen M, Wirsching A, Kharboulit S, Aigner M, Völkl S, et al. CD19 CAR-T cells for treatment-refractory autoimmune diseases: the phase 1/2 CASTLE basket trial. *Nat Med* (2026) 32(3):1142–51. doi: 10.1038/s41591-025-04185-6
- Bucci L, Böltz S, Hagen M, Tur C, Nöthling DM, Rothe T, et al. BCMA T-Cell engager therapy in patients with refractory autoimmune disease. *N Engl J Med* (2025) 393(15):1544–7. doi: 10.1056/NEJM2506740
- Torija A, Matignon M, Vincenti F, Casanova-Ferrer F, Pilon C, Tambur AR, et al. Anti-HLA serologic response to CD38-targeting desensitization therapy is challenged by peripheral memory B cells in highly sensitized kidney transplant candidates. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2025) 25(1):88–101. doi: 10.1016/j.ajt.2024.08.004
- Malvezzi P, Martínez-Lacalle A, Crespo E, Torija A, Imerzoukene F, Meneghini M, et al. Dual targeting of B cell compartments using belatacept and daratumumab in highly sensitized kidney transplant candidates: a mechanistic proof-of-concept desensitization trial. *Am J Transpl* (2026) 0(0):1011–23. doi: 10.1016/j.ajt.2025.12.283
- Leon J, Aubert O, Devriese M, Alameda F, Charbonnier S, Fourgeaud J, et al. B-cell maturation antigen-Targeted T-cell engager therapy combined with B-cell depletion for treatment of refractory HLA sensitization. *Kidney Int* (2026) 109(6):1148–54. doi: 10.1016/j.kint.2026.01.020
- Arana C, Garcia-Busquets A, Nicoli M, Betriu S, Gille I, Heemskerk MHM, et al. Chimeric HLA antibody receptor T cell therapy for humoral transplant rejection. *Nephrol Dial Transpl* (2025) 40(1):19–26. doi: 10.1093/ndt/gfae160
- Dragon AC, Bonifacius A, Lienenklaus S, Verboom M, Gerhards JP, Ius F, et al. Depletion of alloreactive B cells by drug-resistant chimeric alloantigen receptor T cells to prevent transplant rejection. *Mol Ther* (2025) 33(3):1031–47. doi: 10.1016/j.ymthe.2025.01.009
- Cheng Q, Pelz A, Taddeo A, Khodadadi L, Klotzsche J, Hoyer BF, et al. Selective depletion of plasma cells *in vivo* based on the specificity of their secreted antibodies. *Eur J Immunol* (2020) 50(2):284–91. doi: 10.1002/eji.201948144



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# Guiding light: new European guidelines on CLAD prevention and treatment

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### KEYWORDS

chronic lung allograft dysfunction, clinical practice guideline, clinical trial, lung transplantation, prevention

## A Forum discussing

### European society for organ transplantation clinical practice guideline on prevention and treatment of chronic lung allograft dysfunction

by Bos S, Hellemons M, Dave K, Engel MFM, Holm A, Morlacchi L, Müller V, Pavanello S, Picard C, Rossi V, Saez-Gimenez B, Gottlieb J and Vos R (2026). *Transpl. Int.* 39:16959. doi: 10.3389/ti.2026.16959

Chronic lung allograft dysfunction (CLAD) is a challenging clinical entity in lung transplantation. It is common, deadly, difficult to prevent, and almost entirely unresponsive to therapy. As the number one killer of lung transplant patients after the first year [1], it also limits lung transplant as a therapy for advanced lung disease both by reducing its cost-effectiveness and requiring stringent selection criteria to minimize its risks. It was described over four decades ago now after the first lung transplants in the 1980s (then referred to collectively as bronchiolitis obliterans syndrome or BOS) [2], but despite significant advances in the time since in understanding its pathology, clinical course, risk factors, outcome associations, physiologic and radiographic subtypes, and finally the development of a consensus definition [3], the lung transplant community has had only limited success in evolving meaningful strategies to address CLAD.

In recent years though, there have been important developments. The first successful multicenter randomized clinical trial (RCT) of CLAD prevention was recently completed by a consortium of Scandinavian countries (ScanCLAD), demonstrating that tacrolimus is superior to cyclosporine in preventing CLAD [4]. Although only a minority of centers were still using cyclosporine as *de novo* immunosuppression after transplant [5], this new evidence can and should change practice there. It also informs clinicians when making decisions about whether to rotate patients from tacrolimus to cyclosporine and for what indications it is worth the trade-off. The successful completion of a multicenter clinical trial studying the role of anti-fibrotic therapy in patients with the BOS subtype of CLAD—specifically, that it showed no benefit—has also helped move the needle on a perennial question in what is understood to be a fibrotic airways process [6]. A single-center RCT in Vienna has also now shown that extracorporeal photopheresis (ECP) may prevent CLAD, in addition to potentially lowering rates of rejection and infection as well [7]. These works build on a previous successful Belgian clinical trial showing that azithromycin prophylaxis can reduce CLAD risk [8].

Despite these advances though, the evidence for CLAD therapeutics remains mixed or entirely lacking, and this can result in heterogeneity in the clinical approach [9, 10]. CLAD is

a universally feared complication, particularly in its rapidly progressive forms, and clinicians faced with an ailing patient and armed with limited evidence do not tend to stand by. The interventions clinicians impose are well-intentioned and may in some cases be beneficial but can set the stage for patient- and system-level harms, such as adverse drug effects, infectious or malignant complications of immunosuppression, and resource utilization without clear benefit. It is these contexts where clinical practice guidelines can make a difference by evaluating the evidence, providing grounded recommendations, and highlighting the important gaps that need to be addressed.

Enter the European Society for Organ Transplantation (ESOT) CLAD guidelines, reported in this issue of *Transplant International* [11]. A panel of noted European lung transplant experts spanning 8 countries has produced a document that at least in part fills this role. The group conducted a comprehensive literature search resulting in 8 recommendations addressing various CLAD prevention and treatment subjects, including macrolide therapy, maintenance immunosuppressive choices, extracorporeal photopheresis, and anti-rejection therapies, among others. The choice of what topics to address is strategic, as these reflect some of the most widely used and widely available therapies for CLAD, some of which have significant risk such as anti-thymocyte globulin and alemtuzumab. It should be noted that this is not the first guideline on CLAD management. A 2014 joint statement of the International Society for Heart and Lung Transplantation (ISHLT), European Respiratory Society, and American Thoracic Society summarized potential treatment strategies for CLAD (then BOS), in addition to diagnostic recommendations, though there was limited focus on prevention. This document also predated the 2019 definition consensus statement and particularly given the evolution of the evidence-base in the interim, a new guideline statement 12 years later is logical and much needed.

Due to limitations imposed by the ESOT guideline format, which restricts these documents to 8 PICO questions and associated recommendations, the authors acknowledge that they could not delve comprehensively into other important topics which are relevant to CLAD prevention and treatment. These include the role of induction therapy, palliative care strategies, retransplantation, anti-reflux surgery, corticosteroid therapy, and others. While understandable, these are regrettable omissions which render the document non-definitive and mean the community must await either a new iteration of this document or another guideline from another organization. Induction therapy and retransplantation in particular are important aspects of transplant care on which guidance is needed, particularly given practice is heterogeneous [12]. There have been several clinical trials on induction therapy in lung transplantation, but none have demonstrated an effect, and these drugs can have risks for patients with frequent

infections [13, 14]. Meanwhile retransplantation is a critical therapeutic resource for advanced CLAD in an otherwise well patient but one which is difficult to balance against first-time transplants in donor resource-constrained settings. While it is addressed by the ISHLT candidate selection guidelines, this document would seem an ideal place to formally comment on its use.

Considered on its own merits though, this author group has produced a meaningful evidence-based document with measured clinical guidance that will be helpful for smaller/newer lung transplant programs, in training lung transplant clinicians, and in helping existing programs re-evaluate their current approaches. Most importantly, it sheds light on the area most in need of study: CLAD therapeutics. It is this author's hope that with the evolution of the evidence base in recent years, armed with a standard definition and consensus endpoints [15], and with the current trials underway, we are entering an era in which the next iteration of a CLAD management document contains evidence for therapies which are not only effective in preventing CLAD, but in treating patients already affected by it.

## Data availability statement

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

## Author contributions

KH wrote the editorial.

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**Abbreviations:** CLAD, chronic lung allograft dysfunction; BOS, bronchiolitis obliterans syndrome; RCT, randomized clinical trial; ECP, extracorporeal photopheresis; ESOT, European Society for Organ Transplantation; ISHLT, International Society for Heart and Lung Transplantation.



## References

1. Dellgren G, Cherikh WS, Singh TP, Kim KH, Lewis A, Keller BC, et al. The international thoracic organ transplant registry of the international society for heart and lung transplantation: 2026 annual report of heart and lung transplantation. *J Heart Lung Transpl* (2026). doi: 10.1016/j.healun.2026.04.001
2. Burke CM, Theodore J, Dawkins KD, Yousem SA, Blank N, Billingham ME, et al. Post-transplant obliterative bronchiolitis and other late lung sequelae in human heart-lung transplantation. *Chest* (1984) 86(6):824–9. doi: 10.1378/chest.86.6.824
3. 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) 38:1–44. doi: 10.1016/j.healun.2019.03.009
4. Dellgren G, Lund TK, Raivio P, Leuckfeld I, Svahn J, Holmberg EC, et al. Effect of once-per-day tacrolimus versus twice-per-day ciclosporin on 3-year incidence of chronic lung allograft dysfunction after lung transplantation in scandinavia (ScanCLAD): a multicentre randomised controlled trial. *Lancet Respir Med* (2023) 12:34–44. doi: 10.1016/s2213-2600(23)00293-x
5. Combs MP, Walter K, Hixson H, Belloli EA, Najor MS, Chan KM, et al. Impact of tacrolimus vs cyclosporine on chronic lung allograft dysfunction incidence and allograft survival in the international society of heart and lung transplantation registry. *J Heart Lung Transpl* (2025) 44(3):307–17. doi: 10.1016/j.healun.2024.10.013
6. Perch M, Corris P, Lordan J, Bessa V, Magnusson J, Verleden GM, et al. A European multi-center, randomized controlled trial of pirfenidone in bronchiolitis obliterans syndrome after bilateral lung transplantation. *Eur Respir J* (2025) 67:2402154. doi: 10.1183/13993003.02154-2024
7. Benazzo A, Cho A, Auner S, Schwarz S, Kovacs Z, Ramazanova D, et al. Extracorporeal photopheresis for the prevention of rejection after lung transplantation: a prospective randomised controlled trial. *Eur Respir J* (2025) 65(2):2400733. doi: 10.1183/13993003.00733-2024
8. Vos R, Vanaudenaerde BM, Verleden SE, De Vleeschauwer SI, Willems-Widyastuti A, Van Raemdonck DE, et al. A randomised controlled trial of azithromycin to prevent chronic rejection after lung transplantation. *Eur Respir J* (2010) 37(1):164–72. doi: 10.1183/09031936.00068310
9. Kapnadak SG, Milinic T, Morrell ED, Brandon W, Mayer M, Knapp S, et al. Approach to prevention and management of chronic lung allograft dysfunction in North American lung transplant centers. *J Heart Lung Transpl* (2026) 45:1470–3. doi: 10.1016/j.healun.2026.06.004
10. Michaels M, Kaur K, Weatherald J, Nagendran J, Aversa M, Nador R, et al. Variability in lung transplant clinical practices in Canada: results of a national survey. *Transpl Direct* (2026) 12(2):e1897. doi: 10.1097/txd.0000000000001897
11. Bos S, Hellemons M, Dave K, Engel MFM, Holm A, Morlacchi L, et al. European society for organ transplantation clinical practice guideline on prevention and treatment of chronic lung allograft dysfunction. *Transpl Int* (2026) 39:16959. doi: 10.3389/ti.2026.16959
12. Shagabayeva L, Osho AA, Moonsamy P, Mohan N, Wolfe S, Langer NB, et al. Induction therapy in lung transplantation: a contemporary analysis of trends and outcomes. *Clin Transpl* (2022) 36(11):e14782. doi: 10.1111/ctr.14782
13. Snell GI, Westall GP, Levvey BJ, Jaksch P, Keshavjee S, Hoopes CW, et al. A randomized, double-blind, Placebo-controlled, multicenter study of rabbit ATG in the prophylaxis of acute rejection in lung transplantation. *Am J Transpl* (2014) 14(5):1191–8. doi: 10.1111/ajt.12663
14. Jaksch P, Ankersmit J, Scheed A, Kocher A, Muraközy G, Klepetko W, et al. Alemtuzumab in lung transplantation: an open-label, randomized, prospective single center study. *Am J Transpl* (2014) 14(8):1839–45. doi: 10.1111/ajt.12824
15. Greenland JR, Perch M, Halloran K, Levine DJ, Morrell ED, Reed A, et al. Considerations for endpoints in lung transplant clinical trials: an ISHLT consensus statement. *J Heart Lung Transpl* (2025) 45:e104–e128. doi: 10.1016/j.healun.2025.09.017



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# The INTEGRITY guidelines – ethical guidance for research involving deceased donation and transplantation

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The absence of ethical guidance for research that involves actual and prospective deceased donors of organs, cells or tissues for transplantation presents challenges for researchers, donation agencies, and participants in such research. To address this, the European Society for Organ Transplantation (ESOT), together with the International Society for Organ Donation Professionals (ISODP) and The Transplantation Society (TTS), convened an interdisciplinary, multinational team to develop the first international guidelines for ethical practice in research involving deceased donation and transplantation activities (INTEGRITY). The guidelines were developed over twelve months via an international, iterative consultative process. The resultant principles are presented in this article.

### KEYWORDS

deceased donation, ethics of clinical research, guidelines, research ethics, transplantation

## Introduction

The donation of human organs, cells, and tissues after death for use in therapeutic transplantation often presents ethical complexities due to the intersection of end-of-life care with opportunities for donation. Foremost among these are concerns for the validity of decision-making about donation and respect for the wishes of the prospective donor, for the wellbeing of donors prior to death, and for the wellbeing of their families throughout the end-of-life period [1]. The allocation of donated organs and tissues for use in transplantation further entails concern for equity of access to these scarce resources [2], and ethical

challenges can also arise in decision-making by organ transplant candidates about acceptance of offered organs from deceased donors [3].

These ethical challenges are increasingly complicated by the addition of research considerations, as efforts to optimise the recovery and utilisation of organs and tissues in transplantation require trials of new interventions involving deceased donors or donated organs and tissues [4]. There is also growing recognition that individuals in whom donation for transplantation after death is considered represent a valuable potential source of organs, cells and tissues that may be used in various research activities beyond the field of transplantation [5]. Relevant research activities range from pre- or post-mortem donor or donor-organ intervention studies aimed at improving utilisation of organs for transplantation, to use of donor tissue samples in basic sciences research, investigation of family experiences of and decision-making about deceased donation, studies evaluating the properties or outcomes of tissue grafts, and many more [6–13].

Ethical guidance for deceased donation for the purpose of transplantation is well-established at the international level [14], as is ethical guidance for research that involves living persons [15]. However, there is little guidance available to support the ethical design and conduct of research that involves people for whom donation for transplantation is considered during the end-of-life period (henceforth ‘donors’) or recipients of transplants obtained from deceased donors (henceforth ‘recipients’). In the context of deceased donation, the end-of-life period typically extends from the time when death is first anticipated, throughout the dying process and beyond the determination of death. This period may encompass both pre- and also post-mortem interventions in the body of the donor. Following the end-of-life period, materials donated by a deceased donor or data relating to a donor may be used in research even several years later, for example when studying the long-term outcomes of deceased donor transplantation. Ethical guidance for biobanks may address post-mortem use of materials and data but is typically designed to support research use of materials donated by living persons or obtained after death in accordance with an advance directive [8, 9].

Consequently prospective researchers, transplant clinicians, and professionals involved in deceased donation for transplantation (henceforth ‘donation professionals’) face considerable difficulties when developing, evaluating or implementing studies that intersect in various ways with deceased donation for transplantation [16–21]. Some guidance has addressed specific types of research involving deceased donors, such as research involving the recently deceased and donor-intervention studies [20, 22], or regulatory frameworks in specific jurisdictions [23]. However, no comprehensive guidance has been developed that considers the full range of ethical considerations pertinent to the diverse research activities that may indirectly or directly involve donors [16]. Consequently, uncertainty regarding ethical standards and governance processes presents risks of harm for those involved in or impacted by research activities and may also impede or prevent valuable research.

To address this problem, the European Society for Organ Transplantation (ESOT), together with the International Society for Organ Donation Professionals (ISODP) and The Transplantation Society (TTS), convened an interdisciplinary, multinational team to develop the first international guidelines

for ethical practice in research involving deceased donation and transplantation activities (INTEGRITY). In this paper, we report on the INTEGRITY Project, outlining the development process, briefly discussing the significance of the eight core ethical principles which underpin the full guidelines, and presenting the complete set of principles. The complete guidelines are accessible here [<https://esot.org/the-integrity-project/>] in several languages.

## Development of the guidelines

The guidelines were developed over 12 months via an international, iterative consultative process (see [Figure 1](#)). This approach was designed to explore potential ethical considerations from a wide range of perspectives and experiences, as these considerations may be significantly impacted by sociocultural, economic and health system factors in the local context in which research takes place. The development process can be understood in four main phases: initial scoping, iterative drafting with expert input, public consultation, and final validation.

### Initial scoping

Building on the work of a ‘think tank’ group assembled by ESOT as part of the 2021 Milan Congress [16], an initial set of ethical concerns and questions of specific relevance to various types of research involving deceased donation was developed via review of the literature and targeted consultation with members of the ‘core’ 13 member INTEGRITY Project Team. This international group comprised individuals with diverse experience and expertise in health law and ethics, research and clinical practice involving deceased donation and transplantation, and lived experience of transplantation. The resultant list was further expanded and refined in consultation with the larger 27 member ‘collaborator’ team representing a wider range of expertise from around the world, and with stakeholders who participated in a hybrid workshop held in Melbourne, Australia in May 2025.

### Iterative drafting

An initial draft of the guidelines was prepared by members of the core team and revised in response to feedback from collaborators received during a hybrid workshop held during the ESOT Congress in London, United Kingdom in June 2025, and via email circulation.

### Public consultation

A further draft was developed, which was made available for public consultation between 2nd October and 7th November 2025, via an anonymous online survey hosted on the Research Electronic Data Capture software platform [24]. Participation was possible in English, Arabic, Chinese, French, Italian, Japanese, Portuguese and Spanish. The text of the guidelines and the survey was initially translated using Claude (<https://claude.ai>), with all translations subsequently reviewed and validated by native speakers with professional experience in transplantation or ethics. In addition to some basic demographic questions, the feedback questionnaire requested levels of agreement with the proposed principles of the

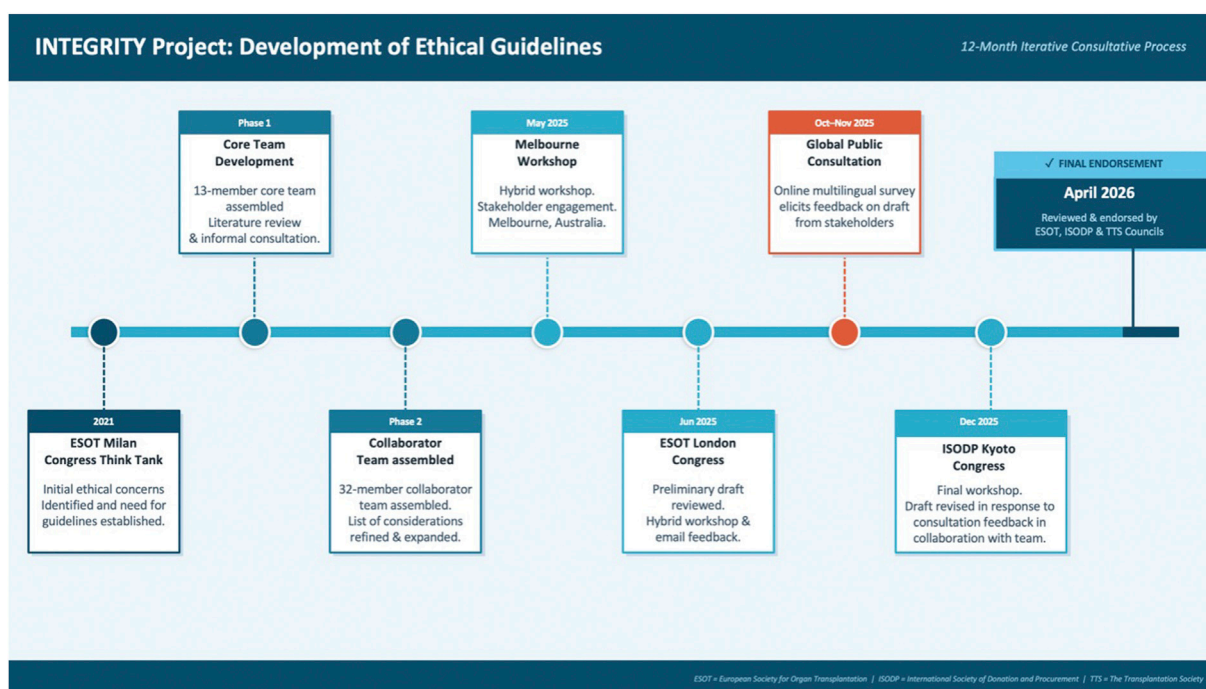


FIGURE 1  
Development process for the INTEGRITY Guidelines.

draft guidelines using a five-point Likert scale and respondents had the option of providing comments to clarify their views or propose additions or revisions.

Any person aged 18 years or older was eligible to participate in the consultation. Participation was encouraged via social media posts on LinkedIn and X and email communications to the members of ESOT, ISODP, and TTS, and by email dissemination among collaborator networks. National and regional professional societies for transplantation and donation, as well as research ethics organizations and representatives of stakeholders such as the World Health Organization (WHO) and the World Medical Association were also contacted via email to invite their participation.

## Final validation

The results of the consultation survey were used to guide further revisions to the draft guidelines by the core project team, which were again reviewed and further revised in response to feedback from the collaborator team. A final workshop was held in Kyoto, Japan during the ISODP Congress in December 2025 at which further feedback was received. The final version was reviewed and endorsed by the ESOT, ISODP, and TTS Councils in April 2026.

## Results of the public consultation

271 complete responses were submitted during the official consultation period, by respondents from 48 countries. Respondents represented a broad range of countries and

professional backgrounds, reflecting the diversity of perspectives included in the consultation process. A majority were female (53%; male 44%; 3% non-binary or undisclosed) and identified as health professionals (54%; 13% were non-clinical organ, cell or tissue banking professionals; 10% ethicists, legal scholars, or members of ethics committees; 6% researchers; 4% donor family members or transplant recipients; 11% other including members of the public). No proposed draft principle received less than 75% agreement (including agree and strongly agree responses), and 74% of the statements including the proposed core principles, received at least 90% agreement.

## The INTEGRITY guidelines

The Guidelines comprise a preamble that defines their scope, a set of eight core ethical principles under which several subprinciples are outlined that provide further specification, and a glossary that defines key terms used in the guidelines.

## Purpose and scope

The guidelines are intended to assist researchers, transplantation and donation professionals, prospective research participants, donation decision-makers (including surrogates making a decision on behalf of a possible donor), and ethics governance bodies in designing, evaluating and conducting research that involves deceased donors or use of organs, cells, or tissues from deceased donors. The guidelines apply to research involving adults and children, including persons who lack decision-making capacity.



TABLE 1 Principle 1.

|                                                                                                                                                                                                                                                                                                                                                                                                 |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Principle 1                                                                                                                                                                                                                                                                                                                                                                                     |
| 1. All research activities involving deceased donors should be consistent with respect for the dignity of human beings and their bodies.                                                                                                                                                                                                                                                        |
| 1.1 The involvement of deceased donors in research should be celebrated and acknowledged as a valuable gift and contribution to society comparable in significance to donation for transplantation.                                                                                                                                                                                             |
| 1.2 Designated custodians of a deceased donor's body, organs, cells or tissues should take responsibility for the respectful care of these materials throughout all research activities until they are transplanted into living persons, returned to donor families, stored, or appropriately disposed of.                                                                                      |
| 1.2.1 Where feasible, the ultimate disposal of human remains should be in accordance with the known or expected wishes of the deceased and/or their family.                                                                                                                                                                                                                                     |
| 1.2.2 Where relevant, those making a decision about deceased donation of organs, cells or tissues in the context of research should receive information about the likely duration of storage of donated organs, cells or tissues, and about arrangements for the disposal of remains (see Principle 7).                                                                                         |
| 1.3 Research activities should respect the cultural, religious, and spiritual needs of people and their families, facilitating where possible preferred practices relating to end-of-life care, the process of death, decision-making, and treatment of the human body before and after death.                                                                                                  |
| 1.4 When research activities involve interventions in the body of a deceased donor, care for the body of the deceased should be consistent with the respectful treatment of a deceased donor for transplantation.                                                                                                                                                                               |
| 1.5 If the body of a deceased donor is physiologically maintained or perfused by machines, e.g., mechanical ventilators or extracorporeal membrane oxygenation (ECMO) devices, for the purpose of research after death:                                                                                                                                                                         |
| 1.5.1 The family of the deceased should receive support consistent with that provided to the families of deceased donors for transplantation during the period leading to organ and tissue removal.                                                                                                                                                                                             |
| 1.5.2 The family of the deceased should be permitted, where feasible, to attend the deceased.                                                                                                                                                                                                                                                                                                   |
| 1.5.3 The duration of time during which the deceased body is physiologically maintained or perfused should be strictly limited to the period necessary to achieve the relevant research aims, with careful consideration of the potential burdens of prolonged protocols on grieving families.                                                                                                  |
| 1.6 All research involving deceased donors should be subject to scientific and ethical review and oversight at an appropriate level.                                                                                                                                                                                                                                                            |
| 1.6.1 Authorities with responsibility for overseeing human research, deceased donation, and transplantation activities in each jurisdiction should establish clear guidance and mechanisms to ensure that research involving deceased donors is reviewed appropriately by competent boards or committees, and that the principles outlined in these guidelines are upheld in the local context. |
| 1.6.2 The level of oversight and review required may differ according to the nature of the research, the populations that may be impacted by the research, and/or the potential burdens or risk of harm associated with research activities.                                                                                                                                                    |
| 1.6.3 Where unclaimed bodies or organs, cells, or tissues removed from unclaimed bodies after death may legally be used in research, there should be a specific process for ethical oversight of such use to avoid the exploitation of vulnerable individuals and communities.                                                                                                                  |
| 1.7 When proposed research activities involving deceased donors are not addressed by current legal or ethical frameworks, such frameworks should, where possible, be revised to provide governance of these activities.                                                                                                                                                                         |
| 1.7.1 Where revision of existing frameworks has not yet occurred, exceptional arrangements for ethical review and oversight of proposed research activities may be implemented, provided that the proposed research adheres to these guidelines and complies with relevant laws and ethical standards governing deceased donation in the relevant jurisdiction.                                 |
| 1.8 Consistent with routine ethical safeguards for human research, when research involves deceased donors:                                                                                                                                                                                                                                                                                      |
| 1.8.1 The scientific merit of proposed research should be evaluated to avoid unnecessary or futile research activities involving deceased donors.                                                                                                                                                                                                                                               |
| 1.8.2 A clear and independent mechanism(s) should be established to communicate with stakeholders, facilitate management of inquiries, and handle complaints or concerns about proposed or actual research activities.                                                                                                                                                                          |

They are applicable to a range of research activities including studies that involve:

1. Interventions in the body of the deceased donor.
2. Collection and/or use of personal information from or about the deceased donor.
3. Removal, interventions in, and/or use of organs, cells, or tissues donated after death—including prior to transplantation, use of stored materials, and studies of transplant recipients.
4. Collection and use of information from donor families, recipients of transplants from deceased donors, or healthcare professionals about their lived experiences of deceased donation.
5. Secondary use of information about deceased donors or donated materials originally collected for clinical or quality assurance purposes.

## Use and limitations of the guidelines

All human research activities require ethical governance, including those that intersect with deceased donation for transplantation. As noted in Principle 1.6, “All research involving deceased donors should be subject to scientific and ethical review and oversight at an appropriate level.” The ethical oversight of deceased donation and transplantation provides a reassuring framework for the protection of donors and transplant recipients. However, research activities require specific consideration of ethical elements that are not within the scope of clinical practice in donation and transplantation. On the other hand, many of the ethical complexities of research that intersects with deceased donation may be unfamiliar to individuals or groups responsible for the ethical oversight of human research.

The INTEGRITY Guidelines are intended to help bridge the gap between donation and transplantation ethics, and routine human

TABLE 2 Principle 2.

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Principle 2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| 2. Research activities should be designed to provide equitable opportunities for individuals and communities to participate in, contribute to, and benefit from research involving deceased donors.                                                                                                                                                                                                                                                                                                                                                               |
| 2.1 Research should promote equity by ensuring fairness in the distribution of opportunities to be a research participant, of potential burdens and risks of research, and of potential benefits from research.                                                                                                                                                                                                                                                                                                                                                   |
| 2.2 No individuals or groups should be systematically excluded from opportunities for involvement in research solely because of actual or perceived vulnerability.                                                                                                                                                                                                                                                                                                                                                                                                |
| 2.2.1 To facilitate opportunities for inclusion of vulnerable populations in research, efforts should be made to address potential vulnerabilities, to reduce risks, and to remove barriers to participation in research activities.                                                                                                                                                                                                                                                                                                                              |
| 2.3 When considering the benefits of research to which individuals and communities should have access, these should be defined as including the scientific knowledge gained or therapeutic products derived from research that may be used to prevent illness or disease progress, or to improve quality of care, e.g., for patients and their families, and/or to improve health outcomes                                                                                                                                                                        |
| 2.4 Where relevant, research involving deceased donors should strive to support equity in healthcare and health outcomes, consistent with the values that underpin deceased donation and transplantation programs.                                                                                                                                                                                                                                                                                                                                                |
| 2.5 Where possible, researchers and research institutions, donation and transplantation professionals, organizations, and registries, healthcare organizations, and other relevant organizations should cooperate and/or collaborate <ul style="list-style-type: none"> <li>• to increase opportunities for research,</li> <li>• to improve efficiency in use of resources for research,</li> <li>• to maximise the relevance and value of research for diverse populations, and</li> <li>• to share information and knowledge for the benefit of all.</li> </ul> |
| 2.6 When data about deceased donation and transplantation activities are collected by donation or transplantation programs or by designated registries for non-research purposes and these data are not routinely made publicly available, mechanisms should be established to enable data sharing and to ensure equitable access to these data for research purposes.                                                                                                                                                                                            |

research ethics, by highlighting key ethical considerations and establishing normative standards for research involving donors.

The Guidelines comprise principles that are designed to be adapted for application in local jurisdictions, ensuring alignment with relevant national laws and existing ethical oversight. They are not intended to replace existing guidance, but rather to address gaps in guidance and reinforce the relevance of existing guidance. The principles highlight ethical considerations of particular relevance to research that intersects with routine practices relating to deceased donation for transplantation and should therefore be used in conjunction with routine ethical guidance for the conduct of human research in general and for deceased donation and transplantation of human organs, cells, and tissues. The guidelines do not provide practical advice on the implementation of principles. Further publications by the INTEGRITY Project team are planned that will provide explanatory content to assist in the interpretation and application of specific principles in particular research contexts.

While efforts were made to engage members of the public—as future possible donors- and donor family members and transplant recipients in the development of the Guidelines, these key stakeholders comprised a disproportionately small number of participants in the consultation and had limited involvement in the drafting process. Future revisions to the Guidelines should ideally be informed by a wider range of stakeholder perspectives from around the world.

## The core principles

In this section, we briefly discuss the significance of the eight core principles that provide the normative foundation for the INTEGRITY Guidelines and present their subsidiary principles in tabular format.

## All research activities involving deceased donors should be consistent with respect for the dignity of human beings and their bodies

Principle 1 (see Table 1) draws attention to the specific ethical considerations of research involving deceased donors or deceased donation. Just as the WHO has designated human organs, cells, and tissues for transplantation “health products of an exceptional nature” [14], such research should be considered ethically exceptional. Many activities within scope of the guidelines may not directly involve a living person as a subject of ethical concern, for example when stored tissue specimens are used in biomedical research. In other research, a living person such as the recipient of a deceased donor transplant may be the focus of ethical concern, and consideration of the donor may be easily forgotten. The moral status and inherent ethical value of a living person are typically regarded as substantially greater than those of a deceased individual. Nevertheless, for many people, the treatment of the dead—including the body and its parts—retains important ethical significance.

This first principle accordingly establishes a requirement to consider the ethical dimensions of research involving donors regardless of whether the donor is imminently dying, recently deceased, or long dead, while allowing that particular ethical considerations may be more or less relevant depending on the time that has elapsed since death and the nature of research activities. The principle is important because most ethical guidance for research frames ethical considerations solely with a focus on how research may affect the living; in the United States of America, regulation of human research ethics notably excludes the dead [23]. Philosophers and others disagree on whether deceased individuals should be considered to have interests, and the extent to which living persons owe respect for such posthumous interests

TABLE 3 Principle 3.

## Principle 3

3. When designing, conducting, or evaluating a research study, ethical consideration should be given to the interests of all individuals who may be involved in or impacted by the research activities.

3.1 The interests of individuals or groups that are most involved in and/or impacted by research activities should receive primary consideration in the ethical design, conduct and evaluation of research.

3.2 The assessment of risks and potential burdens and benefits of research should take into account the perspectives and interests of all individuals and groups who may be affected.

3.2.1 When research interventions in deceased donors target specific organs, cells or tissues, the potential impact of these interventions on non-target ("bystander") organs, cells or tissues used, e.g., in transplantation, should be explicitly considered.

3.2.2 When research considerations influence the donation process and/or allocation of organs, cells or tissues donated for transplantation, the potential impact of these changes on the availability of and access to transplantation for individuals and groups should be explicitly considered. (See Principle 4.)

3.2.3 The likely impact of involvement in research on fulfillment of the deceased donor's known or expected wishes regarding end-of-life care, donation for transplantation, contributing to research, or post-mortem treatment of their body should be considered when evaluating potential benefits, burdens and risks of research.

3.3 Key stakeholders should be included wherever possible in the design and ongoing oversight of research studies, e.g., donor families, transplant candidates or recipients, members of specific cultural communities, or donation and transplantation personnel, with appropriate management of potential conflicts of interests, where relevant.

3.4 Relevant donation agencies should routinely be consulted when designing studies that involve deceased donors.

3.5 Health professionals who are involved in deceased donation or transplantation activities but not engaged as researchers in studies that involve deceased donors should routinely be informed of research activities that may intersect with their clinical duties; they should have the opportunity to seek further information or assurance regarding the research and its potential impact on their practice.

3.6 Where health professionals are granted the right of conscientious objection with regards to performance of clinical duties that are inconsistent with their moral beliefs, this right should be extended to encompass clinical research activities with which they may be asked to assist.

3.6.1 When exercising the right of conscientious objection in the context of research involving deceased donors, health professionals should strive to ensure that their objection does not negatively impact opportunities for patients (or their families) to be involved in and/or benefit from research.

3.7 Research should only proceed when the expected benefits are judged to outweigh any unavoidable risks or burdens.

3.7.1 The risks and potential burdens for all those who may be negatively impacted by a research study should be avoided where possible and otherwise minimised as much as possible.

3.7.2 The standard supports and services provided to deceased donors, donor families, transplant recipients and healthcare professionals in the context of deceased donation for transplantation should also be provided when these individuals are involved in research, or when deceased donation occurs solely for the purpose of research. Additional or extended services may be necessary to ensure appropriate care is provided throughout the course of research activities.

[25]. There is similar disagreement regarding the ethical obligations living persons may have to respect the known or estimated wishes of the dead regarding the treatment of their bodies, personal information, and material property. While laws sometimes provide a legal framework to govern the disposal of property, bodies, and even privacy of personal health data after a person's death, there are often gaps. In many jurisdictions, for example, laws provide for the removal and use of tissues and organs after death in transplantation and research but lack guidance for those tasked with making decisions about involvement of a donor in research activities that may commence immediately prior to or following the determination of death [26].

Several of the subprinciples derived from Principle 1 reaffirm commonly accepted norms such as the importance of considering the cultural, religious and spiritual values and preferences of stakeholders in research, especially in the context of end-of-life care and decision-making. However, others address particular concerns that may be rarely encountered in other fields of research. For example, Principle 1.5.3 states that when the body of a deceased donor is physiologically maintained for the purpose of research, as was done in the Parsons' model for xenotransplantation trials [5], the duration of such trials should be strictly limited. This aligns with the familiar principle of nonmaleficence in human research ethics which entails the minimisation of risks and burdens during research

and limits the duration of trials or interventions. Extended utilisation of a deceased body in research requires careful assessment of the necessity of such use and proportionality of the benefits of research compared with the burdens on families, where relevant, and potential threats to the dignity of the deceased [22].

### Research activities should be designed to provide equitable opportunities for individuals and communities to participate in, contribute to, and benefit from research involving deceased donors

The second principle (see Table 2) affirms the importance of justice in research, as demonstrated through equitable opportunities to participate in and benefit from research. Although this is a well-established principle of human research ethics, it merits inclusion as a core principle in these guidelines given the importance of equity in deceased donation and transplantation. Deceased donation for transplantation is often considered a public good, ideally conducted under the auspices of governmental health authorities who must uphold public trust by ensuring equitable access to the benefits of transplantation for the populations that contribute to donation.

Equitable inclusion in research is especially important if all members of the wider community are to benefit from scientific

TABLE 4 Principle 4.

## Principle 4

4. Research activities should be designed in ways that safeguard the availability of donated organs, cells, and tissues and ensure equitable access to the benefits of donation and transplantation.

4.1 Efforts should be made to maintain the availability of organs, cells, and tissues for use in transplantation while supporting opportunities for involvement of deceased donors in research and use of donations in research.

4.2 Evaluation of the potential benefits and risks of proposed research involving deceased donation must consider the potential impact of research activities on the availability and equitable allocation of organs, cells, and tissues for use in transplantation.

4.2.1 Ensuring the availability of organs, cells, and tissues for use in therapeutic transplantation should usually take priority if there is a conflict between this goal and research goals that require the involvement of deceased donors in research or the use of donated organs, cells, or tissues.

4.3 Clear policies should be established by relevant institutions, in particular donation and transplantation agencies, to guide the management of requests for endorsement of or involvement in research studies, particularly those that require access to and use of deceased donor bodies, organs, cells or tissues, or data about deceased donors, donor families, or transplant recipients.

4.3.1 Policies should enable procedural fairness in decision-making about support for research projects and foster equity in access to donated organs, cells, and tissues for use in research and transplantation.

4.3.2 Representatives of donation authorities, deceased donor and transplant recipient communities, healthcare professionals, custodians of donor organs, cells or tissues, custodians of data in donation and transplant registries, and relevant research communities should be included in the development of policies and guidelines.

4.3.3 Prioritisation of research requests to make use of scarce resources (e.g., donated organs or tissues) or populations (e.g., deceased donors that may be involved in intervention studies) should be guided by the values and goals of relevant stakeholders, (e.g., donation programs and deceased donor communities).

4.4 Decisions about proposed research activities that may temporarily disrupt the routine allocation of organs, cells, and/or tissues for transplantation should be made transparently and clearly documented and disclosed to key stakeholders, including the public, which comprises the population of future deceased donors and transplant recipients.

4.4.1 Where relevant, research protocols should include a mechanism for monitoring and evaluation of the outcomes of any disruptions to routine allocation of organs, cells or tissues for transplantation. The results of this evaluation should inform future decision-making about research and about allocation of donated organs or tissues.

advances. Equitable representation in research activities supports a fair distribution of benefits and burdens and helps to produce therapies that are effective for all relevant populations [27]. Factors contributing to disparities in deceased donation for transplantation and access to transplantation and research participation, such as distrust among some communities, may be exacerbated when research intersects with donation activities [28].

Equity is also considered in the context of Principle 4, which focuses on the potential impact of research activities on the availability of and equity of access to transplantation rather than equitable inclusion in research and equity in the outcomes of research. Inevitably, there is some entanglement between the principles. In particular, Principle 4 addresses procedural justice in decision-making about research which has important implications for equity in access to transplantation as well as opportunities for research.

**When designing, conducting, or evaluating a research study, ethical consideration should be given to the interests of all individuals who may be involved in or impacted by the research activities**

Deceased donation is fundamentally relational, often involving complex connections between various stakeholders that require careful attention to ensure that relevant interests and the broader implications of actions or inactions are considered in decision-making. Exploring an opportunity for deceased donation, for example, can impact not only the possible donor but also their family, other patients requiring healthcare, transplant candidates, as well as public trust and future donation behaviours. When an opportunity for donation involves research activities, there may

be additional or alternative stakeholders involved, and the key considerations in terms of potential benefits, burdens and risks for some or all stakeholders may change.

The scope of the third principle (see Table 3) may initially appear unfeasible, requiring an excess of ethical concern by researchers. However, it is important to note that consideration for the interests of all does not entail exhaustive exploration of every possible interest, nor does it require that all those potentially impacted must be directly involved in decision-making about research. Subprinciple 3.1 notably specifies that “primary consideration” should be given to those most involved in or impacted by research activities. The views of particular stakeholders may be more or less relevant when designing a particular study. Similarly, the level of detail required for consent for involvement in a study will differ according to the nature of research activities in which stakeholders are involved.

**Research activities should be designed in ways that safeguard the availability of donated organs, cells, and tissues and ensure equitable access to the benefits of donation and transplantation**

Research activities have the potential to increase the availability of therapeutic products derived from donated organs, cells, or tissues [29–31]. For example, reconditioning or repair of donor organs prior to transplantation can improve rates of utilisation and/or graft outcomes [32]. However, inclusion of donated organs in some experimental studies could also risk non-utilisation or poorer outcomes if studies are unsuccessful. Research can also disrupt routine allocation systems if some transplant candidates or centres are not eligible, or unwilling, to participate in a study,



TABLE 5 Principle 5.

## Principle 5

5. The privacy and confidentiality of personal information of deceased donors, donor families, and transplant recipients should be protected in all research activities.

5.1 Appropriate mechanisms to ensure ethical governance of research activities that involve collection, storage, sharing or use of personal information should be implemented according to the nature of the activity(ies) and the context in which they occur. Such mechanisms should conform to privacy and data protection laws and standards in the relevant jurisdiction(s).

5.1.1 Specific provisions should be made by researchers and donation and transplantation registries for governance of data collected from Indigenous peoples to respect data sovereignty and the values and preferences of Indigenous peoples [33].

5.2 Information about the collection, storage and use of data in donation and transplant registries should routinely be available to persons making decisions about deceased donation or transplantation; this information should address the possible use of data in research and, where relevant, options to access or limit use of data, withdraw data, or otherwise control data held in registries.

5.3 Access to and use of data held in donation and transplantation registries by researchers should be subject to ethical review and oversight, consistent with other forms of research involving deceased donors.

5.3.1 Registries must establish ethical guidelines for the evaluation of requests to access and use registry data in research.

5.3.2 Registries should maintain publicly available policies governing protection, use and access to data for research purposes.

5.3.3 The level of ethical review required for research involving use of data relating to deceased donation or transplantation activities will depend on several factors including:

- the conditions under which data were collected such as consent provisions relating to use of data in research,
- the nature of the proposed research including associated risks and potential benefits of data use,
- the nature of the data that will be provided to researchers.

5.3.4 Some studies that use only aggregated and anonymised data in research may not require formal research ethics review, whereas others, e.g., those in which potentially sensitive population characteristics or outcomes are the focus of analysis, may require substantive evaluation.

5.3.5 When research involves use of data that are already in the public domain, such as registry data that may be reported in public or governmental websites, formal research ethics review may not be required; researchers and organizations that manage donation and transplantation data should nevertheless strive to ensure that relevant values and principles from these guidelines are applied in the design and implementation of their research.

5.4 Where relevant, research protocols should include plans for the management of personal information about deceased donors that may be discovered, and which could have significance for donor families, transplant recipients, or specific deceased donor communities.

5.4.1 Consent for research may include, where feasible and appropriate, tiered or dynamic consent mechanisms that would permit, for example, relevant individuals to be contacted in future to make a decision about disclosure of new findings or significant health-related information, or to make decisions about future uses of data or donated organs, cells, and tissues (see Principle 7) [34–36].

5.5 Where specific research activities pose a significant risk of privacy breaches or loss of anonymity for individual donors, donor families, or transplant recipients, e.g., publication of case reports or highly innovative translational research, this risk should be clearly disclosed, and explicit consent should be obtained. (see Principle 7)

5.5.1 Additional safeguards should be incorporated in research protocols as necessary to manage risks to privacy, such as provision of guidance or professional support to transplant recipients or donor families involved in high profile studies relating to social media use, or management of interview requests from news media.

5.6 Care should be taken to ensure that necessary safeguards for privacy and data protection in research protocols do not create barriers to inclusion of populations that may benefit from research.

5.6.1 Deceased donation or transplantation programs that have low volumes of activity should not be prevented from conducting or reporting research involving small populations. Strategies should be used to address concerns about anonymity, e.g., with collaboration between programs or centres (see Principle 2) used to pool data and care in the reporting of results (see Principle 6).

potentially undermining equity in transplantation. Safeguarding the availability of donated organs, cells, and tissues for transplant and equity of access to the benefits of donation and transplantation is thus a core concern that must be carefully considered when evaluating the potential benefits and risks of proposed research. The principle does not preclude research that may temporarily disrupt routine allocation or even foreseeably decrease the supply of donated tissues or cells for transplantation, for example, where the longer-term benefits of research are expected to outweigh these harms.

Principle 4 (see Table 4) also highlights the importance of procedural fairness in institutional decision-making about research involving donors, which has implications for safeguarding donation for transplantation as well as equitable opportunities for research (as discussed in the context of Principle 2). Historically, there has been little

transparency regarding the allocation of donated materials, typically those deemed unsuitable for use in transplantation, to researchers. Anecdotal reports suggest that in many countries, allocation decisions for research are often made *ad hoc*, in response to requests from research teams, which may favour those with pre-existing relationships with donation programs or personnel. More work is needed to help institutions and agencies establish procedures to govern these decisions, and those relating to direct involvement of possible donors in research prior to death, not least to improve transparency and accountability for key stakeholders such as donor and recipient communities. Further specification of principles to guide allocation decisions and policies when research activities may temporarily disrupt routine allocation systems, especially of organs for transplantation, may also be helpful.

TABLE 6 Principle 6.

## Principle 6

6. All research activities must be conducted transparently and be open to scrutiny, to uphold public trust in deceased donation and in the integrity of research involving deceased donors.

6.1 Routine data collection is a fundamental safeguard of donation and transplantation programs and should be maintained throughout all research activities involving deceased donors.

6.1.1 Data relating to the involvement of deceased donors in research should be routinely collected and reported in national registries, consistent with the international standards that mandate the routine collection and public reporting of data relating to deceased donation for transplantation [14, 37].

6.1.2 Where national registries do not yet exist, deceased donation and transplantation programs should routinely collect and regularly review information about research activities that involve deceased donors.

6.2 Appropriate mechanisms to ensure the transparency of research activities involving deceased donors should be implemented according to the nature of the research activity(ies) and the context in which they occur. Such mechanisms should conform to privacy and data protection laws and standards in the relevant jurisdiction(s).

6.2.1 A centralised, publicly accessible source of general information about research activities involving deceased donors should be established in association with equivalent sources of information about deceased donation for transplantation; this may provide links to other sources of more detailed information about specific research activities, e.g., online clinical trials registries or research project websites.

6.2.2 Researchers have primary responsibility for ensuring mechanisms are in place to maintain effective communication with research participants or other stakeholders in studies, and to effectively disseminate the findings of research to relevant stakeholder communities.

6.2.3 When the nature of research activities may delay or limit the disclosure of results or activities to stakeholders, e.g., due to the timeline of research protocols or commercial interests, these constraints on disclosure should be communicated to relevant stakeholders and carefully considered when evaluating the potential benefits and burdens of the proposed research.

6.3 Wherever possible, the results of research involving deceased donors should be:

- published in scientific journals and communicated to relevant scientific and professional audiences to inform future research in which the involvement of deceased donors is considered.
- made available to all relevant stakeholders, including donor families, using appropriate mechanisms to support their access to and understanding of research outcomes, and allowing for individuals to opt-out of such communications if desired.

6.4 When submitting results for publication, researchers should be required to attest that research involving deceased donors has been conducted in accordance with relevant ethical frameworks for research, and that any deceased donation activities involved in the research were consistent with the ethical standards outlined in the WHO Guiding Principles, [14] and the Declaration of Istanbul on Organ Trafficking and Transplant Tourism [38].

6.4.1 The publication of research that investigates or documents unethical practices relating to deceased donation or transplantation, e.g., evaluating the outcomes of travel for transplantation that involves organ trafficking, is ethically permissible provided that the research itself adheres to relevant ethical standards.

6.5 Data about research activities should generally be reported in aggregate to reduce the risk of identification of individuals.

6.5.1 Where small numbers, e.g., in low volume donation or transplantation programs, increase the risk of individuals or groups being identified in data reports, consideration should be given to the omission of more granular results in publicly accessible reports.

6.6 When disseminating research findings to relevant audiences, efforts should be made to avoid the stigmatization of individuals and populations and to maintain respect for research participants even when they cannot be directly identified.

6.6.1 Efforts to avoid the stigmatization of populations - for example, when reporting on populations that have a higher probability of requiring organ transplantation and a lower probability of contributing to deceased donation of organs - should not discourage the transparent publication of research that reports specific challenges impacting specific groups, which is essential for informing efforts to address inequities.

Concerns about the potential stigmatisation of populations should be carefully addressed at the stage of protocol refinement rather than considered only at the time of dissemination of results.

## The privacy and confidentiality of personal information relating to deceased donors, donor families, transplant recipients, and, where relevant, healthcare professionals should be protected in all research activities

Principles 5 and 6 (see Tables 5 and 6) are consistent with routine ethical principles that stipulate protections for the privacy of research participants while advocating for transparency in research activities. In general, such considerations should also apply to research involving deceased donors, but they may sometimes be more complicated, especially when tensions arise between frameworks and policies governing data use and publication

in research and non-research settings. Such tensions are discussed in the context of Principle 6 below.

The use of health records data in research is increasingly an ethical concern, as the adoption of electronic records and artificial intelligence have expanded opportunities for research [39]. Data relating to deceased donors may present complexities in addition to those associated with use of routine health records data, or even use of data relating to deceased individuals, which is an emerging area of ethical interest [40]. For example, some data are collected for the purpose of donation for transplantation rather than therapeutic care of the donor, other clinical data may be collected after death, and some datasets will inevitably combine private information about individual donors as well as recipients, or donor family members as well as donors. These and other factors may introduce new ethical

TABLE 7 Principle 7.

## Principle 7

7. Research activities should promote the autonomy of individuals who may be involved in or impacted by research.

7.1 Research activities should respect a person's fundamental interest in

- having control over their body and personal information,
- having the option to be involved in decision-making that may impact them and,
- where relevant, having substitute decision-makers make decisions in accordance with the person's values and preferences.

## Recruitment

7.2 Individuals who are responsible for research recruitment or supporting decision-making about involvement in research should receive appropriate training. Core competencies of such individuals include being able to:

- Understand relevant ethical considerations with respect to deceased donation and transplantation as well as research.
- Provide appropriate care for the decision-makers and/or arrange appropriate referrals to other support services or care providers when necessary.
- Support informed decision-making about research opportunities.

7.3 Potential conflicts of interest in the recruitment of donors, donor families, or transplant recipients for research, such as those that may arise as a result of holding dual roles as clinician-researchers, should be identified and carefully managed in accordance with relevant policies or guidelines.

7.4 Research protocols that involve recruitment of patients currently receiving clinical care -whether deceased donors or transplant recipients - should generally require coordination with relevant clinical teams or services.

7.4.1 Relevant donation and transplantation personnel should be familiar with current research activities and research coordinators should be familiar with relevant donation and transplantation programs, to avoid disclosure of misinformation and to ensure timely referral to donation, transplantation, or research coordinators, as relevant.

7.4.2 Where the recruitment of deceased donors for research is not under the auspices of donation personnel, this should routinely require coordination and consultation with relevant donation personnel or organizations.

7.5 In general, opportunities for deceased donation for transplantation should be introduced to relevant stakeholders prior to discussion of opportunities for involvement of deceased donors in research.

7.6 When decision-making about deceased donation for transplantation takes place, decision-makers should be informed of relevant opportunities for involvement of the donor in research.

7.6.1 If organs, cells, and/or tissues cannot be donated for use in transplantation, the option of donating these for use in research should be presented whenever possible.

7.6.2 When biological specimens and/or data relating to deceased donors for transplantation may be retained for the purpose of research, this should be anticipated and should usually be disclosed to individuals making a decision about donation for transplantation.

7.6.3 Where feasible and desired, it may be appropriate to offer people making a decision about deceased donation more choices about the distribution and use of donations for research purposes than are usually permitted when making decisions about deceased donation for transplantation.

7.7 Where options for involvement in research are present, for example if donated organs or tissues might be used in a range of research activities or transplant candidates might be eligible to participate in more than one clinical trial, individuals making a decision about involvement in research or donation for research should be empowered to choose from among the available options if they wish to do so.

## Authority for decision-making about involvement of deceased donors in research

7.8 Where necessary, legal frameworks should be revised and/or established to provide clear mechanisms for authorising the involvement of deceased donors in research.

7.8.1 In jurisdictions where advance care directives can be used to guide healthcare decision-making for people when they lose decision-making capacity, individuals should be enabled to express values or preferences about future involvement in research in such directives.

7.8.2 In jurisdictions that provide mechanisms for individuals to register their wishes or preferences with regards to donation for transplantation after death, e.g., by joining a donor registry, such mechanisms should include opportunities to express preferences or wishes regarding involvement in research or donation for research.

7.8.3 Where possible, the person(s) with legal authority for substitute decision-making about deceased donation for transplantation should also have authority to make decisions about a deceased donor's involvement in research.

7.8.4 In some cases, legal authority for involvement of individuals in some types of research may appropriately reside with organizations or groups other than the individuals concerned or their substitute decision-makers [42].

7.9 Public education about deceased donation should include both information about donation for transplantation and information about opportunities for individuals to be involved in research at the end of life or to donate organs, cells or tissues for use in research. Individuals should be encouraged to discuss their wishes regarding donation for transplantation and research with their families.

7.10 Clear and accessible guidance should be provided to substitute decision-makers regarding how decisions about involvement of donors in research should be made.

7.10.1 Guidance should address how to manage conflicts among members of donor families regarding the involvement of a deceased donor in research; this should be consistent with the approach to decision-making about donation for transplantation.

7.10.2 Subject to the laws in each jurisdiction, when making decisions about research activities that involve interventions in the body of a deceased donor before or after death, substitute decision-makers should prioritise the deceased donor's known or expected values and preferences.

(Continued)

TABLE 7 Continued

## Consent requirements

7.11 To support voluntary and informed decision-making, research protocols (and protocols for decision-making about deceased donation that are inclusive of donation for research) should take into account:

- The timing, frequency, and location of discussions about involvement in research,
- The mechanisms used to provide information about the research,
- The identity and role of the person involved in requesting or supporting decision-making about research and their roles and relationships with decision-makers,
- The implications of accepting or declining participation in research on opportunities for transplantation or donation for transplantation, where relevant.

7.12 Where appropriate and feasible, the process of decision-making about involvement in research and/or donation for research should be designed to support dynamic and flexible decision-making, and to minimise confusion and burdens on decision-makers while ensuring that:

- key information about the research and any practical implications that may be important are communicated and considered,
- decision-makers are supported and given the option to explore or discuss further information throughout the process,
- the limitations of information provided are made explicit (e.g., uncertainty regarding future uses of donated organs, cells, or tissues in research)
- any options for or limitations on withdrawal from research are clearly communicated.

7.12.1 When designing or evaluating specific research protocols or broader systems for the recruitment of participants in research involving deceased donors, a range of consent models should be considered and adapted where necessary to provide the best fit in the local context, while satisfying legal requirements and the ethical obligations outlined herein.

## Disclosure of information

7.13 The mechanisms used to disclose information to decision-makers about research should be designed to optimise communication and understanding of information and to reduce the burdens of information disclosure.

7.14 The disclosure of information to decision-makers about involvement in research should be designed to ensure that key information is provided, considered, and tailored to meet the preferences and needs of individuals in the context of particular research protocols. Such information should typically include:

- who is conducting the research,
- the goals of the research,
- what the research activities involve,
- the potential benefits, burdens and risks of involvement in the research, and
- how to obtain more information and/or express concerns or complaints about the conduct of the research.

7.14.1 Information about substantive risks or burdens of research involvement for individuals should routinely be explicitly disclosed and considered when seeking consent.

7.14.2 Information that is likely to be considered significant by donors, donor families or transplant recipients and/or could influence their decision-making about involvement in research should be routinely provided to those deciding about such involvement.

7.15 When involvement in research does not present substantive risks or burdens and decision-makers prefer not to receive detailed information about what research involves, it is not always necessary or appropriate to impose detailed information on decision-makers, provided that decision-makers have the opportunity to receive more information at the time of making the decision or in the future.

7.16 Some research may involve considerations that entail specific consent requirements including explicit disclosure of information. Information that is likely to be deemed significant by research participants and that should usually be disclosed during decision-making about research, where relevant, includes:

- a) The extent to which research activities may deviate from standard clinical practice in deceased donation or transplantation of deceased donor organs, cells, or tissues.
- b) The potential effects of research activities on deceased donation for transplantation or access to transplantation.
- c) The potential impact of research involvement on a donor's end-of-life care or transplant recipient's current or future clinical care, including the potential financial implications of any changes to care.
- d) Source(s) of research funding and potential commercial interests in the research, including the possibility of donated organs, cells or tissues becoming saleable products.
- e) The potential collection and/or use of genetic data.
- f) The potential creation of perpetual cell lines from donated materials.
- g) The potential use of donated materials in non-human animal research.
- h) The potential use of donated materials in research that may lead to the creation of children.
- i) The potential distribution of donated organs, cells, tissues, or personal data internationally, and/or transfer of custodianship of donated materials or data.

7.17 Potential assumptions on the part of research decision-makers that may be influenced by expectations of deceased donation for transplantation or of routine transplantation activities, and which may not be applicable in the case of some research activities should be explicitly addressed when obtaining consent.

7.18 Potential or actual conflicts of interest relating to research activities should be avoided, and unavoidable conflicts of interest should be disclosed.

7.19 When transplant candidates are offered organ, cell or tissue transplants that have been obtained from a deceased donor who was involved in an interventional study, they should receive any relevant information that may have clinical implications for their transplant.

7.19.1 If there are plans to collect or use data about the transplant recipient as part of a study that involves interventions in deceased donors, information about the study should usually be disclosed when offering the transplant, even if there is no information of potential clinical significance for the recipient.

## Waiver of consent and presumed consent

(Continued)



TABLE 7 Continued

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| <p>7.20 In some cases, an HREC [Human Research Ethics Committee] or equivalent body may approve a waiver of consent requirements or deem it reasonable to presume consent for the involvement of individuals or groups in research activities. Factors that may influence whether it is ethically reasonable to presume consent include:</p> <ul style="list-style-type: none"> <li>• the relevant legal framework(s) in the jurisdiction,</li> <li>• the practical feasibility of seeking explicit consent,</li> <li>• the level of risk or potential burdens for those involved in the research, and whether these exceed the risks or potential burdens that they would otherwise encounter in their daily life or as part of their previously agreed involvement in donation or transplantation activities,</li> <li>• whether the intended involvement of the individual or use of their data or previously collected or donated organs, cells, or tissues in research is consistent with their known or expected values and preferences,</li> <li>• the presence of any information indicating that the individual or group would not wish to be involved in this type of research, or that they would wish to make an explicit decision about any such involvement.</li> </ul> <p>7.20.1 It is generally reasonable to waive individual consent requirements for research involving use of data about deceased donors or transplant recipients that are routinely collected for the purpose of monitoring quality and safety, e.g., in registries, when the research aims to improve deceased donation and transplantation programs and their outcomes. Such secondary use of data should be consistent with the guidance outlined in Principle 6.</p> <p>7.21 Although it may sometimes be reasonable to infer a person's wishes regarding donation of organs, cells, or tissues for use in research on the basis of their previously expressed wishes regarding donation for transplantation, consent for - or refusal of - donation for research or involvement in research should not be presumed solely on the basis of expressed wishes regarding donation for transplantation.</p> <p>7.21.1 In jurisdictions with an opt-out ('deemed' or 'presumed') model of consent for deceased donation for transplantation, consent or refusal for involvement of deceased donors in research should not be presumed solely on the basis of an individual's decision to opt-out or not to opt-out of donation for transplantation, unless explicitly provided for in legislation.</p> |
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concerns or uncertainty, for example, regarding the purposes for which data may be used and by whom. Use of some data relating to deceased donors may therefore be considered beyond the scope of ethical guidance or legal frameworks established for use of ordinary health records data in research.

Principle 5 and its subsidiary principles affirm the importance of safeguarding privacy and protecting data relating to deceased donors and recipients of deceased donor transplants. The subprinciples stop short of providing nuanced guidance on specific questions of urgent practical importance in some settings, such as when consent may be waived for the use of registry data in research. Further work is needed to establish more specific guidance to support ethical access to and use of data in research activities.

### All research activities must be conducted transparently and be open to scrutiny, to uphold public trust in deceased donation and in the integrity of research involving deceased donors

There is a particular concern for transparency in research activities involving donors and recipients of transplants from deceased donors, due to the need to maintain public trust in deceased donation and transplantation systems. Accountability for the safe and equitable procurement and use of donations, as well as avoidance of exploitation or other harms to donors during the end-of-life period, should be a concern not only for researchers but also for donation programs and personnel who may not be involved in research activities.

Deidentified data relating to deceased donation and transplantation are routinely reported in aggregate to the public at the national and international levels, although there is often less detail and transparency regarding utilisation of

donated tissues and cells compared with organs used in transplantation [41]. However, the frameworks under which data are used and shared for the purpose of accountability or quality assurance in donation for transplantation may not routinely allow for the use of these data in research. Conversely, when publishing data collected or generated as part of research activities, specific protections may be required to minimise the risk of harm to individuals or communities. These considerations highlight a potential tension between the core principles: the protection of privacy may conflict with the requirement for transparency.

The principles make clear that the transparency and accountability considered standard for donation for transplant activities should be equally applicable when donation occurs for research or when donors are involved in research. In combination with Principle 5, Principle 6 and its subsidiaries provide a strong foundation at the procedural level by recommending that mechanisms to facilitate ethical governance and decision-making about data collection, storage, and use are needed.

### Research activities should promote the autonomy of individuals who may be involved in or impacted by research

Principle 7 (see Table 7) stipulates respect for the autonomy of those involved in or impacted by research, and, in numerous subprinciples, articulates several important ethical considerations regarding recruitment for and decision-making about involvement in research. It thus addresses one of the foremost concerns of researchers and donation personnel: consent. Ethical and legal uncertainty regarding consent requirements in the context of particular types of studies involving donors has been widely discussed [21, 43–46]. Key questions include when consent for

TABLE 8 Principle 8.

## Principle 8

8. Research activities must respect the prohibition of financial gain in the human body and its parts and uphold the principle of financial neutrality in donation.

8.1 The prohibition of financial gain in the human body and its parts does not preclude the coverage or reimbursement of necessary costs associated with the conduct of research involving deceased donors, or the recovery, transport, preparation, preservation, storage or use of donated bodies, organs, cells, or tissues in research activities [50].

8.1.1 Costs associated with research activities involving deceased donors should be routinely documented and open to scrutiny by relevant stakeholders.

Financial neutrality in donation.

8.2 Deceased donors and their families should neither lose nor gain financially as a result of donation, whether donation occurs for the purpose of transplantation, research, or both.

8.3 Where additional costs are incurred by deceased donors, donor families, or transplant recipients because of their involvement in research, these should be covered whenever possible by relevant research, healthcare or governmental organizations.

8.4 Financial incentives, including fungible gifts or other rewards, should never be offered to individuals or families in exchange for their agreement to donate the body, organs, cells, or tissues after death for use in research, or to involve a deceased donor in a research study.

8.4.1 The principle of financial neutrality in donation does not preclude the use of incentives for recruitment of transplant recipients or donor family members in research studies, provided that:

- routine ethical concerns regarding the use of incentives in research are appropriately addressed.
- any incentives offered to donor families for their participation in research do not create actual or perceived conflicts of interest in donation decision-making, e.g., where their participation in research is contingent upon the donation decision.

8.5 Individuals with responsibility for recruitment of deceased donors for involvement in research should not be offered incentives or rewards proportionate to their success in obtaining agreement for deceased donation or involvement of donors in research. This does not preclude the payment of salaries or other costs associated with recruitment or research coordination activities.

8.6 Ceremonies and memorials that celebrate deceased donors who have contributed to research and/or transplantation are encouraged and should not be considered inappropriate incentives.

Commercial interests

8.7 The presence of commercial interests in research involving deceased donors should be accepted where necessary for the success of research that offers substantial benefits for the public, especially through the development of new or improved medical products derived from human organs, cells or tissues, or the creation of valuable scientific knowledge relating to health.

8.7.1 When evaluating the potential benefits of research for the public, the potential impact of commercial interests on equity of access to these benefits should be carefully considered.

8.8 When proposed research activities involving deceased donors are intended or likely to generate financial gains for researchers, research institutions, commercial entities, funding agencies, or other parties, or when such gains become apparent in the course of research, relevant parties should be encouraged to invest a proportion of profits in a manner that:

- Benefits public health, e.g., by supporting donation and transplantation programs,
- Improves equity in access to healthcare resources, e.g., by subsidizing the costs of new medical products derived from donated organs, cells, or tissues for disadvantaged populations, and/or,
- Supports research aimed at improving health outcomes for all.

8.9 The management of commercial interests in research activities requires scrupulous attention in the context of research involving deceased donors to avoid undermining public trust in the integrity, values and culture of deceased donation programs and personnel.

8.9.1 When potential, perceived, or actual commercial conflicts of interest relate to research activities that directly involve deceased donors, these should be

- treated as significant risks when evaluating research,
- routinely avoided where possible,
- specifically disclosed to decision-makers about research,
- routinely disclosed in publications reporting on research activities including reports by donation organizations or registries where relevant.

involvement in research should be obtained and from which stakeholders, and how much information should be provided and by whom.

Several authors have previously discussed the complexities that may arise when recipients of deceased donor organ transplants are involved in research due to the logistical challenges of providing relevant information and obtaining consent for research during decision-making about acceptance of a deceased donor organ offer [21, 44]. Researchers and donation personnel may be more anxious,

however, about the implications of principle 7 and its subprinciples regarding decision-making about involvement of donors themselves in research. The families of prospective donors typically bear responsibility for decision-making about donation and donor involvement in research, where relevant. Donation decision-making often follows stressful and emotionally exhausting decision-making about end-of-life care and may itself be overwhelming for some families [47]; the additional cognitive load of information about research opportunities may be

considered excessive. Some families may find detailed decision-making about donation or research distressing, and donation personnel and researchers may question the feasibility of informed decision-making in this context. Donation personnel may also worry that discussion of research opportunities could negatively impact consent for donation for transplantation.

The relevance of autonomy is also more complex when the individuals concerned are deceased, as occurs when decision-making about research takes place after the determination of death. Wicclair, for example, suggests that “it makes no sense to protect the autonomy of the dead” [48]. However, he also argues that respecting the known or estimated wishes and preferences of the dead with regard to involvement in research may be ethically justifiable. A key justification for doing so in the case of research involving donors is that this would be consistent with the approach to decision-making about deceased donation for transplantation.

Although legal frameworks in some jurisdictions allow consent to be presumed for the removal of organs for use in transplantation, in practice families (or equivalent) are routinely approached to make or confirm a decision about donation on behalf of the donor, assuming the donor is unable to do so. While family preferences sometimes override the known or estimated wishes of a possible donor, it is increasingly accepted that surrogate decisions about donation for transplantation should ideally follow a substituted judgment approach. That is, decisions should be based on what the possible donor would choose if they were able to make the decision [49]. Similarly, decisions about end-of-life care, such as the withdrawal of life-sustaining measures that may precede donation after circulatory determination of death, are increasingly made according to a person’s known or expected preferences and values, provided these are considered clinically appropriate and not disproportionately burdensome or harmful. It is thus reasonable to infer that decisions about donation for research, or involvement of donors in research during the perimortem period, should also be made using a substituted judgement approach where possible, as indicated in Principle 7.10.2.

The guidelines make clear the importance of tailoring consent processes and information disclosure to support effective decision-making, while minimising burdens and distress for decision-makers, whether they are possible donors providing first person consent, donor family members, or transplant candidates. Communicating key information for decision-making about research may require innovative strategies that differ from the detailed informed consent models commonly associated with clinical research or the blanket consent or “tick-box” options typically included in donation for transplantation forms to allow for use of donated materials in research.

The risks of relying on a “tick-box” option regarding involvement in research on the consent form for donation for transplantation should be self-evident: some decision-makers may decline important research opportunities due to lack of knowledge of the nature and potential value of the research, while others may agree without realising the extent to which research activities and their potential outcomes differ from those of routine donation for transplantation. In the absence of clear guidance regarding consent requirements and feasible mechanisms to support disclosure of information and decision-making about research opportunities, there is a risk that research may proceed without appropriate consent from or engagement with stakeholders, and/or that

opportunities for valuable research will be missed. The principles outlined in these Guidelines provide grounds for the development of more specific guidance and practical advice regarding support for decision-making about research in particular settings.

## **Research activities must respect the prohibition of financial gain in the human body and its parts and uphold the principle of financial neutrality in donation**

Finally, principle eight (see Table 8) reaffirms one of the core ethical tenets of donation and transplantation, which prohibits trade in the human body and holds that donors and donor families should neither lose nor gain financially as a consequence of donation [14, 38, 51, 52]. Regardless of the purpose for which donated organs, cells, or tissues are used—in transplantation or research—the underlying ethical concerns about financial interests influencing donation decisions, undermining equity, and exploiting the vulnerable remain relevant. The subprinciples outlined under principle eight do not preclude, where necessary and ethically appropriate, the provision of financial payment to living persons involved in research to cover the costs associated with their participation in research activities, or coverage of other research-related costs. Coverage of any costs associated with donation, whether for transplantation or research, is also consistent with the principle of financial neutrality [53, 54]. If a deceased donor’s involvement in research, for example, imposes additional financial burdens on their family, these should be alleviated wherever possible.

The prevailing ethos of donation for transplant is that of a public good, with equitable access to the benefits of deceased donor transplantation ideally ensured by public institutions. This may appear threatened when deceased donation intersects with commercial interests in research. In fact, commercial interests are already prevalent in the donation and transplantation sectors in many countries, especially in the context of the tissue sector [55]. While commercial interests may be necessary to bring innovative therapies to patients, such interests in research may raise concerns about the injustice of industry profiting from materials that are gifted without financial gain by donors. This is especially relevant if it is unclear or unlikely that the public will be able to freely or equitably access the benefits of the research, for example in the form of new and expensive medical products derived from donated materials. Addressing these concerns prospectively is important to maintain public support for donation and involvement in research; this requires particular care when research may involve the development of perpetual cell lines or use of genetic material [56]. The case of Henrietta Lacks, for example, has heavily influenced trust and attitudes towards use of biospecimens in research [57]. Further guidance will be required to address concerns in the context of specific types of research and jurisdictions. The subprinciples in this final section again provide high-level guidance that may be useful in grounding more specific principles or policy with regard to commercial interests in research involving deceased donors. In particular, the principles affirm the importance of upholding the fundamental ethical values embedded in donation for

transplantation programs and highlight the need for caution and care to ensure that research activities do not jeopardise these values and the goals of such programs.

## Conclusion

In 2024, the World Health Assembly called on member states to “promote research and innovation to maximize the use and optimize the outcomes of transplantation of human cells, tissues and organs, as well as enable development of alternative therapies to those based on the clinical use of human cells, tissues and organs” [58]. These inaugural international guidelines provide a strong foundation for current research practice but require further work at the local level for adaptation and implementation, including training in their use for researchers, donation and transplantation professionals, and research ethics governance bodies. Further work is also required on specific issues at the normative and practical levels, including consent requirements and mechanisms, allocation issues, use of registry data, and the management of commercial interests in research.

Cultural change may also be needed within donation and transplant programs to address historical beliefs and practices regarding the role of research within the context of deceased donation activities. Where opportunities for involvement in research arise, these should be considered an option for donors and transplant recipients, rather than a competing interest. Timely consultation and collaboration between researchers and donation and transplantation programs can help integrate research opportunities into donation pathways efficiently, while maintaining high standards of care for donors, donor families and transplant recipients, and can help identify and address potential ethical concerns.

## Data availability statement

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

## Author contributions

DEM conceived and led the study. DM, AA, RF, JF, ML, JEL, EM, GCO, HO, KR, MS, S-NT, and ET participated in the design of the study, analysis of the data, drafting and review of the guidelines and this manuscript. Members of the INTEGRITY Collaborator Team contributed to the analysis of the data and drafting of the guidelines. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

Author JEL is employed by United Therapeutics Corporation as Vice President and Chief Medical & Surgical Officer, Global Clinical Product Development - Xenotransplantation. Author GCO has been a consultant to Organox, Getinge, and XVivo. He is a member of the Scientific Advisory Board of iCoat Medical.

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

## References

- Grossi AA, Baiardo Redaelli M, Procaccio F, Picozzi M, Citerio G, Cabrini L. Ethical tensions and professional attitudes toward circulatory death organ donation in the ICU: a systematic review. *Intensive Care Med* (2025) 51: 1833–54. doi: 10.1007/s00134-025-08100-y
- Bartling T, Oedingen C, Kohlmann T, Schrem H, Krauth C. Comparing preferences of physicians and patients regarding the allocation of donor organs: a systematic review. *Transpl Rev* (2020) 34:100515. doi: 10.1016/j.trre.2019.100515
- Cutting RB, Muscat DM, Patel P, De La Mata NL, Irish GL, Wyld M, et al. Values, preferences, and risk tolerance of people waitlisted for a kidney transplant regarding potential deceased donor organ profiles: a systematic review. *Transplantation* (2025) 109:e326–39. doi: 10.1097/TP.0000000000005267
- Oniscu GC, Rockell K, Martin DE. Challenges in undertaking research in transplantation. *The Lancet* (2024) 405:681–3. doi: 10.1016/S0140-6736(24)00931-0
- Porrett PM, Orandi BJ, Kumar V, Houpp J, Anderson D, Killian AC, et al. First clinical-grade porcine kidney xenotransplant using a human decedent model. *Am J Transplant* (2022) 22:1037–53. doi: 10.1111/ajt.16930
- Wells SB, Rainbow DB, Mark M, Szabo PA, Ergen C, Caron DP, et al. Multimodal profiling reveals tissue-directed signatures of human immune cells altered with age. *Nat Immunol* (2025) 26:1612–25. doi: 10.1038/s41590-025-02241-4
- Ashton DM, Blaker CL, Hartnell N, Haubruck P, Heffernan SA, Little CB, et al. Challenging the perceptions of human tendon allografts: influence of donor age, sex, height, and tendon on biomechanical properties. *Am J Sports Med* (2023) 51:768–78. doi: 10.1177/03635465221143385
- 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: 438–48. doi: 10.1097/TP.0000000000004280
- Avilés L, Kean S, Tocher J. Ambiguous loss in organ donor families: a constructivist grounded theory. *J Clin Nurs* (2023) 32:6504–18. doi: 10.1111/jocn.16574
- Marelli S, Querci L, Pozzi F, Cipolla C, Piccolo G, Sacchi M, et al. Understanding organ donation refusal in the next of kin: a fifteen-year retrospective study in ten thousand potential donors. *J Anesth Analgesia Crit Care* (2025) 5:60. doi: 10.1186/s44158-025-00282-7
- Sharma VJ, Starkey G, D'costa R, James F, Mouhtouris E, Davis L, et al. Australian donation and transplantation biobank: a research biobank integrated within a deceased organ and tissue donation program. *Transpl Direct* (2022) 9:E1422. doi: 10.1097/TXD.0000000000001422
- Tingle SJ, Chilvers N, Kourounis G, Thompson ER, Dark J, Sheerin NS, et al. *Ex-situ* machine perfusion across the organs; shared principles and transferable knowledge from clinical trials. *Transpl Rev* (2026) 40:101027. doi: 10.1016/j.trre.2026.101027
- Hashemian MN, Zia MJ, Khorrami-Nejad M, Abed QS, Hashemian H. Long-term outcomes of corneal transplantation: a review of 8,378 patients. *BMC Ophthalmol* (2025) 25:39. doi: 10.1186/s12886-024-03826-7
- World Health Organization (WHO). WHO guiding principles on human cell, tissue and organ transplantation: sixty-third world health assembly. *Cell Tissue Bank* (2010) 11: 229–33. doi: 10.1097/TP.0b013e3181ec29f0
- World Medical Association. WMA declaration of helsinki - principles for medical research involving human participants (2024). Available online at: <https://www.wma.net/policies-post/wma-declaration-of-helsinki/> (Accessed February 21, 2025).
- Martin DE, Cronin AJ, Dalle Ave A, van Haren FMP, Locke JE, Miñambres E, et al. Addressing ethical confusion in deceased donation and transplantation research: the need for dedicated guidance. *Transpl Int* (2021) 34:2459–68. doi: 10.1111/tri.14108
- Escoto M, Issa F, Cayouette F, Consolo H, Chaudhury P, Dhanani S, et al. Research and innovation in organ donation: recommendations from an international consensus forum. *Transpl Direct* (2023) 9:E1446. doi: 10.1097/TXD.0000000000001446
- Parent B, Kates OS, Arap W, Caplan A, Childs B, Dickert NW, et al. Research involving the recently deceased: ethics questions that must be answered. *J Med Ethics* (2024) 50:622–5. doi: 10.1136/jme-2023-109413
- Rodrigue JR, Feng S, Johansson AC, Glazier AK, Abt PL. Deceased donor intervention research: a survey of transplant surgeons, organ procurement professionals, and institutional review board members. *Am J Transpl* (2016) 16:278–86. doi: 10.1111/ajt.13482
- Slessarev M, Bain KL, Basmaji J, Blydt-hansen TD, Cooper J, Aragon FD, et al. Developing guidance for donor intervention randomized controlled trials: initial discussions from the canada-united kingdom 2022 workshop. *Transplantation* (2024) 00:1–6. doi: 10.1097/TP.0000000000004983
- Gordon EJ, Knopf E, Phillips C, Mussell A, Lee J, Veatch RM, et al. Transplant candidates' perceptions of informed consent for accepting deceased donor organs subjected to intervention research and for participating in posttransplant research. *Am J Transpl* (2020) 20:474–92. doi: 10.1111/ajt.15607
- Pentz R, Cohen C, Wicclair M, DeVita MA, Flamm AL, Youngner SJ, et al. Ethics guidelines for research with the recently dead. *Nat Med* (2005) 11:1145–9. doi: 10.1038/nm1105-1145
- Glazier AK, Heffernan KG, Rodrigue JR. A framework for conducting deceased donor research in the United States. *Transplantation* (2015) 99:2252–7. doi: 10.1097/TP.0000000000000841
- Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—A metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* (2009) 42:377–81. doi: 10.1016/j.jbi.2008.08.010
- Wilkinson TM. Last rights: the ethics of research on the dead [1]. *J Appl Philos* (2002) 19:521–31. doi: 10.4324/9781315237367
- Then SN, Martin DE, Opdam HI. Ante-mortem interventions for deceased donation: legal barriers and uncertainty in Australia's decision-making frameworks. *Med J Aust* (2025) 223:236–40. doi: 10.5694/mja2.70020
- Lu B, Okonofua S, Farahbakhsh F, Hatkar R, Sano L, Conway M, et al. Addressing racial disparities in representation across blood, stem cell and organ and tissue donor pools. *Vox Sang* (2026) 121:710–21. doi: 10.1111/vox.70214
- Toro C, Eromosele OB, Flynn DB, Wilson AA, Kotton DN, Hughes TM, et al. Organ donation for research biobanking among historically marginalized racial and ethnic groups: a systematic review. *JAMA Netw Open* (2025) 8:e2512133. doi: 10.1001/jamanetworkopen.2025.12133
- Ohira M, Hotta R, Tanaka Y, Matsuura T, Tekin A, Selvaggi G, et al. Pilot study to determine the safety and feasibility of deceased donor liver natural killer cell infusion to liver transplant recipients with hepatocellular carcinoma. *Cancer Immunol Immunother* (2022) 71:589–99. doi: 10.1007/s00262-021-03005-3
- Williams AM, Trahanas J, Bommareddi S, McGann KC, Ahmad A, Lima B, et al. Donation after circulatory death heart transplant without preimplant reanimation using rapid ultraoxygenated recovery. *JAMA* (2026) 335:885. doi: 10.1001/jama.2025.25169
- Johnstone BH, Woods JR, Goebel WS, Gu D, Lin CH, Miller HM, et al. Characterization and function of cryopreserved bone marrow from deceased organ donors: a potential viable alternative graft source. *Transpl Cel Ther* (2023) 29(95): e1–95.e10. doi: 10.1016/j.jct.2022.11.010
- Cai X, Johnston EK, Cook KE, Abbott RD, Taner B, Yang L. Reconditioning steatotic liver grafts: advances in pharmacological defatting approaches for

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- expanding the donor pool. *Liver Transplant* (2025) 32:901–12. doi: 10.1097/lvt.0000000000000789
33. FNIG (First Nations Information Governance). *The First Nations Principles of OCAP®* (2025). Available online at: <https://fnigc.ca/ocap-training/> (Accessed June 11, 2026).
34. Wiertz S, Boldt J. Ethical, legal, and practical concerns surrounding the implementation of new forms of consent for health data research: qualitative interview study. *J Med Internet Res* (2024) 26:e52180. doi: 10.2196/52180
35. Lay W, Gasparini L, Siero W, Hughes EK. A rapid review of the benefits and challenges of dynamic consent. *Res Ethics* (2025) 21:180–202. doi: 10.1177/17470161241278064
36. Nembaware V, Johnston K, Diallo AA, Kotze MJ, Matimba A, Moodley K, et al. A framework for tiered informed consent for health genomic research in Africa. *Nat Genet* (2019) 51:1566–71. doi: 10.1038/s41588-019-0520-x
37. United Nations General Assembly. Resolution 75/195 strengthening and promoting effective measures and international cooperation on organ donation and transplantation to prevent and combat trafficking in persons for the purpose of organ removal and trafficking in human organs. *A/Res/75/195* (2020). Available online at: <https://documents.un.org/doc/undoc/gen/n20/374/15/pdf/n2037415.pdf?token=ZhkSbu0QGgljwPjOR&fe=true> (Accessed June 11, 2026).
38. The declaration of istanbul on organ trafficking and transplant tourism (2018 edition). *Transplantation* (2019) 103:218–9. doi: 10.1097/TP.0000000000002540
39. Piasecki J, Walkiewicz-Żarek E, Figas-Skrzypulec J, Kordecka A, Dranseika V. Ethical issues in biomedical research using electronic health records: a systematic review. *Med Health Care Philos* (2021) 24:633–58. doi: 10.1007/s11019-021-10031-6
40. Bak MAR, Willems DL. Contextual exceptionalism after death: an information ethics approach to post-mortem privacy in health data research. *Sci Eng Ethics* (2022) 28:32. doi: 10.1007/s11948-022-00387-0
41. Martin D, Van Assche K, Cervantes L, Forsythe JLR, Muller T, Perez-Blanco A, et al. Towards equity in global access to SoHO-based therapies: recommendations for action. *Transplantation* (2025) 109:60–72. doi: 10.1097/TP.0000000000005106
42. Woolfall K, Paddock K, Watkins M, Kearney A, Neville K, Frith L, et al. Guidance to inform research recruitment processes for studies involving critically ill patients. *J Intensive Care Soc* (2024) 25:95–101. doi: 10.1177/17511437231197293
43. Rey MM, Ware LB, Matthay MA, Bernard GR, McGuire AL, Caplan AL, et al. Informed consent in research to improve the number and quality of deceased donor organs. *Crit Care Med* (2011) 39:280–3. doi: 10.1097/CCM.0b013e3181feeb04
44. Gordon EJ, Veatch RM, Abt P, Reese PP. Organ donor intervention research informed consent – timing and risk. *Am J Transpl* (2020) 20:906. doi: 10.1111/ajt.15758
45. Abt PL, Marsh CL, Dunn TB, Hewitt WR, Rodrigue JR, Ham JM, et al. Challenges to research and innovation to optimize deceased donor organ quality and quantity. *Am J Transpl* (2013) 13:1400–4. doi: 10.1111/ajt.12243
46. Cooper J, Harvey D, Gardiner D. Examining consent for interventional research in potential deceased organ donors: a narrative review. *Anaesthesia* (2020) 75:1229–35. doi: 10.1111/anae.15039
47. Potter JE, Perry L, Elliott RM. Bereaved family members' perspectives of their organ donation decision at 3 months post death of the donor-eligible patient in critical care: a dual-method study. *Aust Crit Care* (2025) 38:101132. doi: 10.1016/j.aucc.2024.10.001
48. Wicclair MR. Ethics and research with deceased patients. *Camb Q Healthc Ethics* (2008) 17:87–97. doi: 10.1017/S0963180108080092
49. Martin DE, Then SN. *Ethical Guidelines for Cell, Tissue and Organ Donation and Transplantation in Australia*. Canberra: National Health and Medical Research Council (2025). Available online at: <https://www.nhmrc.gov.au/research-policy/ethics/ethical-guidelines-cell-tissue-and-organ-donation-and-transplantation> (Accessed June 11, 2026).
50. Oveido. 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 (1997). Available online at: <https://rm.coe.int/168007cf98> (Accessed June 11, 2026).
51. World Medical Association (WMA). WMA statement on organ and tissue donation (2020). Available online at: <https://www.wma.net/policies-post/wma-statement-on-organ-and-tissue-donation/> (Accessed June 11, 2026).
52. Dominguez-Gil B, López-Fraga M, Muller E, Potena L, Martin DE, Pérez Blanco A, et al. Santander global summit in transplantation: supporting global convergence in the shared goals of sufficiency, transparency and oversight. *Transplantation* (2024) 109:2–6. doi: 10.1097/TP.0000000000005222
53. Delmonico FL, Martin D, Domínguez-Gil B, Muller E, Jha V, Levin A, et al. Living and deceased organ donation should be financially neutral acts. *Am J Transplant* (2015) 15:1187–91. doi: 10.1111/ajt.13232
54. Martin DE, Capron AM, Fadhil RAS, Forsythe JLR, Padilla B, Pérez-Blanco A, et al. Supporting financial neutrality in donation of organs, cells, and tissues. *Transplantation* (2024) 109:48–59. doi: 10.1097/TP.0000000000005197
55. Pirnay JP, Vanderkelen A, Ectors N, Delloye C, Dufrane D, Baudoux E, et al. Beware of the commercialization of human cells and tissues: situation in the european union. *Cell Tissue Bank* (2012) 13:487–98. doi: 10.1007/s10561-012-9323-3
56. Lee S, Cho MK, Kraft SA, Varsava N, Gillespie K, Ormond KE, et al. “I don't want to be henrietta lacks”: diverse patient perspectives on donating biospecimens for precision medicine research. *Genet Med* (2019) 21:107–13. doi: 10.1038/s41436-018-0032-6
57. Galasso I, Geiger S. Genetic research and the collective good: participants as leaders to reconcile individual and public interests. *J Med Ethics* (2025) 51:710–8. doi: 10.1136/jme-2022-108867
58. World Health Assembly. EB154(7) increasing availability, ethical access and oversight of transplantation of human cells, tissues and organs (2024). Available online at: [https://apps.who.int/gb/ebwha/pdf\\_files/EB154/B154\(7\)-en.pdf](https://apps.who.int/gb/ebwha/pdf_files/EB154/B154(7)-en.pdf) (Accessed August 27, 2025).



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# European society for organ transplantation clinical practice guideline on prevention and treatment of chronic lung allograft dysfunction

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Chronic lung allograft dysfunction (CLAD) is the primary cause of late graft loss after lung transplantation, affecting up to half of lung transplant recipients within 5 years and presenting with heterogeneous phenotypes, most commonly bronchiolitis obliterans syndrome (BOS) and restrictive allograft syndrome. Prevention and treatment of CLAD are challenging. This European Society for Organ Transplantation (ESOT) clinical practice guideline was developed by a multidisciplinary Task Force, including patient and allied healthcare representation, using systematic reviews and GRADE methodology. Eight key PICO questions addressing prevention and treatment of CLAD were evaluated. In terms of prevention, recommendations are in favor of using tacrolimus over cyclosporine, either mycophenolate or azathioprine, and azithromycin to reduce the risk of CLAD. Regarding treatment, montelukast could be considered in early-stage BOS. Current data are inconclusive in demonstrating benefits of anti-thymocyte globulin, alemtuzumab or extracorporeal photopheresis compared with standard of care, and do not support the use of antifibrotics in BOS. The Task Force emphasizes the urgent need for high-quality clinical trials and standardized care pathways in CLAD management.

### KEYWORDS

chronic lung allograft dysfunction, prevention, treatment, GRADE, practice guideline

## Introduction

Chronic lung allograft dysfunction (CLAD) is the leading long-term complication after lung transplantation and remains the major cause of death beyond the first post-transplant year [1]. CLAD is characterized by gradual and irreversible lung function decline, which severely affects quality of life after lung transplantation and may eventually be fatal [2].

CLAD develops in approximately one-third to one-half of lung transplant recipients within 5 years of transplantation and can manifest in different phenotypes [2–4]. The most common are bronchiolitis obliterans syndrome (BOS), characterized by an obstructive spirometric pattern, and restrictive allograft syndrome (RAS), characterized by a restrictive spirometric pattern and radiological opacities. Mixed or undefined forms also occur. The disease course is highly heterogeneous, but mostly progressive over time, necessitating timely intervention to stabilize graft function, alleviate symptoms and prevent graft loss [2, 5].

A range of alloimmune and non-alloimmune factors contribute to CLAD development and progression. Key pathogenic alloimmune drivers include acute cellular rejection, antibody-mediated rejection, human leukocyte antigen (HLA) mismatches, and donor-specific anti-HLA antibodies (DSAs). Non-alloimmune drivers include inflammatory and infectious injuries to the lung allograft, due to ischemia-reperfusion injury, bacterial, viral, or fungal respiratory infections, gastroesophageal reflux with micro-aspiration, and environmental exposures [6].

After lung transplantation, immunosuppressive therapy is used to prevent graft rejection and associated functional decline, graft loss, and mortality, while preserving the best possible quality of life. Standard maintenance immunosuppression includes a calcineurin inhibitor (CNI), a cell-cycle inhibitor and corticosteroids [7]. Although the optimal immunosuppressive regimen remains unknown, practice has changed over time, and the most commonly used combination worldwide is currently tacrolimus, mycophenolate mofetil (MMF), and prednisolone [8–10]. Immunomodulatory drugs, including preventive azithromycin to delay CLAD onset, are used in some centers but are not universally supported or adopted. Other preventive strategies for CLAD focus on reducing modifiable risks and intervening early to preserve graft function. Vigilant monitoring and management of known risk factors, including prompt treatment of acute rejection, pulmonary infections, and gastroesophageal reflux, are considered essential to preserve graft function.

Once CLAD is established, no therapies have been definitively proven to halt or reverse disease progression, and retransplantation remains the only curative option for carefully selected patients. A variety of treatment modalities are suggested and used, ranging from

changes in maintenance immunosuppression to lymphocyte depleting and modulatory therapies (e.g., azithromycin, anti-thymocyte globulin (ATG), alemtuzumab, extracorporeal photopheresis (ECP)), as well as other adjunctive therapies (e.g., montelukast) [11, 12]. Most of these drugs are used off-label in the management of CLAD. Considering the limited available evidence and lack of consensus guidelines on therapeutic strategies in CLAD, practice patterns show substantial inter-center variability [10], with no universally accepted standard of care. This clinical practice guideline therefore aims to provide evidence-based recommendations for prevention and treatment strategies for CLAD in lung transplant recipients.

## Methods

This guideline was developed by a European Society for Organ Transplantation (ESOT) Task Force, including specialists in respiratory and transplant medicine, with recognized expertise in the management of CLAD, as well as an allied healthcare specialist and patient representative. The patient representative was actively involved in all discussions as a full member of the panel and provided input into the final recommendation. Between June 2024 and November 2025, the panel met 15 times (all videoconferences and one hybrid meeting). In addition, a smaller methodology subgroup met frequently and regular discussions on individual topics were held via email.

## PICO and narrative questions

Key clinical questions for both clinicians and patients regarding prevention and treatment of new-onset or progressive CLAD were discussed. Following ESOT methodology, we formulated eight questions in accordance with the PICO format (Patient, Intervention, Comparison, Outcomes). Due to the limited number of PICO questions that could be included, it was not possible to address all therapeutic options for CLAD prevention and treatment (e.g., induction immunosuppression, anti-reflux surgery, pulse corticosteroid therapy, total lymphoid irradiation). The final selection of PICO questions was determined based on voting by the Task Force members ([Supplementary Material 1](#)).

All Task Force committee members decided on the outcomes of interest for each PICO question, based on their relative importance to adults with CLAD and clinical decision-making [13]. Critical outcomes were graft loss, overall survival and serious adverse events for all PICO questions, as well as CLAD onset for the PICO questions regarding prevention (PICO questions 1–3).

Systematic reviews were performed to answer these PICO questions.

## Disclosure of potential conflicts of interest

Committee members disclosed all potential conflicts of interest prior to the start of the Task Force ([Supplementary Material 1](#)). Members with potential conflicts abstained from voting on recommendations in which there was potential conflict. The

**Abbreviations:** ATG, anti-thymocyte globulin; AZA, azathioprine; BOS, bronchiolitis obliterans syndrome; CLAD, chronic lung allograft dysfunction; CMV, cytomegalovirus; CNI, calcineurin inhibitor; DSA, donor-specific antibody; EBV, Epstein-Barr virus; ECP, extracorporeal photopheresis; ESOT, European Society for Organ Transplantation; EtD, Evidence to decision; FEV1, forced expiratory volume in one second; HLA, human leukocyte antigen; MMF, mycophenolate mofetil; PICO, Patient, Intervention, Comparison, Outcomes; PTLD, post-transplant lymphoproliferative disease; RAS, restrictive allograft syndrome; RCT, randomized controlled trial.



librarian and medical writer were non-voting members of the Task Force.

## Systematic review

A systematic review was performed according to ESOT methodology. An experienced information specialist (ME) designed and ran the search strategies on the electronic databases of PubMed, EMBASE and Cochrane Library from inception using MeSH terms and keywords for each clinical question. The initial searches undertaken in October 2024 were updated in December 2025. Results of the searches were sent to panel member pairs and the title/abstract and full text were independently screened using predefined inclusion and exclusion criteria. A detailed description of the methods and the PRISMA flow diagrams [14] for all PICO questions are summarized in [Supplementary Material 1](#).

Studies were summarized using the GRADE approach (Grading of Recommendations, Assessment, Development and Evaluation) for guideline development, including both systematic (for PICO questions) and narrative (for additional sources) reviews of the evidence [15–19].

## Assessment of the level of evidence and degree of recommendations

The GRADE approach was used to assess the certainty of evidence and the degree of recommendations [14, 18, 19]. Recommendations were graded as strong or conditional after considering the certainty of evidence, balance between desirable and undesirable outcomes, assumptions about the relative importance of outcomes, implications for resource use, and acceptability and feasibility of implementation. Key considerations correlated with these gradings are summarized in [Table 1](#).

Evidence profiles and evidence-to-decision (EtD) frameworks were generated for each PICO question [16] ([Supplementary Material 2, 3](#)). Based on these formats, the Task Force Committee formulated clinical recommendations and decided on their strength by consensus or voting, if required. Following the

GRADE approach, strong recommendations are phrased as “we recommend”, while conditional recommendations are phrased as “we suggest” [14, 18, 19].

## Results

The number of studies identified and selected for each PICO question is displayed in the PRISMA flow diagrams ([Supplementary Material 1](#)). The EtD frameworks for all questions are summarized here, with complete versions in the [Supplementary Material](#), and grouped into prevention and treatment.

### Prevention

| PICO question 1                                                                                                                                                                                                                           |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| In adult lung transplant recipients, should azithromycin be used to prevent the onset of CLAD?                                                                                                                                            |
| Recommendation                                                                                                                                                                                                                            |
| <ul style="list-style-type: none"><li>In adult lung transplant recipients, we suggest using azithromycin to prevent the onset of CLAD (Conditional recommendation in favor of the intervention, very low certainty of evidence)</li></ul> |

### Summary of evidence

Two single-center randomized placebo-controlled trials (RCT) evaluated azithromycin for CLAD prevention (203 patients) [20, 21]. Azithromycin or placebo was administered either from discharge (initiated on average at 36 days post-transplant) for 2 years [16] or preoperatively until 31 days post-transplant [21], respectively. In the latter study, open-label azithromycin was prescribed after 90 days post-transplant and continued in 91% (prior placebo) and 97% (prior azithromycin) of included patients. Long-term outcomes (>2 years) of the first trial were later assessed in a retrospective *post-hoc* analysis (n = 83) [22].

Azithromycin significantly reduced CLAD incidence at 2 years compared with placebo (13% vs. 44%;  $p = 0.017$ ) [20]. At a median

TABLE 1 GRADE-based recommendations used in this document, based on GRADE [15] and used in accordance with the European Society for Organ Transplantation methodology.

| Target group | Strong recommendation <sup>#</sup> (“we recommend”)                                                  | Conditional recommendation (“We suggest”)                                                                                                                                                                                                                             |
|--------------|------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Patients     | All or almost all informed people would follow the recommended advice for or against an intervention | Most informed people would choose the recommended course of action, but a substantial number would not                                                                                                                                                                |
| Clinicians   | Most patients should receive the recommended course of action                                        | The health professional should acknowledge that different choices may be appropriate for individual patients and should devote time to the process of shared decision-making by which they ensure that the informed choice reflects individual values and preferences |
| Policymakers | The recommendation can be adopted as a health policy in most situations                              | Policy making will require substantial debate and involvement of many stakeholders                                                                                                                                                                                    |

<sup>#</sup>Strong recommendations based on high-quality evidence will apply to most patients for whom these recommendations are made, but they may not apply to all patients in all conditions; no recommendation can take into account all of the unique features of individual patients and clinical circumstances.

follow-up of 5.5 years, azithromycin-treated patients from Vos et al. still showed a lower incidence of CLAD (28% vs. 51%;  $p = 0.048$ ) and significantly longer CLAD-free survival [22].

## Other supportive evidence

Two additional retrospective single-center studies assessed the effects of azithromycin prophylaxis. One reported increased overall survival ( $p = 0.002$ ) and reduced risk of BOS in patients receiving azithromycin, although not significant ( $p = 0.07$ ) [23]. The other study found that azithromycin prophylaxis, initiated at week 3 post-transplant or later than 3 weeks post-transplant, compared to patients not receiving azithromycin, was associated with a significantly lower risk of CLAD, with the earliest initiation showing the lowest incidence [24].

In summary, data suggest that azithromycin delays and may prevent the onset of CLAD post-transplant; but there is yet no clear evidence from RCTs that prophylactic azithromycin reduces graft loss and improves overall survival, although longer follow-up data are needed for these outcomes. Evidence on optimal initiation time is limited, though early initiation post-transplant may be preferred [24]. It is currently unclear whether the beneficial effects of azithromycin prophylaxis are more pronounced in specific patient populations.

Across the RCTs and *post-hoc* analysis, adverse effects, including serious allergic, cardiac, neurological, gastrointestinal events (requiring treatment discontinuation in 5% in the azithromycin group vs. 0% in the placebo group), and QT interval changes, were comparable between groups or clinically insignificant [20–22]. Similarly, the two retrospective single-center studies found no increased risk of adverse events, renal dysfunction, or malignancy associated with azithromycin [23, 24]. Of note, azithromycin prophylaxis did not affect the bronchial bacterial microbiota of lung transplant recipients [25].

In contrast, clinical experience indicates that gastrointestinal intolerance to azithromycin, related to its gastropkinetic properties, may necessitate dose reduction or discontinuation. Ototoxicity was not assessed in the included studies, but prior data from patients with chronic lung disease have shown an association between long-term azithromycin use and increased risk of hearing impairment, which physicians should be aware of [26].

Overall, azithromycin is generally well tolerated. However, some adverse effects, such as ototoxicity, have not been systematically studied, and the long-term impact of continuous use remains uncertain.

## Justification of recommendation

The Task Force supports the use of azithromycin for CLAD prevention, based on evidence demonstrating a clinically meaningful reduction in CLAD incidence and a generally favorable safety profile with mild and infrequent adverse events.

## Implementation considerations

Oral administration of azithromycin can be initiated during index admission or at routine follow-up visits. No

additional monitoring beyond standard post-transplant care is required.

## Future research

Future research on azithromycin should address the optimal timing of initiation after lung transplantation and whether treatment should be applied universally or targeted to specific risk groups. Studies should also incorporate patient-reported outcome measures and further evaluate long-term effects, including impact on graft and overall survival.

Task Force members had mixed opinions on whether a large multi-center randomized trial of azithromycin to prevent CLAD is needed. Better evidence would be useful, especially on giving the drug to all patients versus only higher-risk ones, and on the best timing. However, a placebo-controlled trial raises ethical and practical problems: the drug is already widely used, recruiting patients would be difficult and the study would need long follow-up. The main uncertainty is therefore not whether azithromycin works overall, but how and when it should be used, including which patients to treat, how long to treat them, and when to stop. Pragmatic randomized trials or large multi-center observational studies may be more realistic ways to answer these questions.

### PICO question 2

In adult lung transplant recipients, should tacrolimus be used over cyclosporine to prevent the onset of CLAD?

### Recommendation

- In adult lung transplant recipients, we recommend using tacrolimus over cyclosporine in the prevention of CLAD. (Strong recommendation in favor of the intervention, low certainty of evidence)

## Summary of evidence

Four RCTs were identified (662 patients; 329 tacrolimus, 333 cyclosporine), with follow-up duration ranging from 507 days to 3 years [4, 27–29].

Across included trials, tacrolimus was associated with a significantly lower incidence of CLAD with moderate certainty of evidence [4, 27–29]. No statistically significant difference between groups was observed for overall survival (RR 0.91, 95% CI 0.68–1.21,  $p = 0.63$ –52) [4, 24–26] and graft loss (RR 0.70, 95% CI 0.6–1.06,  $p = 0.10$ ) [4, 27, 29], but studies were not primarily powered for these outcomes.

Serious adverse events were only reported in the ScanCLAD trial [4] and were comparable between groups. Pooled data showed similar rates of infection [4, 27–29]; kidney dysfunction (serum creatinine >2 mg/dL) occurred significantly more often with tacrolimus [27–29] and this was also observed in the ScanCLAD trial [4]. In this study, however, upon switching from extended-release tacrolimus to immediate-release tacrolimus (protocol change during the study), the incidence of kidney dysfunction was no longer higher than with cyclosporine. No significant difference in malignancy was reported with tacrolimus compared to cyclosporine treatment [4, 27, 28].

## Other supportive evidence

In addition to the RCTs, three quasi-randomized trials [30–32], three observational studies [8, 33, 34] and a recent large registry study [35] were identified.

Reported overall survival was similar [34] or higher [8, 33] with tacrolimus compared to cyclosporine. In the quasi-randomized trials, one-year survival did not differ significantly in two studies [30, 31], while it was higher in the tacrolimus group in the other [27]. CLAD incidence was lower with tacrolimus compared with cyclosporine [31, 32]. The large registry study demonstrated that receiving cyclosporine for maintenance immunosuppression (compared with tacrolimus) was associated with an increased risk of developing CLAD (HR 1.16, 95% CI 1.08–1.23,  $p < 0.001$ ) and with an increased overall risk of death/retransplant (HR 1.16, 95% CI 1.09–1.23,  $p < 0.001$ ).

It should be noted that trough levels varied across studies, and there is currently no consensus on optimal trough targets at different time points after transplantation. The target trough levels for tacrolimus used in the ScanCLAD trial were 10–14 ng/mL at 0–3 months, 8–12 ng/mL at 3–6 months, 8–10 ng/mL at 6–12 months, and 6–8 ng/mL beyond 12 months [4].

Across solid organ transplantation, tacrolimus provides superior early rejection control and short-term graft survival compared with cyclosporine in kidney [36] and liver [37] transplants and is at least non-inferior in heart transplants [38]. However, robust, direct evidence that tacrolimus prevents late chronic allograft dysfunction beyond these surrogate benefits is limited.

No differences in infection rates and kidney dysfunction between tacrolimus and cyclosporine were reported [32, 33]. Tacrolimus was associated with a higher risk of post-transplant diabetes, neurotoxicity and gastrointestinal side effects in liver and kidney transplants [36, 37] but lower risk of hypertension [39].

Conversion from twice-daily, immediate release to once-daily, extended-release tacrolimus formulation appears safe for renal, metabolic, and allograft function and may improve adherence [40], but additional benefits regarding adverse effects remain uncertain. The ongoing Revolution Trial (NCT05001074) compares extended- and immediate-release tacrolimus in lung transplant recipients, assessing renal function and other clinical outcomes [41]. Results expected in 2026 may give insights into the optimal formulation.

## Justification of recommendation

Data supports the use of maintenance tacrolimus over cyclosporine in reducing CLAD incidence. Mortality and re-transplantation appeared neutral within the limited time frame of most studies, which were not adequately powered to detect a clinically meaningful effect. The registry study, which included a longer follow-up up to 14 years post-transplant, demonstrated an increased overall risk of death/retransplant in cyclosporine-treated patients. Overall certainty of evidence was considered low based on the low certainty of evidence for the critical outcomes graft loss and overall survival, despite moderate certainty of evidence for the outcome CLAD,

necessitating recommendation downgrading for risk of bias and imprecision according to GRADE. The moderate certainty of evidence for CLAD is in line with other Refs. [12, 42, 43], but our overall certainty was rated low because of GRADE methodology [44]. However, given the moderate certainty of evidence for the CLAD outcome, the Task Force issued a strong recommendation for the use of tacrolimus even though data on its effect on long-term graft and overall survival are limited.

## Implementation considerations

There are no major implementation concerns, as both tacrolimus and cyclosporine are widely available and included on the [WHO Model List of Essential Medicines](#) [35]. It remains insufficiently clear whether once-daily dosing with extended-release formulation offers advantages over twice-daily dosing with immediate-release formulation in terms of adverse events and side effects. Ongoing and future studies may help clarify this issue. Cyclosporine continues to serve as an essential therapeutic option for patients who experience unacceptable side effects with tacrolimus.

## Future research

Future research should prioritize long-term patient and graft survival, renal outcomes and adverse effects of different tacrolimus formulations and dosing schedules, and management of severe adverse events. Further study is needed on quality of life, optimal CNI trough levels, therapeutic drug monitoring and dose reduction strategies. Cost-effectiveness of tacrolimus formulations and *versus* cyclosporine could also be important.

### PICO question 3

In adult lung transplant recipients, should mycophenolate mofetil be used over azathioprine to prevent the onset of CLAD?

### Recommendation

- In adult lung transplant recipients, we suggest using either mycophenolate mofetil or azathioprine to prevent the onset of CLAD. (Conditional recommendation for either the intervention or the comparison, low certainty of evidence)

### Remarks

There may be specific patient subgroups in which selecting one agent over the other is clinically justified. For example, in patients planning pregnancy, azathioprine (AZA) is preferred due to the teratogenic potential of mycophenolate mofetil (MMF). The choice of cell cycle inhibitor may also be influenced by individual susceptibility. AZA may be preferred in patients susceptible to or with persistent gastrointestinal side effects due to mycophenolic acid. Conversely, in patients with or susceptible to myelotoxicity, MMF might be preferred. Similarly, patients with gout requiring treatment with allopurinol (which may cause a serious drug interaction with AZA) may benefit from MMF. Finally, patients with genetic mutations in the thiopurine S-methyltransferase (TPMT) gene should avoid AZA (when homozygous deficient) or require reduced dosing (when heterozygous), as AZA treatment may induce severe, potentially fatal, bone marrow suppression by accumulation of toxic metabolites (6-thioguanine nucleotides) in these patients, which does not occur when treated with MMF.

## Summary of evidence

Two multicenter, randomized prospective open-label studies (399 patients) compared post-lung transplant outcomes between AZA and MMF [45, 46], with just one study reporting CLAD onset [45].

Six-month overall survival rates were similar in one study (86% vs. 82%;  $p = 0.57$ ) [40], while the other study reported an overall three-year patient survival of 75% in the MMF group and 69% in the AZA group ( $p = 0.18$ ) [45]. CLAD incidence at 3 years post-randomization, CLAD grade, time to onset and survival did not differ between groups, but analysis of the composite endpoint (CLAD, death, re-transplantation for graft failure, or withdrawal due to lack of therapeutic response) showed a significant advantage for MMF over AZA, largely driven by a higher rate of treatment withdrawal in the AZA group [45].

With respect to side effects, no significant differences between AZA and MMF were reported in the incidence of CMV infection or disease [46], rates of opportunistic infections, sepsis, abnormal kidney function, or malignancy [45].

## Other supportive evidence

Several retrospective studies assessed CLAD incidence, yielding inconsistent results [8, 33, 47–51].

One study found a numerically, but not statistically, higher incidence of CLAD in the MMF group (58% MMF vs. 49% AZA) [48]. Three studies reported similar rates of CLAD incidence, graft loss, and survival between MMF and AZA [8, 49, 51]. By contrast, Speich et al. reported a significantly lower graft loss rate and a trend toward better survival in the MMF group; however, interpretation of these findings is limited because of substantial cross-over (42% of patients receiving AZA were switched to MMF) [50].

According to the 2022 consensus on maintenance immunosuppression in solid organ transplantation [7], mycophenolic acid may be more effective than AZA in preventing acute rejection across solid organ transplants, including kidney, pancreas, liver, and heart. Although not a predefined outcome of our guideline, it is worth mentioning that the RCTs found no significant difference in the incidence of acute cellular rejection at 6 months, 1 year and 3 years between MMF and AZA [45, 46]. Conversely, a lower risk with MMF was reported in some retrospective studies [47, 50–52].

Similar adverse events were generally reported across studies, although a trend toward increased gastrointestinal problems with MMF and a higher incidence of anemia with AZA were observed [47, 50]. Rates of infections, malignancy and diabetes were comparable between groups [8, 49]. By contrast, one study reported lower CMV-related disease with MMF [50].

Regarding drug discontinuation, one study found a higher frequency in the MMF group (not significant,  $p = 0.19$ ), mainly due to leukopenia and gastrointestinal issues [46], while another showed higher rates in the AZA group ( $p = 0.026$ ) [50]. Gastrointestinal tolerance may be improved by spreading daily dose throughout the day (e.g., three times daily instead of twice daily) or switching to enteric-coated mycophenolate

sodium, which delays release of mycophenolic acid until the small intestine. The latter has been associated with improved gastrointestinal symptoms, health-related quality of life, and psychological wellbeing in case series of solid organ transplant recipients, and may limit dose reductions, interruptions or discontinuation [53, 54].

It should be noted that a higher incidence of cutaneous squamous cell carcinoma has been observed in solid organ transplant recipients exposed to AZA [55].

Switching drugs (e.g., MMF to AZA for fertility reasons) or omitting cell cycle inhibitors for a longer time may increase the risk of developing *de novo* DSAs and acute rejection [56].

Finally, MMF warrants caution in patients (both females and males) planning pregnancy due to its teratogenic potential [57].

## Justification of recommendation

Evidence shows no significant difference in effectiveness between AZA and MMF, and both have acceptable safety profiles. Accordingly, the Task Force does not suggest the use of one agent over the other. Overall, there has been an increased utilization of MMF over time. The Task Force additionally reported a modest clinical preference for MMF, primarily attributed to its perceived lower risk of acute cellular and antibody-mediated rejection [7].

## Implementation considerations

MMF is considered easy to manage in clinical practice, although, unlike AZA, it is not included in the 2025 WHO Model List of Essential Medicines [58]. Regarding individual susceptibility, the U.S. Food and Drug Administration advise thiopurine S-methyltransferase (TPMT) genotyping/phenotyping prior to commencing AZA, due to the risk of severe myelotoxicity in TPMT-deficient individuals [59]. MMF effectiveness and side effects are influenced by polymorphisms in UDP-glucuronosyltransferases enzymes, which regulate bioavailability of its active metabolite; routine pharmacogenetic testing is not yet standard but may guide individualized MMF dosing [60]. Prescribers should be aware that cyclosporine can significantly lower MMF blood levels, necessitating higher doses to achieve the same area under the curve dose level, by inhibiting its enterohepatic recirculation. Finally, caution is advised with both AZA and MMF in patients with documented short telomere syndrome due to increased myelotoxicity, which may necessitate a dose reduction or discontinuation of the cell cycle inhibitor.

Out of the scope of this systematic review is the role of proliferation signal inhibitors, which may represent an alternative in patients intolerant to both MMF and AZA.

## Future research

Long-term real-world data may complement RCTs by comparing cell cycle inhibitors in lung transplantation. Key unresolved questions include DSA development with MMF vs. AZA, optimal and individualized dosing, identification of patient subgroups that may benefit preferentially from one agent, long-term



drug effects, management of MMF during infections, and guidance on treatment interruption around vaccinations.

## Treatment

| PICO question 4                                                                                                                                                                                                                                                       |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| In adults with (progressive) CLAD, should extracorporeal photopheresis be used?                                                                                                                                                                                       |
| Recommendation                                                                                                                                                                                                                                                        |
| <ul style="list-style-type: none"> <li>In adults with (progressive) CLAD, we suggest either using extracorporeal photopheresis or not using it. (Conditional recommendation for either the intervention or the comparison, very low certainty of evidence)</li> </ul> |
| Remarks                                                                                                                                                                                                                                                               |
| There may be specific patient populations that respond preferentially to ECP (e.g., BOS patients, non-rapid decliners, and/or patients with early CLAD stages), but responder profiles need to be consolidated by more data                                           |

## Summary of evidence

Currently, there are no published RCTs on the use of ECP in CLAD. We identified seven cohort studies with a control group assessing 182 CLAD patients treated with ECP compared to 364 controls [61–67]. All studies suffered from significant selection or indication bias; the ECP group often included patients with more advanced CLAD stages or rapid decliners. Treatment protocols varied with different numbers of cycles per month and in total.

Concerning critical outcomes, one study found a higher re-transplantation rate in the ECP group compared with controls (35% vs. 15%;  $p = 0.004$ ) [63]; of note, in this study, the ECP group included more patients with higher CLAD stages and more rapid decliners than the control group. Two studies showed 31%–55% graft loss during follow-up between 12 and 36 months in both ECP and control groups [62, 67]. Four studies found no difference in overall survival compared with controls [61, 62, 65, 67], whereas the study by Pecoraro et al. documented significantly higher survival in the ECP group (155 vs. 114 months;  $p = 0.03$ ) [66]. In Jaksch et al., survival benefit for ECP was only found in responders, not in the overall ECP group, compared to controls [63].

Different methods were used to measure the effect on forced expiratory volume in one second (FEV1) evolution. One study reported an overall positive effect on FEV1 of ECP compared to controls [66], while two studies found no differences [64, 65]. Greater FEV1 decline in the ECP group was observed in two studies [61, 63], and in one study assessment was not possible due to a high rate of early mortality in the ECP group [62].

Response rate—defined as FEV1 stabilization and/or improvement—ranged from 33% to 80%. This variation likely reflects differences in patient populations or CLAD stages. For instance, Del Fante et al. found 60% stable ECP vs. 70% stable controls, but with less CLAD stage I patients in the ECP group; over time, overall treatment failure occurred in 67% of patients in the ECP group compared with 93% of controls [61].

Regarding undesirable effects, no difference in infection rates between groups was reported in three studies [63, 65, 66], but one study observed a significantly lower incidence of CMV infections in

the ECP group [63]. A decrease in bacterial and CMV infections after ECP initiation compared with the pre-ECP period has also been reported, despite lack of direct comparison to the control group [61]. Serious complications associated with ECP were infrequent across studies.

## Other supportive evidence

Thirteen additional observational studies evaluating ECP in CLAD were identified, with sample sizes ranging from 8 to 631 patients and some patient overlap among larger cohorts [68–80].

Survival outcomes varied widely. The largest study (631 patients) reported overall survival of 86% at 6 months and 77% at 12 months, with 5-year post-ECP graft survival ranging from 70% in responders, to 56% in stable patients and 35% in non-responders [79], while another large cohort (373 patients) reported 46% overall survival and 41% graft survival at a median follow-up of 92 months [73]. Other studies reported a median overall survival of 7 years, with 50% 10-year graft survival [71], 66% 5-year overall survival [75], or median survival ranging from 2.6 years post-ECP initiation [72] to a mean of 15 months [80].

Most studies focused on graft function, particularly FEV1 evolution before and after ECP initiation. In the largest cohort [79], FEV1 stabilization or improvement was observed in 62% of patients at 6 months and 51% at 12 months, while other studies reported response rates of 19%–80% [68–70, 72, 74, 79, 80]. Interestingly, a recent randomized trial evaluated the preventive use of ECP in the early post-operative course and found a significantly lower incidence of CLAD at year 3 in the ECP group [81].

Several subgroup analyses attempted to identify responder profiles. Patients with a BOS phenotype generally demonstrated better responses than those with a RAS phenotype [65, 74, 79], although findings were inconsistent [61]. Similarly, patients with earlier CLAD stages (I–II) tended to respond better than those with advanced disease (III–IV) [63, 73, 75, 79], although this association was not uniformly observed [61, 66, 74]. Rapid FEV1 decliners showed reduced or trending toward reduced responses in some studies [63, 74], while others reported no effect [64] or even better responses [77].

Adverse effects were generally infrequent. Since ECP is thought to have mainly immunomodulatory rather than immunosuppressive effects, it may cause fewer infections [79] compared to other treatments. Treatable line-related infections occurred in 13%–14% of patients requiring central venous access [69, 72]. Venous access is a notable concern and may pose a significant barrier to treatment with ECP; based on our experience, the use of central lines varies considerably across centers. No ECP-specific long-term toxicities were reported, with long-term follow-up studies showing reassuring safety profiles [71, 75]. Fatigue and iron deficiency anemia, though underreported, are observed in clinical practice and warrant monitoring. In a RCT where ECP was used as a preventive immunomodulatory treatment to reduce the risk of CLAD, the main adverse event attributable to ECP was anemia, reported as a reason for discontinuation in 38% [81].

In conclusion, the actual efficacy of ECP in the treatment of CLAD remains uncertain. Multiple sources of bias were identified

TABLE 2 Dosing and costs of various CLAD treatments.

| Treatment    | Typical CLAD regimen                                                 | Costs* | Notes                                                                                                                             |
|--------------|----------------------------------------------------------------------|--------|-----------------------------------------------------------------------------------------------------------------------------------|
| Azithromycin | 250 mg orally 3×/week or 250 mg every other day                      | €      | Generic; very low cost across EU. Often <€10 for a month's supply                                                                 |
| Montelukast  | 10 mg orally daily                                                   | €      | Generic; inexpensive in most EU countries                                                                                         |
| Pirfenidone  | 801 mg 3×/day (standard dose)                                        | €€     | Generic; widely available. Actual reimbursed costs vary                                                                           |
| ECP          | Initial: 2 days every 1–2 weeks; Maintenance: 2 days every 4 weeks** | €€€    | Varying availability. Highly variable by center, frequency, and technology (online vs. offline). Reimbursement differs by country |
| ATG          | Short course (3–10 doses, total 3–9 mg/kg)                           | €€€    | Varying availability. Vial costs vary; total depends on weight and protocol                                                       |
| Alemtuzumab  | Usually 1–few 10–30 mg IV doses                                      | €€€    | Varying availability                                                                                                              |

\*Monthly costs in Europe € - < 100€, €€ 100–1.000€, €€€ = >1.000€.

\*\*Differing schedules exist.

ATG, anti-thymocyte globulin; CLAD, chronic lung allograft dysfunction; ECP, extracorporeal photopheresis.

across studies, including logistical and co-intervention bias, and the lack of adequately matched control groups limited the ability to distinguish treatment effects from the natural history of CLAD. Results from the ongoing randomized eCLAD trial (UK, NCT05721079) are awaited to provide more robust evidence [82].

Quality of life and patient-reported outcome measures were not assessed in the published studies, representing an important gap. Given the burden of long-term, repeated ECP sessions, patient engagement, counseling, and stopping protocols are essential.

## Justification of recommendation

Although ECP shows few severe side effects, the limited and not well-established desirable effects, along with the significant therapeutic and logistical burden for patients and hospitals, preclude the group from making a recommendation either for or against the use of ECP in the management of CLAD. This recommendation may require revision when additional data become available, especially following the results of the ongoing multicenter randomized controlled eCLAD trial.

## Implementation considerations

High procedure costs (see Table 2), requirement for specialized equipment and trained personnel at hospitals, potential need for a central line, and coordination of travel logistics for the patients should be considered when assessing ECP indication. Reimbursement policies across healthcare systems are variable.

## Future research

Further research on ECP in established CLAD should clarify treatment response, define responder subgroups, standardize treatment schedules, and assess biological samples to elucidate its mechanism of action, patient-reported outcomes, side effects, and

cost-effectiveness. Additional studies are also needed to evaluate the role of ECP in CLAD prevention.

### PICO question 5

In adults with (progressive) CLAD, should anti-thymocyte globulin be used?

### Recommendation

- In adults with (progressive) CLAD, we suggest either using anti-thymocyte globulin or not using it. (Conditional recommendation for either the intervention or the comparison, very low certainty of evidence)

### Remarks

We suggest using ATG with caution in patients with a history of or risk for malignancy (e.g., recent malignancy, malignancy with a high risk of recurrence, Epstein-Barr virus (EBV) mismatch), or a history of infections (especially difficult-to-treat or fungal infections)

## Summary of evidence

We identified nine retrospective observational studies assessing ATG as a treatment for CLAD (390 treated patients, 10–108 per study) [83–91].

Graft survival ranged from 77% to 80% at a median follow-up of 10–63 months [84, 88]; in one study with an ATG-free control group, median graft survival was lower in ATG-treated patients (26 vs. 45 months;  $p = 0.021$ ), possibly reflecting more severe baseline disease and residual confounding despite propensity matching [90]. Overall survival was 95% at 6 months [87], 90% at 10 months [84], 54% at 12 months [89] and 56% at 17 months [86]. The study by Padhye et al. showed a lower 1-year post-CLAD mortality compared with CLAD patients who did not receive ATG, although not significant (SHR 0.66, 95%CI 0.39–1.14,  $p = 0.134$ ) [91].

Across studies, FEV1 response rates varied depending on the definition applied. Improvement in FEV1 was observed in 13%–40% of patients, while improvement or stabilization was reported in 23%–52% [83, 85, 86, 88, 89]. A >20% reduction in FEV1 decline

occurred in 40%–65% of cases [87, 90]. Padhye et al. reported no difference in rate of FEV1 decline post- vs. pre-ATG and compared with non-ATG treated patients, although no pre-defined lung function period was assessed; *i.e.*, the rate of decline 3 months after CLAD diagnosis to last follow-up was compared with lung function evolution from transplant up to 3 months before CLAD diagnosis [91].

Reported undesirable effects included skin cancer (9%–19%) and other types of malignancy (7%–8%) [88, 90]. Post-transplant lymphoproliferative disorder (PTLD) occurred in 0%–10% of cases [83, 84].

## Other supportive evidence

The rate of FEV1 decline as a predictor of ATG response yielded inconsistent results. A trend toward a better response in rapid decliners [90] and CLAD stage I-II patients [88, 89] was observed, although this association was not consistently reported [87, 90]. No association between response and CLAD phenotype was reported in three studies [87, 88, 90].

There was wide variability across studies in terms of follow-up and time between CLAD diagnosis and ATG treatment. Studies also differed in total dose and type of ATG administered, as well as monitoring of lymphopenia. ATG was generally used as a second- or third-line therapy, with prior treatments varying across studies.

The Task Force judged that the evidence for a positive effect was limited, mostly because most studies lacked control groups. However, clinical experience suggests that ATG may contribute to lung function stabilization in some cases.

Treatment tolerance varied across studies. Fever and chills were common [84, 86, 87, 89], while most adverse events were mild [84, 89]. Infusion-related reactions ranged from 4% [86] to 15% cytokine release syndrome and 22% serum sickness [87]. Overall, severe reactions appeared rare, and premedication with corticosteroids, acetaminophen, and/or antihistamines may reduce symptoms.

Bacterial and/or viral infection rates ranged from 12% to 35%, with serious infections occurring in 8%–14% of patients [88, 89]. Cytopenia was inconsistently reported: severe leukopenia occurred in 4% of patients [86] and neutropenia in up to 14% [89].

Additional safety information can be derived from ATG use as an induction therapy. A systematic review of six small RCTs found no clear evidence of a higher risk of adverse outcomes associated with T-cell antibody induction (including ATG) compared with no induction or with each other, in lung transplantation [92].

Although ATG increases overall immunosuppressive burden, retrospective studies showed no clear increase in the risk of PTLD in lung or other solid organ transplantation [93]. Current expert opinion favors use of the lowest effective dose, with caution in EBV-seronegative recipients and other high-risk populations.

## Justification of recommendation

There is no high-quality data supporting the use of ATG in the management of CLAD, although existing studies and Task Force members' clinical experience suggest potential for lung function stabilization. The main limitation of ATG therapy is an increased risk

of infections. Overall, ATG may be considered as a treatment option in selected CLAD patients, including those with concomitant ACR.

## Implementation considerations

Availability of this therapy may be limited in certain countries, and treatment necessitates hospitalization and intravenous access. Administration of ATG via a central line or large-bore intravenous access is generally preferred given the risk of local irritation and thrombophlebitis. At present, no definitive recommendations exist on the optimal dosing, duration of treatment, and monitoring of its effects.

## Future research

RCTs are warranted to better define efficacy and safety. Additional evidence is required to determine optimal dosing, treatment duration, and appropriate monitoring strategies. Furthermore, data is lacking for high-risk groups, such as EBV-mismatched recipients at increased PTLD risk, and for specific clinical subgroups, including different CLAD phenotypes.

### PICO question 6

In adults with (progressive) CLAD, should alemtuzumab be used?

### Recommendation

- In adults with (progressive) CLAD, we suggest either using alemtuzumab or not using it. (Conditional recommendation for either the intervention or the comparison, very low certainty of evidence)

### Remarks

Alemtuzumab should be used with caution in patients with a history of or risk for malignancy (*e.g.*, recent cancer, malignancy with a high risk of recurrence, EBV mismatch) or a history of infections, particularly difficult-to-treat or fungal infections

## Summary of evidence

Three small retrospective observational studies were identified (41 patients; 10–17 per study) [65, 94, 95], including one with a control group of 78 untreated patients [65].

No significant difference in overall survival was observed between the treatment group and controls at 6–12 months [65]. The other studies reported graft and patient survival of 69% at 1 and 2 years [94] and 5-year overall survival of 76% [95].

In one study, mean FEV1 did not improve following alemtuzumab treatment [94]. In another cohort, a >10% increase in FEV1 was observed at 30 days, followed by a 10% decline by day 120 [95]. In the controlled study, alemtuzumab was associated with a slower decline in FEV1 at 1 month, but not at later time points [65]. Reams et al. observed improvement in BOS grade in 40% of patients, stability in 30%, and worsening in 30% [94]. Ensor et al. reported 53% freedom from BOS progression at 6 months [95].

Overall, alemtuzumab was associated with short-term attenuation of spirometric decline, mainly in BOS and early-stage disease patients, but no survival benefit was shown, and effects

TABLE 3 Overview of recommendations.

| PICO number | PICO question                                                                                                             | Recommendation                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|-------------|---------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| PICO 1      | In adult lung transplant recipients, should azithromycin be used to prevent the onset of CLAD?                            | In adult lung transplant recipients, we suggest using azithromycin to prevent the onset of CLAD<br><i>Conditional recommendation in favor of the intervention, very low certainty of evidence</i>                                                                                                                                                                                                                                                    |
| PICO 2      | In adult lung transplant recipients, should tacrolimus be used over cyclosporine to prevent the onset of CLAD?            | In adult lung transplant recipients, we recommend using tacrolimus over cyclosporine in the prevention of CLAD<br><i>Strong recommendation in favor of the intervention, low certainty of evidence</i>                                                                                                                                                                                                                                               |
| PICO 3      | In adult lung transplant recipients, should mycophenolate mofetil be used over azathioprine to prevent the onset of CLAD? | In adult lung transplant recipients, we suggest using either mycophenolate mofetil or azathioprine to prevent the onset of CLAD<br><i>Conditional recommendation for either the intervention or the comparison, low certainty of evidence</i>                                                                                                                                                                                                        |
| PICO 4      | In adults with (progressive) CLAD, should extracorporeal photopheresis be used?                                           | In adults with (progressive) CLAD, we suggest either using extracorporeal photopheresis or not using it<br><i>Conditional recommendation for either the intervention or the comparison, very low certainty of evidence</i>                                                                                                                                                                                                                           |
| PICO 5      | In adults with (progressive) CLAD, should anti-thymocyte globulin be used?                                                | In adults with (progressive) CLAD, we suggest either using anti-thymocyte globulin or not using it<br><i>Conditional recommendation for either the intervention or the comparison, very low certainty of evidence</i>                                                                                                                                                                                                                                |
| PICO 6      | In adults with (progressive) CLAD, should alemtuzumab be used?                                                            | In adults with (progressive) CLAD, we suggest either using alemtuzumab or not using it<br><i>Conditional recommendation for either the intervention or the comparison, very low certainty of evidence</i>                                                                                                                                                                                                                                            |
| PICO 7      | In adults with (progressive) CLAD, should antifibrotics be used?                                                          | In adults with (progressive) CLAD phenotype BOS, we suggest not using antifibrotic therapy with pirfenidone<br><i>Conditional recommendation against the intervention, low certainty of evidence</i><br>In adults with (progressive) CLAD phenotype RAS, we suggest either using antifibrotic therapy with pirfenidone or not using it<br><i>Conditional recommendation for either the intervention or the comparison, low certainty of evidence</i> |
| PICO 8      | In adults with (progressive) CLAD, should montelukast be used?                                                            | In adults with (progressive) CLAD, we suggest either using montelukast or not using it<br><i>Conditional recommendation for either the intervention or the comparison, low certainty of evidence</i>                                                                                                                                                                                                                                                 |

BOS, bronchiolitis obliterans syndrome; CLAD, chronic lung allograft dysfunction; RAS, restrictive allograft syndrome.

beyond 3–6 months remain unclear [65, 94, 95]. Interpretation of the results is limited by selection bias, as alemtuzumab was preferentially used for rapidly progressive disease. Further data is needed to determine the true effect size. Nonetheless, experience from some Task Force centers suggests possible stabilization of lung function, and alemtuzumab may be a potential option in patients with prior DSAs.

## Other supportive evidence

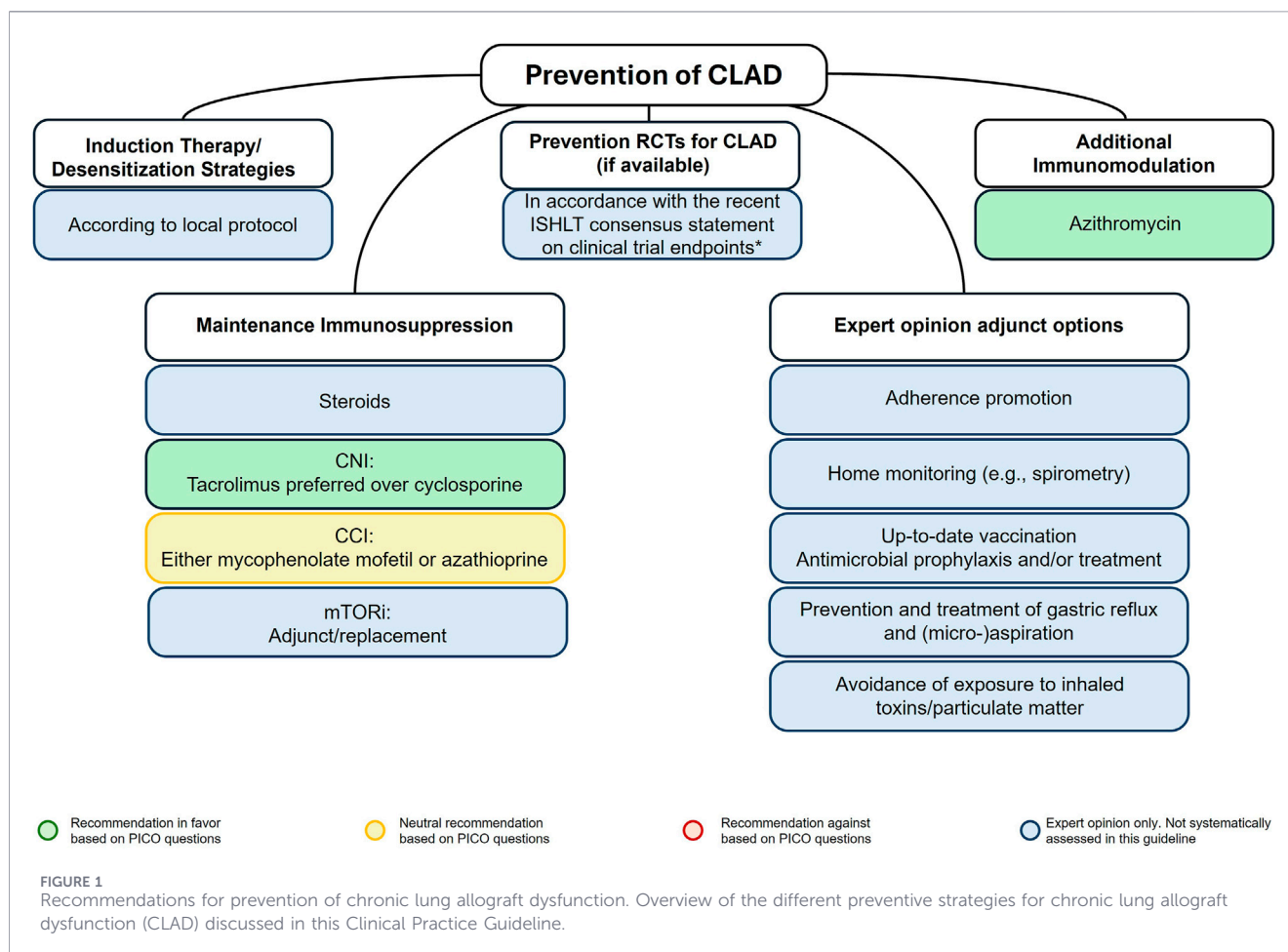
In the identified studies, infection rates ranged from 50% to 77% while infection-related mortality ranged from 10% to 30%

[65, 94, 95]. Patients with CLAD already have an increased baseline infection risk; alemtuzumab may further increase the incidence.

In kidney transplant studies, alemtuzumab or ATG treatment for acute T-cell-mediated or glucocorticoid-resistant rejection was associated with good long-term graft survival and function, but frequent infections and reduced patient survival were reported compared with patients not requiring alemtuzumab [96–98]. Infection rates were comparable between the alemtuzumab- and ATG-treated groups [97].

In a study of alemtuzumab induction therapy in lung transplantation, 44% of patients developed (mostly





viral) infections under prophylaxis (27% hospitalized, 4% ICU), nearly 50% occurring within 6 months. Malignancies occurred in 9% of patients (2% PTLT, 7% non-PTLT) [99].

Although data does not demonstrate a clear increase in post-transplant malignancies with alemtuzumab in kidney or lung recipients, clinical experience supports cautious use in patients with a prior history of cancer.

## Justification of recommendation

Alemtuzumab may stabilize lung function in selected patients, but its effect size remains uncertain, and further data is warranted. Its use is supported by limited clinical experience, and potential infection risks should be carefully weighed in clinical decision-making.

## Implementation considerations

Alemtuzumab may have limited availability in some regions, potentially affecting access. It is generally administered intravenously, though subcutaneous or intramuscular routes are alternative options. The costs of the drug and management of potential adverse events should be carefully evaluated during treatment planning.

## Future research

RCTs are necessary to establish definitive evidence regarding the efficacy, safety, optimal dosing and scheduling of alemtuzumab. Future research should additionally assess risk factors for adverse

### PICO question 7

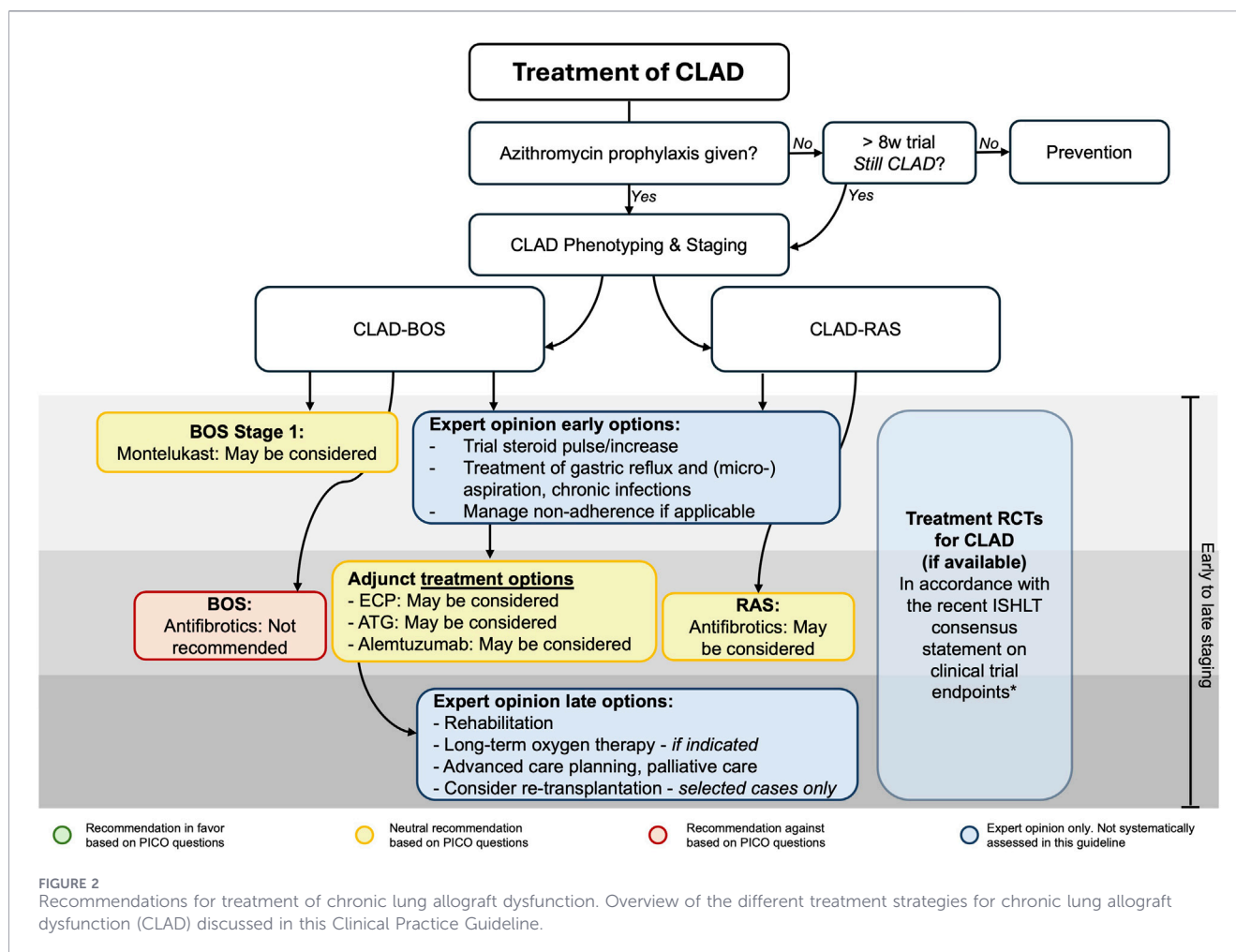
In adults with (progressive) CLAD, should antifibrotics be used?

### Recommendation

- In adults with (progressive) CLAD phenotype BOS, we suggest not using antifibrotic therapy with pirfenidone. (Conditional recommendation against the intervention, low certainty of evidence)
- In adults with (progressive) CLAD phenotype RAS, we suggest either using antifibrotic therapy or not using it. (Conditional recommendation for either the intervention or the comparison, low certainty of evidence)

### Remarks

We do not suggest using antifibrotic therapy with pirfenidone in BOS patients given the lack of efficacy. However, antifibrotic therapy might be considered in RAS patients, as – in theory – it might be more effective in this CLAD phenotype demonstrating more extensive fibrosis, although more data are needed to better understand the effect size in this patient population



events (e.g., PTLT risk in patients with EBV mismatch) and focus on specific subgroups, such as different CLAD phenotypes and patients with DSAs.

## Summary of evidence

Two RCTs have evaluated pirfenidone use in lung transplant recipients [100, 101]. No published studies of nintedanib were identified at the time of writing, although there is a recently completed RCT in BOS (NCT03283007) [102].

The STOP-CLAD trial did not meet enrollment goals and was prematurely ended; therefore, it was underpowered for the primary outcome, which was change in radiographic assessment of small airways disease. The study included 23 patients (14 BOS and 9 RAS; 12 pirfenidone and 11 placebo), and there was no significant difference in FEV1 change over the 24-week study period between pirfenidone and placebo (−3.1% vs. −3.7%;  $p = 0.90$ ). No life-threatening adverse events were reported. Five serious adverse events requiring medical intervention occurred, all in the placebo group, including four infections requiring antibiotic treatment and one case of hyponatremia [100].

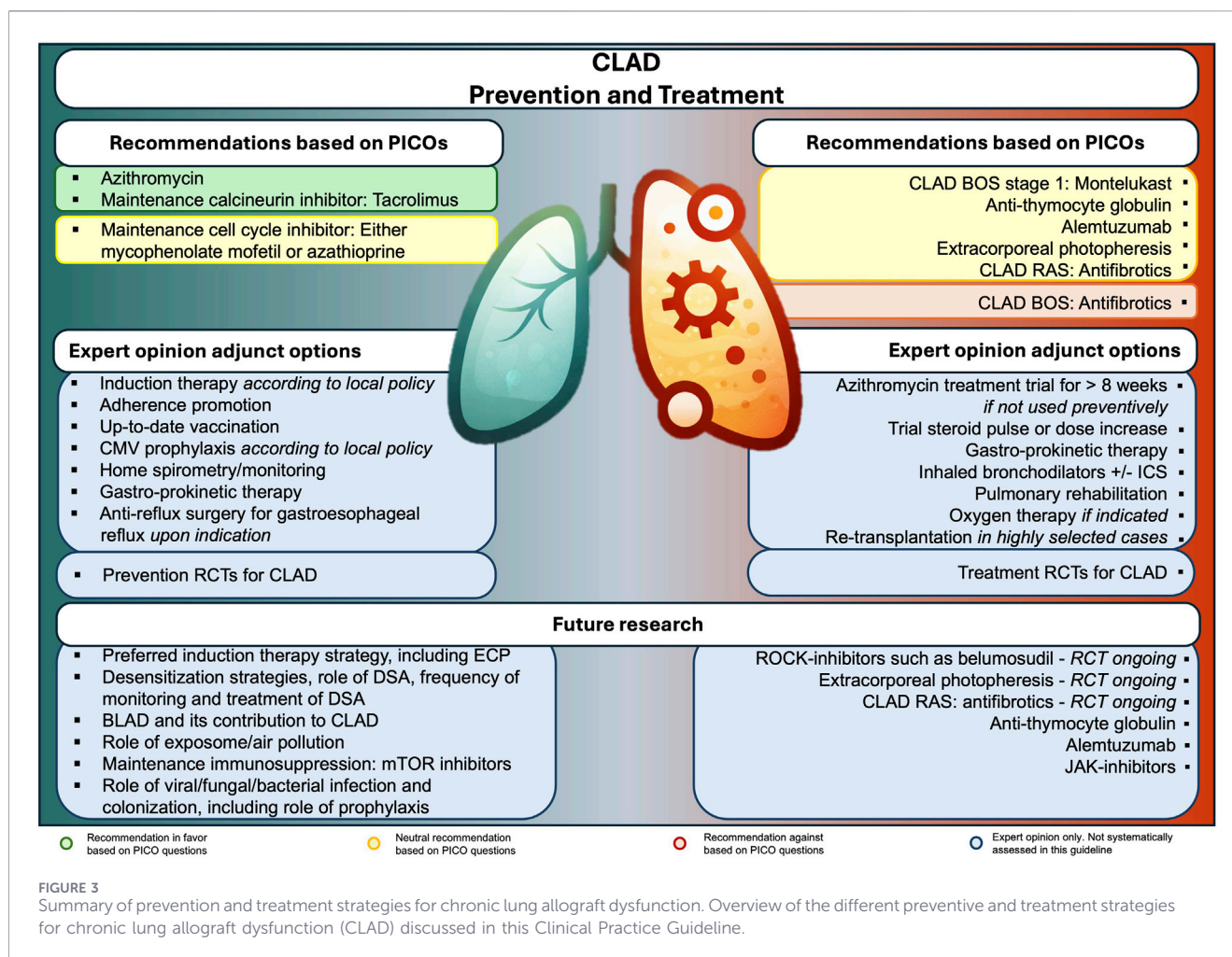
In the EPOS trial (90 BOS patients; 48 pirfenidone and 42 placebo), both the pirfenidone and control groups showed a similar continued FEV1 decline. Secondary endpoints (graft loss, death, re-transplantation) and the incidence of serious adverse events were similar between groups [101].

As pirfenidone did not demonstrate superiority over placebo and standard care, we do not recommend it for treating BOS.

## Other supportive evidence

In addition to serious adverse events, the RCTs reported a similar overall number of adverse events in the pirfenidone and placebo groups [100, 101]; with more gastrointestinal events [100, 101] and fewer infections [100] in patients receiving pirfenidone. Adverse events judged by blinded adjudication to be attributable to the study drug were more common in the pirfenidone group [100].

Two small additional observational studies (9–11 patients) assessed the role of pirfenidone in lung transplant recipients with CLAD [103, 104]. In Vos et al. median 3-year overall survival following RAS diagnosis was 55% and graft loss occurred in 73% of patients, with a median graft loss-free survival of 1.8 years [103]. In the other study, median overall survival from treatment initiation was 7 months [104].



Across the two studies, a non-significant reduction in the rate of FEV1 decline was observed post- vs. pre-pirfenidone at 3 and 6 months [103, 104], with a significant decrease at 12 months in the study by Vos et al. [103] (−50 to −10 mL/month;  $p < 0.05$ ). However, only 6/11 (55%) patients could still be evaluated at that time point.

Antifibrotics may cause notable gastrointestinal symptoms, requiring dose reduction or treatment discontinuation. Dose adjustment due to anorexia or nausea was reported in 55% of patients [103], while a single case required treatment interruption for gastrointestinal toxicity in the other study [104]. Additional risks include phototoxicity and hepatotoxicity.

The final results of the Pirfenidone for Restrictive Chronic Lung Allograft Dysfunction (PIRCLAD) study, a single-center study on safety and tolerability of pirfenidone in restrictive CLAD, are not available yet (NCT03359863) [105]. A multicenter RCT evaluating nintedanib in lung transplant recipients with BOS Grade 0p-1-2 (INFINITx BOS) was recently completed, and the results are awaited (NCT03283007) [102]. Other novel antifibrotics, such as nerandomilast [106], may warrant investigation in CLAD. Inhaled antifibrotics, currently studied in interstitial lung diseases, could be explored for future research in CLAD-RAS [107].

## Justification of recommendation

Upon review of the current scientific evidence, we do not suggest using pirfenidone for CLAD-BOS, as there is no substantial indication of efficacy. Conversely, data are insufficient to draw conclusions on antifibrotic therapy for CLAD-RAS and further research is warranted.

## Implementation considerations

Limited availability and reimbursement may restrict implementation. In some countries, nintedanib may be prescribed for CLAD-RAS when classified as progressive pulmonary fibrosis and/or as an off-label treatment. Patients should be monitored for adverse effects, including gastrointestinal symptoms, hepatotoxicity, and skin toxicity. Additionally, trough levels of CNI should be assessed due to the potential for drug interactions [103].

## Future research

Based on the anticipated outcomes from the PIRCLAD study involving patients with restrictive CLAD, further investigation in this patient population might be recommended. Studies with other antifibrotics, such as nerandomilast, could be considered.

| PICO question 8                                                                                                                                                                                                                                 |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| In adults with (progressive) CLAD, should montelukast be used?                                                                                                                                                                                  |
| Recommendation                                                                                                                                                                                                                                  |
| <ul style="list-style-type: none"> <li>In adults with (progressive) CLAD, we suggest either using montelukast or not using it. (Conditional recommendation for either the intervention or the comparison, low certainty of evidence)</li> </ul> |
| Remarks                                                                                                                                                                                                                                         |
| Montelukast may be effective in cases of early BOS. In patients with new-onset BOS stage 1, a trial of montelukast can be considered                                                                                                            |

## Summary of evidence

One RCT (30 patients; 15 montelukast, 15 placebo) assessed montelukast in lung transplant recipients with BOS and slowly progressive FEV1 decline despite prior azithromycin [108].

Graft loss at 1 and 2 years was similar in montelukast and control groups. Overall, montelukast had no significant effect on FEV1 decline, but a *post-hoc* subgroup analysis demonstrated a decrease in FEV1 decline in BOS stage 1 patients, which was not seen in BOS stage 2–3 patients.

Serious adverse events were similar between groups, and treatment discontinuation rates did not differ significantly. Rates of respiratory infections were also comparable.

## Other supportive evidence

Two additional observational studies assessed montelukast in CLAD patients [109, 110]; all studies were from the same center.

In the largest study (153 patients; 115 BOS, 38 RAS), montelukast stabilized or improved FEV1 in 81% of cases at 3 months, with benefits persisting up to 12 months, and

TABLE 4 Research recommendations regarding prevention.

| Future research recommendations: prevention |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|---------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Azithromycin                                | <ul style="list-style-type: none"> <li>Optimal timing of initiation after lung transplantation</li> <li>Whether treatment should be applied universally or targeted to specific risk groups</li> <li>Patient-reported outcome measures</li> <li>Evaluation of long-term effects, including impact on graft and overall survival</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Tacrolimus vs. cyclosporine                 | <ul style="list-style-type: none"> <li>Long-term data on overall survival and graft survival; updated results from the ScanCLAD study are anticipated</li> <li>Comparative effects of once-daily, extended release vs. twice-daily, immediate-release dosing and of different tacrolimus formulations on renal outcomes and other side effects</li> <li>Management of unacceptable side effects, including thrombotic microangiopathy, posterior reversible encephalopathy syndrome</li> <li>Comparative quality of life outcomes with tacrolimus vs. cyclosporine in lung transplantation; follow-up reports from the ScanCLAD study are expected</li> <li>Optimal trough levels for CNIs at different time points after transplantation, including feasibility and safety of dose reduction</li> <li>Adjustment of CNI target levels based on kidney function, immunological risk, risk factors, and drug–drug interactions</li> <li>CNI-reduction or CNI-free immunosuppressive strategies, such as mTOR inhibitor-based quadruple low CNI maintenance immunosuppressive regimen vs. standard triple maintenance immunosuppressive regimen</li> <li>Best practices for therapeutic drug monitoring, including frequency, methodology, and dose adjustment</li> <li>Cost-effectiveness of tacrolimus compared with cyclosporine in lung transplantation</li> </ul> |
| MMF vs. AZA                                 | <ul style="list-style-type: none"> <li>Optimal dosing, considering pharmacogenetic factors beyond lymphopenia</li> <li>Definition of patient subgroups (e.g., those with telomeropathies) that may benefit preferentially from one agent</li> <li>Development of DSAs in patients receiving MMF vs. AZA</li> <li>Long-term effects of each cell cycle inhibitor</li> <li>Alternatives of cell cycle inhibitors, such as the use of mTOR inhibitors instead of MMF or AZA</li> <li>Timing and management of MMF discontinuation during infections and its impact on CLAD incidence</li> <li>Guidance on pausing cell cycle inhibitors around vaccinations</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Other                                       | <ul style="list-style-type: none"> <li>Clinical trials with novel drugs for preventing CLAD are an unmet need, and the Task Force recommends conducting well-designed RCTs (ideally multicenter RCTs and the use of CLAD adjudication) for CLAD prevention, in accordance with the recent ISHLT consensus statement on Lung Transplant Clinical Trials [105]</li> <li>Further studies on long-term outcomes of organ preservation strategies are needed to inform about the effects on CLAD incidence</li> <li>The use of specific HLA antibody/DSA-directed treatments and whether they reduce the risk of later CLAD development</li> <li>In patients with AMR, more data are needed on the efficacy of AMR treatments as a prevention of CLAD</li> <li>The role of inhaled immunosuppressive drugs, such as inhaled cyclosporine and mTOR inhibitors, in the prevention of CLAD</li> <li>Whether the addition of other immunomodulating or immunosuppressive drugs, such as belumosudil, ECP or tocilizumab, to standard triple immunosuppression has an impact on CLAD incidence</li> </ul>                                                                                                                                                                                                                                                                      |

AMR, antibody-mediated rejection; AZA, azathioprine; CLAD, chronic lung allograft dysfunction; CNI, calcineurin inhibitor; DSA, donor-specific antibody; ECP, extracorporeal photopheresis; HLA, human leukocyte antibody; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin.



TABLE 5 Research recommendations regarding treatment.

| Future research recommendations: treatment |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|--------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ECP                                        | <ul style="list-style-type: none"> <li>- Definition and prediction of treatment response</li> <li>- Consolidation of subgroup analyses to better define responder profiles</li> <li>- Harmonization of practices concerning the frequency and duration of ECP, including RCTs on continuation vs. discontinuation in stable patients under treatment</li> <li>- Evaluation of novel treatment targets, including the use of biomarkers, patient-reported outcome measures (e.g., quality of life and patient burden), acceptable and unacceptable side effects/adverse events, and central line requirement</li> <li>- Development of comparative cost-effectiveness studies</li> <li>- Beyond its role in CLAD management, more data is needed on the role of ECP in CLAD prevention</li> </ul> |
| ATG                                        | <ul style="list-style-type: none"> <li>- RCTs are warranted to better define efficacy and safety</li> <li>- Subgroup analyses to better define responder profiles, including different CLAD phenotypes</li> <li>- Determination of optimal dosing, treatment duration, and appropriate monitoring strategies</li> <li>- Data on high-risk groups, such as EBV-mismatched recipients at increased PTLTD risk</li> </ul>                                                                                                                                                                                                                                                                                                                                                                           |
| Alemtuzumab                                | <ul style="list-style-type: none"> <li>- RCTs are necessary to better define efficacy and safety</li> <li>- Subgroup analyses to better define responder profiles, including different CLAD phenotypes and patients with DSAs</li> <li>- Determination of optimal dosing and scheduling of alemtuzumab</li> <li>- Better risk assessment for adverse events (e.g., PTLTD risk in patients with EBV mismatch)</li> </ul>                                                                                                                                                                                                                                                                                                                                                                          |
| Antifibrotics                              | <ul style="list-style-type: none"> <li>- For the RAS phenotype, further investigation should be decided based on the results from the PIRCLAD study</li> <li>- Studies with other antifibrotics, such as nerandomilast</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Other                                      | <ul style="list-style-type: none"> <li>- Clinical trials with novel drugs in CLAD are an unmet need, and the Task Force recommends conducting well-designed RCTs (ideally multicenter RCTs) for CLAD treatment, in accordance with the recent ISHLT consensus statement on Lung Transplant Clinical Trials [105]</li> <li>- More data is needed on the efficacy and safety of JAK inhibitors in CLAD</li> <li>- More data is needed on the efficacy and safety of ROCK inhibitors in CLAD</li> <li>- More data is needed on the efficacy and safety of mesenchymal stem cells in CLAD</li> <li>- Data are required on other novel potential disease-modifying drugs</li> </ul>                                                                                                                   |

ATG, anti-thymocyte globulin; DSAs, donor-specific antibodies; ECP, extracorporeal photopheresis; JAK, Janus kinase; PTLTD, post-transplant lymphoproliferative disease; RCT, randomized controlled trials; ROCK, Rho kinase.

responders showing superior progression-free and overall survival compared with non-responders [110]. A smaller study ( $n = 22$ ) reported slower FEV1 decline with montelukast compared to standard therapy (13 vs. 114 mL/month) [109].

Overall, these data suggest montelukast may stabilize lung function and delay disease progression in selected CLAD patients. Rapid decliners were less likely to respond [110] and were excluded from the randomized trial [108].

Based on available data on pulmonary chronic graft-versus-host disease (BOS-phenotype) following allogeneic hematopoietic stem cell transplantation, montelukast is recommended as part of the standard first-line “FAM” therapy, which includes inhaled corticosteroids, azithromycin, and montelukast [111]. Nevertheless, the magnitude of montelukast’s therapeutic effect in this context remains uncertain.

No severe adverse events were attributed to montelukast; some patients reported vivid dreams [110]; such sleep disturbances, including nightmares, are known from clinical experience. Rare neuropsychiatric adverse events are recognized from broader use in asthma and allergic disease, leading the U.S. Food and Drug Administration to issue a Boxed Warning for montelukast regarding serious mental health-related adverse effects [112]. Although data largely derive from non-transplant populations,

they are relevant to transplant recipients, who experience neuropsychiatric vulnerability. No severe neuropsychiatric events were reported in the study by Vos et al. [110], and such effects appear uncommon from clinical experience.

Overall, data suggest an acceptable safety profile with a low likelihood of serious harm in the transplant population.

## Justification of recommendation

Overall, the RCT did not demonstrate an effect of montelukast on FEV1; however, some benefit was observed in patients with early-stage BOS (BOS stage 1). Therefore, montelukast may be considered in this group, with discontinuation advised in the event of disease progression.

## Implementation considerations

The advantage of montelukast compared with other treatments (e.g., ECP or ATG) is its oral administration and low costs. Treatment response should be monitored and discontinuation should be considered in the absence of response. Patients should also be monitored for neurological and psychiatric adverse effects, and changes in sleep patterns.

## Future research

The Task Force did not formulate specific future research recommendations for montelukast, as they perceive priorities rather lie elsewhere.

## Treatment dosages and schedules

The most common dosages and schedules of CLAD therapeutics are summarized in [Table 2](#). Since many studies in this field are single center, the dosages used also vary. Regarding tacrolimus, different target levels have been used even in RCTs. The target levels for tacrolimus from the ScanCLAD trial were 10–14 ng/mL at 0–3 months, 8–12 ng/mL at 3–6 months, 8–10 ng/mL at 6–12 months, and 6–8 ng/mL beyond 12 months [4]. A recently published guideline for German-speaking countries for follow-up care after lung transplantation summarizes the target levels for tacrolimus used in other studies [12].

## Conclusion

The ESOT Task Force recommendations on prevention and treatment of CLAD are summarized in [Table 3](#) and displayed in [Figures 1–3](#). Future research recommendations for prevention and treatment are provided in [Tables 4–5](#).

CLAD remains the primary obstacle to long-term success after lung transplantation. It imposes a heavy disease burden, leading to substantial morbidity, diminished quality of life, and premature graft loss in affected recipients. CLAD prevention and treatment are challenging, and guidelines on the management of CLAD are lacking. This ESOT clinical practice guideline on the prevention and treatment of CLAD—developed by a multidisciplinary Task Force including patient and allied healthcare representation, using systematic literature reviews and GRADE methodology—offers evidence-based recommendations to guide contemporary clinical practice.

For prevention, we recommend tacrolimus over cyclosporine as the preferred CNI and conditionally suggest prophylactic azithromycin to delay or reduce the onset of CLAD. MMF and AZA are regarded as comparable cell cycle inhibitors; the choice between them and, potentially, other agents (*e.g.*, mTOR inhibitors) should be individualized according to gastrointestinal tolerance, risk of myelotoxicity, fertility considerations, and other patient-specific factors.

For established (progressive) CLAD, no standardized or highly effective treatment currently exists. Clinical studies are largely limited to surrogate endpoints, such as preservation of graft function measured by FEV1, while data on hard clinical outcomes, including patient survival and patient-relevant outcomes such as quality of life, remain limited. Most interventions, including ECP, ATG, alemtuzumab, and montelukast, received only conditional recommendations owing to very low to low certainty of evidence. Antifibrotic treatment with pirfenidone is not suggested in patients with CLAD-BOS phenotype, but antifibrotics could be considered in patients with the RAS phenotype, pending more robust data. No therapy has been

conclusively shown to halt or reverse disease progression. Retransplantation, while potentially curative, is unfortunately available to only a small minority of patients due to organ scarcity, procedural complexity and patient-specific comorbidities limiting candidacy.

These largely conditional recommendations highlight major evidence gaps. High-quality RCTs, conducted in accordance with the recent International Society for Heart and Lung Transplantation clinical trials consensus statement [113], standardized management pathways, responder identification, and inclusion of patient-reported outcomes are urgently required to better inform future recommendations on the management of CLAD. In the interim, individualized risk-benefit assessment, shared decision-making, prevention and treatment of modifiable risk factors (*e.g.*, non-adherence, infections, gastroesophageal reflux), and close monitoring remain essential to preserve the best possible graft function after lung transplantation.

## Data availability statement

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

## Author contributions

All task force members, except the information specialist ME, were involved in designing the PICO questions, outcomes of interest, screening of the literature, data extraction, creation of EtD tables and recommendations. SB and MH were responsible for the methodology (overview of the process, GRADEing, creation of evidence profiles). SB, MH, JG and RV wrote the manuscript. Everyone reviewed the final version of the manuscript. ME performed the literature searches. 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

The author(s) declared that generative AI was not used in the creation of this manuscript.

## References

- Chambers DC, Cherikh WS, Harhay MO, Hayes D, Hsieh 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
- 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
- Kulkarni HS, Cherikh WS, Chambers DC, Garcia VC, Hachem RR, Kreisel D, et al. Bronchiolitis obliterans syndrome-free survival after lung transplantation: an international society for heart and lung transplantation thoracic transplant registry analysis. *J Heart Lung Transpl* (2019) 38(1):5–16. doi: 10.1016/j.healun.2018.09.016
- Dellgren G, Lund TK, Raivio P, Leuckfeld I, Svahn J, Holmberg EC, et al. Effect of once-per-day tacrolimus versus twice-per-day ciclosporin on 3-year incidence of chronic lung allograft dysfunction after lung transplantation in Scandinavia (ScanCLAD): a multicentre randomised controlled trial. *Lancet Respir Med* (2024) 12(1):34–44. doi: 10.1016/S2213-2600(23)00293-X
- Glanville AR, Verleden GM, Todd JL, Benden C, Calabrese F, Gottlieb J, et al. Chronic lung allograft dysfunction: definition and update of restrictive allograft syndrome-A consensus report from the pulmonary council of the ISHLT. *J Heart Lung Transpl* (2019) 38(5):483–92. doi: 10.1016/j.healun.2019.03.008
- Bos S, Milross L, Filby AJ, Vos R, Fisher AJ. Immune processes in the pathogenesis of chronic lung allograft dysfunction: identifying the missing pieces of the puzzle. *Eur Respir Rev* (2022) 31(165):220060. doi: 10.1183/16000617.0060-2022
- Nelson J, Alvey N, Bowman L, Schulte J, Segovia MC, McDermott J, et al. Consensus recommendations for use of maintenance immunosuppression in solid organ transplantation: endorsed by the American college of clinical pharmacy, American society of transplantation, and international society for heart and lung transplantation: an executive summary. *Pharmacotherapy* (2022) 42(8):594–8. doi: 10.1002/phar.2718
- Erdman J, Wolfram J, Nimke D, Croy R, Wang X, Weaver T, et al. Lung transplant outcomes in adults in the United States: retrospective cohort study using real-world evidence from the SRTTR. *Transplantation* (2022) 106(6):1233–42. doi: 10.1097/TP.0000000000004011
- Valapour M, Skeans MA, Smith JM, Edwards LB, Cherikh WS, Callahan ER, et al. Lung. *Am J Transpl* (2016) 16(Suppl. 2):141–68. doi: 10.1111/ajt.13671
- Gottlieb J, Vos R, Jaksch P, Hellemons M, Holm AM, Morlacchi LC, et al. Chronic lung allograft dysfunction after lung transplantation: prevention, diagnosis and treatment in 44 European centres. *ERJ Open Res* (2025) 11(3):00675–2024. doi: 10.1183/23120541.00675-2024
- Bos S, Pradère P, Beeckmans H, Zajacova A, Vanaudenaerde BM, Fisher AJ, et al. Lymphocyte depleting and modulating therapies for chronic lung allograft dysfunction. *Pharmacol Rev* (2023) 75(6):1200–17. doi: 10.1124/pharmrev.123.000834
- Gottlieb J, Wald A, Bucher B, Tapp B, Hirsch C, Schneider C, et al. Follow-Up care after adult lung transplantation: summary of an evidence-based clinical practice guideline for german-speaking countries. *Respiration* (2026) 16:1–27. doi: 10.1159/000553086
- Guyatt GH, Oxman AD, Kunz R, Atkins D, Brozek J, Vist G, et al. GRADE guidelines: 2. Framing the question and deciding on important outcomes. *J Clin Epidemiol* (2011) 64(4):395–400. doi: 10.1016/j.jclinepi.2010.09.012
- Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* (2021) 372:n71. doi: 10.1136/bmj.n71
- Brozek JL, Akl EA, Compalati E, Kreis J, Terracciano L, Focchi A, et al. Grading quality of evidence and strength of recommendations in clinical practice guidelines part 3 of 3. The GRADE approach to developing recommendations. *Allergy* (2011) 66(5):588–95. doi: 10.1111/j.1398-9995.2010.02530.x
- Alonso-Coello P, Schünemann HJ, Moher J, Brignardello-Petersen R, Akl EA, Davoli M, et al. GRADE evidence to decision (EtD) frameworks: a systematic and transparent approach to making well informed healthcare choices. 1: introduction. *BMJ* (2016) 353:i2016. doi: 10.1136/bmj.i2016
- Schünemann H, Brozek J, Guyatt G, Oxman A, editors. *GRADE Handbook for Grading Quality of Evidence and Strength of Recommendations*. The GRADE Working Group (2013) Available online at: [guidelinedevelopment.org/handbook](http://guidelinedevelopment.org/handbook).
- Andrews J, Guyatt G, Oxman AD, Alderson P, Dahm P, Falck-Ytter Y, et al. GRADE guidelines: 14. Going from evidence to recommendations: the significance and presentation of recommendations. *J Clin Epidemiol* (2013) 66(7):719–25. doi: 10.1016/j.jclinepi.2012.03.013
- Santesso N, Glenton C, Dahm P, Garner P, Akl EA, Alper B, et al. GRADE guidelines 26: informative statements to communicate the findings of systematic reviews of interventions. *J Clin Epidemiol* (2020) 119:126–35. doi: 10.1016/j.jclinepi.2019.10.014
- Vos R, Vanaudenaerde BM, Verleden SE, De VSI, Willems-Widyastuti A, Raemdonck DEV, et al. A randomised controlled trial of azithromycin to prevent chronic rejection after lung transplantation. *Eur Respir J* (2010) 37(1):164–72. doi: 10.1183/09031936.00068310
- Van Herck A, Frick AE, Schaevers V, Vranckx A, Verbeken EK, Vanaudenaerde BM, et al. Azithromycin and early allograft function after lung transplantation: a randomized, controlled trial. *The J Heart Lung Transpl* (2019) 38(3):252–9. doi: 10.1016/j.healun.2018.12.006
- Rutten D, Verleden SE, Vandermeulen E, Bellon H, Vanaudenaerde BM, Somers J, et al. Prophylactic azithromycin therapy after lung transplantation: post hoc analysis of a randomized controlled trial. *Am J Transpl* (2016) 16(1):254–61. doi: 10.1111/ajt.13417
- Li D, Duan Q, Weinkauff J, Kapasi A, Varughese R, Hirji A, et al. Azithromycin prophylaxis after lung transplantation is associated with improved overall survival. *The J Heart Lung Transpl* (2020) 39(12):1426–34. doi: 10.1016/j.healun.2020.09.006
- Cristeto PM, Mora Cuesta VM, Iturbe Fernández D, Tello Mena S, Alonso Lecue P, Sánchez Moreno L, et al. Early onset of azithromycin to prevent CLAD in lung transplantation: promising results of a retrospective single centre experience. *Clin Transpl* (2023) 37(1):e14832. doi: 10.1111/ctr.14832
- Spence CD, Vanaudenaerde B, Einarsson GG, McDonough J, Lee AJ, Johnston E, et al. Influence of azithromycin and allograft rejection on the post-lung transplant microbiota. *The J Heart Lung Transpl* (2020) 39(2):176–83. doi: 10.1016/j.healun.2019.11.007
- Li H, Liu DH, Chen LL, Zhao Q, Yu YZ, Ding JJ, et al. Meta-analysis of the adverse effects of long-term azithromycin use in patients with chronic lung diseases *Antimicrob agents chemother*. *Antimicrob Agents Chemother* (2014) 58(1):511–7. doi: 10.1128/AAC.02067-13
- Zuckermann A, Reichenspurner H, Birsan T, Treede H, Deviatko E, Reichart B, et al. Cyclosporine A versus tacrolimus in combination with mycophenolate mofetil and steroids as primary immunosuppression after lung transplantation: one-year results of a 2-center prospective randomized trial. *J Thorac Cardiovasc Surg* (2003) 125(4):891–900. doi: 10.1067/jmtc.2003.71
- Hachem RR, Yusen RD, Chakinala MM, Meyers BF, Lynch JP, Aloush AA, et al. A randomized controlled trial of tacrolimus versus cyclosporine after lung transplantation. *J Heart Lung Transpl* (2007) 26(10):1012–8. doi: 10.1016/j.healun.2007.07.027
- Treede H, Glanville AR, Klepetko W, Abooun C, Vettorazzi E, Lama R, et al. Tacrolimus and cyclosporine have differential effects on the risk of development of bronchiolitis obliterans syndrome: results of a prospective, randomized international trial in lung transplantation. *J Heart Lung Transpl* (2012) 31(8):797–804. doi: 10.1016/j.healun.2012.03.008

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## Supplementary material

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30. Treede H, Klepetko W, Reichenspurner H, Zuckermann A, Meiser B, Birsan T, et al. Tacrolimus versus cyclosporine after lung transplantation: a prospective, open, randomized two-center trial comparing two different immunosuppressive protocols. *J Heart Lung Transpl* (2001) 20(5):511–7. doi: 10.1016/s1053-2498(01)00244-3
31. Griffith BP, Bando K, Hardesty RL, Armitage JM, Keenan RJ, Pham SM, et al. A prospective randomized trial of FK506 versus cyclosporine after human pulmonary transplantation. *Transplantation* (1994) 57(6):848–51. doi: 10.1097/00007890-199403270-00013
32. Keenan RJ, Konishi H, Kawai A, Paradis IL, Nunley DR, Iacono AT, et al. Clinical trial of tacrolimus versus cyclosporine in lung transplantation. *Ann Thorac Surg* (1995) 60(3):580–4. doi: 10.1016/0003-4975(95)00407-C
33. Reichenspurner H, Kur F, Treede H, Meiser BM, Deutsch O, Welz A, et al. Optimization of the immunosuppressive protocol after lung transplantation. *Transplantation* (1999) 68(1):67–71. doi: 10.1097/00007890-199907150-00013
34. Slebos DJ, Kauffman HF, Koöeter GH, Verschuuren EAM, van der Bij W, Postma DS. Airway cellular response to two different immunosuppressive regimens in lung transplant recipients. *Clin Transpl* (2005) 19(2):243–9. doi: 10.1111/j.1399-0012.2005.00330.x
35. Combs MP, Walter K, Hixson H, Belloli EA, Najor MS, Chan KM, et al. Impact of tacrolimus vs cyclosporine on chronic lung allograft dysfunction incidence and allograft survival in the international society of heart and lung transplantation registry. *J Heart Lung Transpl* (2025) 44(3):307–17. doi: 10.1016/j.healun.2024.10.013
36. Webster AC, Taylor RR, Chapman JR, Craig JC. Tacrolimus versus cyclosporin as primary immunosuppression for kidney transplant recipients. *Cochrane Database Syst Rev* (2005) (4):CD003961. doi: 10.1002/14651858.CD003961.pub2
37. Haddad E, McAlister V, Renouf E, Malthaner R, Kjaer MS, Gluud LL. Cyclosporin versus tacrolimus for liver transplanted patients. *Cochrane Database Syst Rev* (2006) 2010(1):CD005161. doi: 10.1002/14651858.CD005161.pub2
38. Penninga L, Möller CH, Gustafsson F, Steinbrüchel DA, Gluud C. Tacrolimus versus cyclosporine as primary immunosuppression after heart transplantation: systematic review with meta-analyses and trial sequential analyses of randomised trials. *Eur J Clin Pharmacol* (2010) 66(12):1177–87. doi: 10.1007/s00228-010-0902-6
39. Muduma G, Saunders R, Odeyemi I, Pollock RF. Systematic review and meta-analysis of tacrolimus versus cyclosporin as primary immunosuppression after liver transplant. *PLoS One* (2016) 11(11):e0160421. doi: 10.1371/journal.pone.0160421
40. Godinas L, Dobbels F, Hulst L, Verbeek I, De Coninck I, Berrevoets P, et al. Once daily tacrolimus conversion in lung transplantation: a prospective study on safety and medication adherence. *The J Heart Lung Transplant* (2021) 40(6):467–77. doi: 10.1016/j.healun.2021.02.017
41. Grootjans H, Verschuuren E, Kerstjens H, Bakker S, Steenhuis W, Berger S, et al. Randomized controlled trial comparing immediate versus extended release tacrolimus; reducing calcineurin inhibitor related toxicity in lung transplantation patients; the revolution trial. *The J Heart Lung Transplant* (2024) 43(4):S36. doi: 10.1016/j.healun.2024.02.071
42. Pitre T, Gurupatham S, Desai K, Binnie M, Martinu T, Juvet S, et al. Tacrolimus versus cyclosporine immunosuppression in lung transplantation: a systematic review and meta-analysis. *BMJ Open Respir Res* (2025) 12(1):e002672. doi: 10.1136/bmjresp-2024-002672
43. Penninga L, Penninga EI, Möller CH, Iversen M, Steinbrüchel DA, Gluud C. Tacrolimus versus cyclosporin as primary immunosuppression for lung transplant recipients. *Cochrane Database Syst Rev* (2013) 2013(5):CD008817. doi: 10.1002/14651858.CD008817.pub2
44. Guyatt G, Oxman AD, Sultan S, Brozek J, Glasziou P, Alonso-Coello P, et al. GRADE guidelines: 11. Making an overall rating of confidence in effect estimates for a single outcome and for all outcomes. *J Clin Epidemiol* (2013) 66(2):151–7. doi: 10.1016/j.jclinepi.2012.01.006
45. McNeil K, Glanville AR, Wahlers T, Knoop C, Speich R, Mamelok RD, et al. Comparison of mycophenolate mofetil and azathioprine for prevention of bronchiolitis obliterans syndrome in *de novo* lung transplant recipients. *Transplantation* (2006) 81(7):998–1003. doi: 10.1097/01.tp.0000202755.33883.61
46. Palmer SM, Baz MA, Sanders L, Miralles AP, Lawrence CM, Rea JB, et al. Results of a randomized, prospective, multicenter trial of mycophenolate mofetil versus azathioprine in the prevention of acute lung allograft rejection. *Transplantation* (2001) 71(12):1772–6. doi: 10.1097/00007890-200106270-00012
47. Zuckermann A, Klepetko W, Birsan T, Taghavi S, Artemiou O, Wisser W, et al. Comparison between mycophenolate mofetil- and azathioprine-based immunosuppressions in clinical lung transplantation. *The J Heart Lung Transplant* (1999) 18(5):432–40. doi: 10.1016/S1053-2498(99)00004-2
48. Tague LK, Byers DE, Hachem R, Kreisel D, Krupnick AS, Kulkarni HS, et al. Impact of SLC01B3 polymorphisms on clinical outcomes in lung allograft recipients receiving mycophenolic acid. *Pharmacogenomics J* (2019) 20(1):69–79. doi: 10.1038/s41397-019-0086-0
49. Bhorade SM, Jordan A, Villanueva J, Yu A, Kramer H, Vigneswaran WT, et al. Comparison of three tacrolimus-based immunosuppressive regimens in lung transplantation. *Am J Transplant* (2003) 3(12):1570–5. doi: 10.1046/j.1600-6135.2003.00260.x
50. Speich R, Schneider S, Hofer M, Irani S, Vogt P, Weder W, et al. Mycophenolate mofetil reduces alveolar inflammation, acute rejection and graft loss due to bronchiolitis obliterans syndrome after lung transplantation. *Pulm Pharmacol Ther* (2010) 23(5):445–9. doi: 10.1016/j.pupt.2010.04.004
51. Sanborn DM, Friesen JN, Yadav H, Kennedy CC, Wylam ME, Peters SG, et al. Outcomes in lung transplant recipients on azathioprine compared to mycophenolate. *Respir Med* (2025) 239:107991. doi: 10.1016/j.rmed.2025.107991
52. Ross DJ, Waters PF, Levine M, Kramer M, Ruzevich S, Kass RM. Mycophenolate mofetil versus azathioprine immunosuppressive regimens after lung transplantation: preliminary experience. *J Heart Lung Transpl* (1998) 17(8):768–74.
53. Sterneck M, Settmacher U, Ganten T, Sarrazin C, Speidel N, Broering D, et al. Improvement in gastrointestinal and health-related quality of life outcomes after conversion from mycophenolate mofetil to enteric-coated mycophenolate sodium in liver transplant recipients. *Transpl Proc* (2014) 46(1):234–40. doi: 10.1016/j.transproceed.2013.09.026
54. Hwang HS, Hyoung BJ, Kim S, Oh HY, Kim YS, Kim JK, et al. Improved gastrointestinal symptoms and quality of life after conversion from mycophenolate mofetil to enteric-coated mycophenolate sodium in renal transplant patients receiving tacrolimus. *J Korean Med Sci* (2010) 25(12):1759–65. doi: 10.3346/jkms.2010.25.12.1759
55. Jiyad Z, Olsen CM, Burke MT, Isbel NM, Green AC. Azathioprine and risk of skin cancer in organ transplant recipients: systematic review and meta-analysis. *Am J Transpl* (2016) 16:3490–503. doi: 10.1111/ajt.13863
56. Grieves K, Keller BC, Waldman G, Clark JE. Impact of reduced mycophenolate exposure on chronic lung allograft dysfunction incidence after lung transplant. *JHLT Open* (2024) 6:100156. doi: 10.1016/j.jhlto.2024.100156
57. fda. Available online at: [https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2008/065410s000lbl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2008/065410s000lbl.pdf) (Accessed March 27, 2026).
58. WHO. *Model List of Essential Medicines* (2025). doi: 10.2471/B09474
59. Eichelbaum M, Ingelman-Sundberg M, Evans WE. Pharmacogenomics and individualized drug therapy. *Annu Rev Med* (2006) 57:119–37. doi: 10.1146/annurev.med.56.082103.104724
60. Betonico GN, Abudd-Filho M, Goloni-Bertollo EM, Pavarino-Bertelli E. Pharmacogenetics of mycophenolate mofetil: a promising different approach to tailoring immunosuppression? *J Nephrol* (2008) 21(4):503–9.
61. Fante CD, Scudeller L, Oggionni T, Viarengo G, Cemmi F, Morosini M, et al. Clinical investigations long-term off-line extracorporeal photochemotherapy in patients with chronic lung allograft rejection not responsive to conventional treatment: a 10-Year single-center analysis. *Respiration* (2015) 90:118–28. doi: 10.1159/000431382
62. Hage CA, Klesney-Tait J, Wille K, Arcasoy S, Yung G, Hertz M, et al. Extracorporeal photopheresis to attenuate decline in lung function due to refractory obstructive allograft dysfunction. *Transfus Med* (2021) 31(4):292–302. doi: 10.1111/tme.12779
63. Jaksch P, Scheed A, Keplinger M, Ernst MB, Dani T, Just U, et al. A prospective interventional study on the use of extracorporeal photopheresis in patients with bronchiolitis obliterans syndrome after lung transplantation. *J Heart Lung Transplant* (2012) 31(9):950–7. doi: 10.1016/j.healun.2012.05.002
64. Leroux J, Hirschi S, Essaydi A, Bohbot A, Degot T, Schuller A, et al. Initiation of extracorporeal photopheresis in lung transplant patients with mild to moderate refractory BOS: a single-center real-life experience. *Respir Med Res* (2022) 81:100913. doi: 10.1016/j.resmer.2022.100913
65. Moniodis A, Townsend K, Rabin A, Aloum O, Stempel J, Burkett P, et al. Comparison of extracorporeal photopheresis and alemtuzumab for the treatment of chronic lung allograft dysfunction. *The J Heart Lung Transplant* (2018) 37(3):340–8. doi: 10.1016/j.healun.2017.03.017
66. Pecoraro Y, Carillo C, Diso D, Mantovani S, Cimino G, De Giacomo T, et al. Efficacy of extracorporeal photopheresis in patients with bronchiolitis obliterans syndrome after lung transplantation. *Transpl Proc* (2017) 49(4):695–8. doi: 10.1016/j.transproceed.2017.02.035
67. Salerno CT, Park SJ, Kreykes NS, Kulick DM, Savik K, Hertz MI, et al. Adjuvant treatment of refractory lung transplant rejection with extracorporeal photopheresis. *J Thorac Cardiovasc Surg* (1999) 117(6):1063–9. doi: 10.1016/S0022-5223(99)70241-2
68. O'Hagan AR, Stillwell PC, Armliga A, Koo A. Photopheresis in the treatment of refractory bronchiolitis obliterans complicating lung transplantation. *Chest* (1999) 115(5):1459–62. doi: 10.1378/chest.115.5.1459
69. Villanueva J, Bhorade SM, Robinson JA, Husain AN, Garrity ER. Extracorporeal photopheresis for the treatment of lung allograft rejection. *Ann Transpl* (2000) 5(3):44–7.
70. Meloni F, Cascina A, Miserere S, Perotti C, Vitolo P, Fietta AM. Peripheral CD4+CD25+ TREG cell counts and the response to extracorporeal photopheresis in lung transplant recipients. *Transpl Proc* (2007) 39(1):213–7. doi: 10.1016/j.transproceed.2006.10.227
71. Benden C, Speich R, Hofbauer GF, Irani S, Eich-Wanger C, Russi EW, et al. Extracorporeal photopheresis after lung transplantation: a 10-Year single-center experience. *Transplantation* (2008) 86(11):1625–7. doi: 10.1097/TP.0b013e31818bc024



72. Morrell MR, Despotis GJ, Lublin DM, Patterson GA, Trulock EP, Hachem RR. The efficacy of photopheresis for bronchiolitis obliterans syndrome after lung transplantation. *The J Heart Lung Transplant* (2010) 29(4):424–31. doi: 10.1016/j.healun.2009.08.029
73. Greer M, Liu B, Magnusson JM, Fuehner T, Schmidt BMW, Deluca D, et al. Assessing treatment outcomes in CLAD: the hannover-extracorporeal photopheresis model. *J Heart Lung Transplant* (2023) 42(2):209–17. doi: 10.1016/j.healun.2022.09.022
74. Greer M, Dierich M, De Wall C, Suhling H, Rademacher J, Welte T, et al. Phenotyping established chronic lung allograft dysfunction predicts extracorporeal photopheresis response in lung transplant patients. *Am J Transplant* (2013) 13(4):911–8. doi: 10.1111/ajt.12155
75. Isenring B, Robinson C, Buergi U, Schuurmans MM, Kohler M, Huber LC, et al. Lung transplant recipients on long-term extracorporeal photopheresis. *Clin Transpl* (2017) 31(10). doi: 10.1111/ctr.13041
76. Chionis L, Grossman BJ, Hachem R, Commean P, Derfler MC, Vedantham S, et al. The efficacy of extracorporeal photopheresis to arrest bronchiolitis obliterans in lung allograft recipients was compared between two automated photopheresis instruments. *Transfusion (Paris)* (2018) 58(12):2933–41. doi: 10.1111/trf.14913
77. Karnes HE, Schindler EI, Morrell M, Hachem RR, Berman K, Vedantham S, et al. Factors associated with mortality and response to extracorporeal photopheresis in lung allograft recipients with bronchiolitis obliterans syndrome. *Transplantation* (2019) 103(5):1036–42. doi: 10.1097/TP.0000000000002430
78. Vazirani J, Routledge D, Snell GI, Watson D, Paraskeva M, Westall GP, et al. Outcomes following extracorporeal photopheresis for chronic lung allograft dysfunction following lung transplantation: a single-center experience. *Transpl Proc* (2021) 53(1):296–302. doi: 10.1016/j.transproceed.2020.09.003
79. Benazzo A, Bagnera C, Ius F, Fante CD, Gottlieb J, Hoetzenecker K, et al. A European multi-center analysis of extracorporeal photopheresis as therapy for chronic lung allograft dysfunction. *Transpl Int* (2024) 36:11551. doi: 10.3389/ti.2023.11551
80. Gautschi F, Vogelmann T, Ortmanns G, Knörr F, Steinack C, Hage R, et al. Early extracorporeal photopheresis treatment is associated with better survival in patients with chronic or recurrent acute lung allograft dysfunction. *J Clin Apher* (2024) 39(3):e22128. doi: 10.1002/jca.22128
81. Benazzo A, Cho A, Auner S, Schwarz S, Kovacs Z, Ramazanov D, et al. Extracorporeal photopheresis for the prevention of rejection after lung transplantation: a prospective randomised controlled trial. *Eur Respir J* (2025) 65(2):2400733. doi: 10.1183/13993003.00733-2024
82. Fisher AJ, White M, Goudie N, Kershaw A, Phillipson J, Bardgett M, et al. Extracorporeal photopheresis (ECP) in the treatment of chronic lung allograft dysfunction (CLAD): a prospective, multicentre, open-label, randomised controlled trial studying the addition of ECP to standard care in the treatment of bilateral lung transplant patients with CLAD (E-CLAD UK). *BMJ Open Respir Res* (2024) 11(1):e001995. doi: 10.1136/bmjresp-2023-001995
83. Kesten S, Rajagopalan N, Maurer J. Cytolytic therapy for the treatment of bronchiolitis obliterans syndrome following lung transplantation. *Transplantation* (1996) 61(3):427–30. doi: 10.1097/00007890-199602150-00019
84. Snell GI, Esmore DS, Williams TJ. Cytolytic therapy for the bronchiolitis obliterans syndrome complicating lung transplantation. *Chest* (1996) 109(4):874–8. doi: 10.1378/chest.109.4.874
85. Date H, Lynch JP, Sundaresan S, Patterson GA, Trulock EP. The impact of cytolytic therapy on bronchiolitis obliterans syndrome. *J Heart Lung Transpl* (1998) 17(9):869–75.
86. Izhakian S, Wasser WG, Fox BD, Vainshelboim B, Reznik JE, Kramer MR. Effectiveness of rabbit antithymocyte globulin in chronic lung allograft dysfunction. *Transpl Proc* (2016) 48(6):2152–6. doi: 10.1016/j.transproceed.2016.04.024
87. January SE, Fester KA, Bain KB, Kulkarni HS, Witt CA, Byers DE, et al. Rabbit antithymocyte globulin for the treatment of chronic lung allograft dysfunction. *Clin Transpl* (2019) 33(10):e13708. doi: 10.1111/ctr.13708
88. Kotecha S, Paul E, Ivulich S, Fuller J, Paraskeva M, Levvey B, et al. Outcomes following ATG therapy for chronic lung allograft dysfunction. *Transpl Direct* (2021) 7(4):E681. doi: 10.1097/TXD.0000000000001134
89. Margallo Iribarnegaray J, De Pablo Gafas A, Alonso Moralejo R, Quezada Loaiza CA, Revuelta Salgado F, Pina Maizquez I, et al. Antithymocyte globulin treatment for chronic lung allograft dysfunction in lung transplant recipients: experience from a national reference transplant center. *Transpl Proc* (2021) 53(9):2710–7. doi: 10.1016/j.transproceed.2021.08.039
90. Dunn CT, Brandon W, Jacob A, Zhang S, Gao A, Torres F, et al. Anti-thymocyte globulin as a therapy for chronic lung allograft dysfunction: a single-center experience. *Clin Transpl* (2024) 38(10):e15461. doi: 10.1111/ctr.15461
91. Padhye AA, Guffey D, Leon-Pena A, Segraves J, Fernandez R, Loo G, et al. Antithymocyte globulin therapy in chronic lung allograft dysfunction. *Front Transplant* (2025) 4:1607678. doi: 10.3389/frtra.2025.1607678
92. Penninga L, Möller CH, Penninga EI, Iversen M, Gluud C, Steinbrüchel DA. Antibody induction therapy for lung transplant recipients. *Cochrane Database Syst Rev* (2013) 2013(11):CD008927. doi: 10.1002/14651858.CD008927.pub2
93. Hertig A, Zuckermann A. Rabbit antithymocyte globulin induction and risk of post-transplant lymphoproliferative disease in adult and pediatric solid organ transplantation: an update. *Transpl Immunol* (2015) 32(3):179–87. doi: 10.1016/j.trim.2015.04.003
94. Reams BD, Musselwhite LW, Zaas DW, Steele MP, Garantzios S, Eu PC, et al. Alemtuzumab in the treatment of refractory acute rejection and bronchiolitis obliterans syndrome after human lung transplantation. *Am J Transplant* (2007) 7(12):2802–8. doi: 10.1111/j.1600-6143.2007.02000.x
95. Ensor CR, Rihtarchik LC, Morrell MR, Hayanga JWA, Lichvar AB, Pilewski JM, et al. Rescue alemtuzumab for refractory acute cellular rejection and bronchiolitis obliterans syndrome after lung transplantation. *Clin Transpl* (2017) 31(4):e12899. doi: 10.1111/ctr.12899
96. van Vugt LK, van der Zwan M, Clahsen-van Groningen MC, van Agteren M, Hullege-Peelen DM, De Winter BCM, et al. A decade of experience with alemtuzumab therapy for severe or glucocorticoid-resistant kidney transplant rejection. *Transpl Int* (2023) 36:11834. doi: 10.3389/ti.2023.11834
97. van der Zwan M, Clahsen-Van Groningen MC, van den Hoogen MWF, Kho MML, Roodnat JJ, Mauff KAL, et al. Comparison of Alemtuzumab and anti-thymocyte globulin treatment for acute kidney allograft rejection. *Front Immunol* (2020) 11:1332. doi: 10.3389/fimmu.2020.01332
98. van Vugt LK, Tegzess E, van der Zwan M, Clahsen-van Groningen MC, de Winter BCM, Vart P, et al. Multicenter, real-world clinical evaluation of Alemtuzumab and anti-thymocyte globulin for severe acute T cell-mediated kidney transplant rejection. *Clin Transpl* (2024) 38(12):e70046. doi: 10.1111/ctr.70046
99. Benazzo A, Auner S, Boehm PM, Morscher A, Schwarz S, Vidmar B, et al. Outcomes with alemtuzumab induction therapy in lung transplantation: a comprehensive large-scale single-center analysis. *Transpl Int* (2021) 34(12):2633–43. doi: 10.1111/tri.14153
100. Combs MP, Belloli EA, Gargurevich N, Flaherty KR, Murray S, Galbán CJ, et al. Results from randomized trial of pirfenidone in patients with chronic rejection (STOP-CLAD study). *The J Heart Lung Transplant* (2024) 43(9):1468–77. doi: 10.1016/j.healun.2024.05.013
101. Perch M, Corris P, Lordan J, Bessa V, Magnusson J, Verleden GM, et al. A European multi-center, randomized controlled trial of Pirfenidone in bronchiolitis obliterans syndrome after bilateral lung transplantation. *Eur Respir J* (2025) 13:2402154. doi: 10.1183/13993003.02154-2024
102. NCT03283007. Nintedanib in lung transplant recipients with bronchiolitis obliterans syndrome grade 0p-1-2 | ClinicalTrials.gov. Available online at: <https://clinicaltrials.gov/study/NCT03283007> (Accessed March 27, 2026).
103. Vos R, Wuyts WA, Gheysens O, Goffin KE, Schaevers V, Verleden SE, et al. Pirfenidone in restrictive allograft syndrome after lung transplantation: a case series. *Am J Transplant* (2018) 18(12):3045–59. doi: 10.1111/ajt.15019
104. Bennett D, Lanzarone N, Fossi A, Perillo F, de Vita E, Luzzi L, et al. Pirfenidone in chronic lung allograft dysfunction: a single cohort study. *Panminerva Med* (2020) 62(3):143–9. doi: 10.23736/S0031-0808.19.03840-0
105. NCT03359863. Pirfenidone for restrictive chronic lung allograft dysfunction (PIRCLAD) | ClinicalTrials.gov. Available online at: <https://clinicaltrials.gov/study/NCT03359863> (Accessed March 27, 2026).
106. Richeldi L, Azuma A, Cottin V, Kreuter M, Maher TM, Martinez FJ, et al. Nintedanib in patients with idiopathic pulmonary fibrosis. *New Engl J Med* (2025) 392(22):2193–202. doi: 10.1056/NEJMoa2414108
107. West A, Chaudhuri N, Barczyk A, Wilsher ML, Hopkins P, Glaspole I, et al. Inhaled pirfenidone solution (AP01) for IPF: a randomised, open-label, dose-response trial. *Thorax* (2023) 78(9):882–9. doi: 10.1136/thorax-2022-219391
108. Rutters D, Verleden SE, Demeyer H, Van Raemdonck DE, Yserbyt J, Dupont LJ, et al. Montelukast for bronchiolitis obliterans syndrome after lung transplantation: a randomized controlled trial. *PLoS One* (2018) 13(4):e0193564. doi: 10.1371/journal.pone.0193564
109. Verleden GM, Verleden SE, Vos R, De Vleeschauwer SI, Dupont LJ, Van Raemdonck DE, et al. Montelukast for bronchiolitis obliterans syndrome after lung transplantation: a pilot study. *Transpl Int* (2011) 24(7):651–6. doi: 10.1111/j.1432-2277.2011.01248.x
110. Vos R, Eynde RV, Rutters D, Verleden SE, Vanaudenaerde BM, Dupont LJ, et al. Montelukast in chronic lung allograft dysfunction after lung transplantation. *J Heart Lung Transplant* (2019) 38(5):516–27. doi: 10.1016/j.healun.2018.11.014
111. Bos S, Murray J, Marchetti M, Cheng GS, Bergeron A, Wolff D, et al. ERS/EBMT clinical practice guidelines on treatment of pulmonary chronic graft-versus-host disease in adults. *Eur Respir J* (2024) 63(3):2301727. doi: 10.1183/13993003.01727-2023
112. Clarridge K, Chin S, Eworuke E, Seymour S. A boxed warning for montelukast: the FDA perspective. *J Allergy Clin Immunol In Pract* (2021) 9(7):2638–41. doi: 10.1016/j.jaip.2021.02.057
113. Greenland JR, Perch M, Halloran K, Levine DJ, Morrell ED, Reed A, et al. Considerations for endpoints in lung transplant clinical trials: an ISHLT consensus statement. *J Heart Lung Transpl* (2026) 45(2):e104–28. doi: 10.1016/j.healun.2025.09.017



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# Donor-derived viral infections: the usual suspects and the new kids on the block

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The expansion of the transplant donor pool, driven by the need to reduce the disparity between supply and demand, carries the risk of use of marginal donors with viral infections, including classic viruses and emerging threats. This narrative review analyzes these challenges, including new opportunities in managing well known viruses such as Hepatitis B, Hepatitis C and Human Immunodeficiency Virus in both donors and recipients, thanks to new effective antivirals. Furthermore, we highlight the importance of monitoring emerging viral pathogens, such as Hepatitis E and Human Herpesvirus 8 (HHV-8) implementing both targeted pre-transplant donor screening and timely post-transplant diagnosis in the recipient, enabling earlier therapeutic intervention and potentially reducing recipient morbidity and mortality. Moreover, respiratory viral infections have gained significant attention as potential causes of donor derived infection (DDI) transmitted to the recipient, particularly within specific widespread and seasonal contexts, and require further attention and investigation. Continuous updates to surveillance and screening protocols are essential to optimize outcomes of recipients, ensuring transplant safety.

### KEYWORDS

donor derived infection/transplant infection, HHV-8, HIV, respiratory viral infections, viral hepatitis

## Introduction

The number of individuals awaiting transplant continues to overcome donor availability. Several efforts are ongoing to expand the donor pool to include donors at increased infectious risk [1, 2].

A paradigm shift has occurred in the utilization of organs from HBV- and HCV-positive donors, with increasing acceptance of their use in both infected and uninfected recipients. The opioid epidemic has climbed sharply in specific settings (primarily North America), ascending rates of drug abusers with overdose-related deaths has led to the use of available organs from young donors with limited comorbidities, but with higher risk of transmission of HCV, HBV and Human Immunodeficiency Virus (HIV) [3–5]. Of interest that a significant increase in mortality waitlist has been described for individuals who decline these marginal organs compared with those who accept the risk reducing wait time [6, 7].

The potential for donor-derived transmission of previously underestimated viruses (Hepatitis E, Human herpesvirus 8) highlights the importance of surveillance and screening protocols in specific geographical settings [8, 9]. Respiratory viral infections have received increased attention in recent years, including as a potential cause of donor derived infection (DDI), especially in specific seasonal settings [10]. The complex and intricate relationship between climate change, human migration, and infectious disease transmission presents a global challenge that can impact the risk of DDI [11, 12].

The aim of this narrative review is to offer an update on old and new challenges in managing donor derived viral infections. DDI caused by Arboviruses will be discussed in a separate review focused on risk for infectious tropical disease transmission.

## Hepatitis C

In the past, organs from HCV positive donors were commonly discarded or restricted to recipients with chronic HCV due to poor outcomes with interferon-based therapy and high risk of transmission to uninfected recipients. The advent of effective direct-acting antivirals (DAA) has led to HCV eradication and a shift toward the use of HCV NAT positive organs. Moreover, interest in using HCV-positive donors has grown in association with the opioid epidemic in specific geographical settings, reflecting a significant expansion of the donor pool [13].

The risk of transmission of the virus varies significantly. The transmission rates among all HCV seropositive liver allografts (HCV Ab positive, HCV RNA negative) may be relatively low ranging from 9% to 16% [14]. HCV viremic donors (HCV Ab positive, HCV RNA positive) transmit HCV almost universally [15].

Optimal follow up, DAA regimen and timing of therapy initiation, has not been established and factors such as genotype, renal function, drug-drug interactions and drug access should be considered [16].

Initiation of empiric therapy with pangenotypic regimens (Glecaprevir/Pibrentasvir or Sofosbuvir/Velpatasvir) is strongly favored, because the donor genotype is usually unknown at the time of organ allocation and these regimens feature a high barrier to resistance, which successfully overcomes potential baseline mutations, that are more common in Genotype 1a and Genotype 3. Once post-transplant testing confirms the specific genotype, clinicians can consider to safely switch the patient to targeted, genotype-specific options on the basis of drug interaction profiles and local cost-effectiveness [17]. In seronegative patients who receive seropositive viremic allografts, detectable HCV RNA levels as early as 3 days post-transplant have been observed [15]. Peri transplantation (before or soon after transplant within the first week) DAA therapies, that fully prevent the development of viremia, are likely to further mitigate any HCV-associated risks for adverse outcomes, but delayed treatment (after detectable HCV RNA or at 1 month after transplant) and shortened duration approach is common and might be feasible [18, 19]. HCV surveillance and treatment protocol for the recipients of HCV Ab positive, HCV RNA negative/positive donors based on expert opinion are summarized in Table 1.

However, despite a clear decrease in discard rates, HCV positive allografts remain an underutilized resource, especially for liver transplantation [20]. Main barriers include insurance consent for DAA therapy, that is a primarily a challenge in specific countries like United States, or logistical issues through national centralized or local procedures [21]. Current studies have demonstrated similar short-term and long-term outcomes in patients transplanted with HCV viremic donors with sustained virologic response after DAA therapy, compared to non-HCV donors, even for patients undergoing re-transplantation and multivisceral transplantation [22–26]. Recent studies on HCV discordant liver transplants demonstrated the excellent survival of these organs, with low rates of relapse and similar graft survival regardless of HCV viremia or allograft fibrosis [27, 28]. Although late recurrence of HCV is rare with modern DAA, it remains a threat to necessitating surveillance for potential relapse.

Altogether current literature shows a significant increase of HCV D+/R– transplantation, underscore the safety and cost-effectiveness of this practice and support the expansion of this practice further [29, 30]. However, it is essential to continue to provide full informed consent and ensure adequate access to DAA therapy in the peri-transplant period.

## Hepatitis B and hepatitis D

HBV positive donors are another potential opportunity to expand the donor pool. However, organ discard is frequent due to concerns for recipient HBV infection post-transplant and outcomes.

The risk of transmission must be stratified based on donor and recipients HBV serologic status as well as the organ transplanted, with liver being at higher risk [31, 32]. Transplantation into a recipient without serological evidence of prior HBV infection or vaccination carries the highest risk of transmission. Therefore, administration of HBV vaccination before transplantation is strongly recommended, but vaccination rates fell short of recommendations [33]. Moreover, effective nucleos(t)ide analog (NA) therapies are being used as posttransplant prophylaxis to decrease the rate of transmission depending on the donor and recipient serological profile (Table 2). Currently tenofovir disoproxil fumarate, tenofovir alafenamide, and entecavir are currently preferred over lamivudine due to their higher genetic barrier to resistance and to their superior antiviral potency [34].

Liver transplantation from HBV core antibody (antiHBc Ab) donor carries a high risk (on average around 50%) of transmitting hepatitis B to the recipient. A serological profile indicative of previous contact with HBV is preferable in the recipient. However, liver transplantation in HBsAg/HBsAb negative patients is possible, but prophylactic treatment and constant post-transplant monitoring is recommended. Similar short- and long-term graft and patient survival rates have been observed when compared with HBcAb negative grafts [5, 35].

Non hepatic transplantation of HBV core positive organs does not pose any additional risk to patients with a serological profile indicative of previous contact with HBV. Non liver transplantation from antiHBc Ab positive donors in HBsAg/HBsAb negative

**Abbreviations:** anti-HBc, Hepatitis B anti-core antibody; anti-HBs, Hepatitis B surface antibody; DDI, donor derived infection; DAA, direct-acting antivirals; HBsAg, Hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, Human Immunodeficiency Virus; KS, Kaposi Sarcoma; KICS, Kaposi Sarcoma Inflammatory Cytokine Syndrome; MSM, men who have sex with men; NA, nucleos(t)ide analog; NAT, nucleic acid amplification test; PCR, polymerase chain reaction; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2.

TABLE 1 HCV surveillance and treatment protocol for the recipient of HCV Ab positive, HCV RNA negative/positive donors for liver and non-liver organs.

| Donor                   | Recipient                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|-------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                         | <ul style="list-style-type: none"> <li>• HCV Ab (+), HCV RNA (–)</li> <li>• HCV Ab (–), HCV RNA (–)</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| HCV Ab (+), HCV RNA (+) | <ul style="list-style-type: none"> <li>• Baseline recipient HCV RNA before transplant</li> <li>• check HCV RNA early after transplant (3–7 days after transplant)</li> <li>• Repeat HCV RNA frequently (weekly or per center protocol) up to 4–6 weeks after transplant</li> <li>• Start DAA therapy with a prophylactic or preemptive strategy as soon as the recipient is clinically stable and able to take oral medications</li> <li>• Continue treatment for the recommended course and check HCV RNA every 4 weeks until confirm that sustained virologic response at 12 weeks is achieved</li> </ul> |
| HCV Ab (+), HCV RNA (–) | <ul style="list-style-type: none"> <li>• Baseline recipient HCV RNA before transplant</li> <li>• check HCV RNA after transplantation at 1- 3 and 6 months</li> <li>• If HCV RNA positive: Start DAA therapy</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                      |

Abbreviations: HCV, ab, HCV: Hepatitis C Virus antibody; DAA, Direct-Acting Antiviral; RNA, ribonucleic acid.

patients poses a particularly low risk of transmitting HBV to the recipient, but this risk is not absent and deserve monitoring and/or prophylaxis according to the center policy [35, 36].

Use of organs from donors with HBV infection, defined as detection of hepatitis B surface antigen (HBsAg) or detectable HBV DNA is a potential opportunity to increase organ availability, including in naïve non vaccinated recipients [37, 38]. Successful liver transplants have been performed using HBV-infected donors with similar outcomes as HBsAg negative liver grafts without excess mortality, graft loss, or post-transplant HBV-related complications. There are limited studies reporting on the safety and outcomes using HBV-positive donors for non-hepatic organ transplants, but strongly supporting this practice [39]. Of note that fulminant hepatitis in recipients of hepatic and non-hepatic grafts has been described [38, 39].

Quantifying HBV viral loads has proven to support optimization of recipient risk stratification and management in the prevention of DDI [39]. There is a lack of standardized antiviral prophylaxis and long term follow up, that should be personalized based on expert opinion (Table 2). Post-transplant monitoring of liver function, HBsAg/HBcAb seroconversion and HBV quantitative detection is recommended. Pre-transplant HBV vaccination is strongly recommended. Transplant recipients of HBsAg+ organs should receive human HBV immunoglobulin (Ig) against HBsAg, starting during the intraoperative phase, in association with a high barrier NA regardless of the immune status. The initial objective is to maintain an anti-HBs titer >50–500 IU/L, depending on the recipient's specific risk stratification for recurrence. The duration of HB Ig therapy varies according to the transplanted organ and the presence of other coinfections, since recipients of non-liver organs from HBsAg-positive donors generally require short (1–7 days) HB Ig maintenance, whereas long-term (at least 1 year) or lifelong HB Ig combination therapy remains the standard of care for liver transplant recipients, particularly those with HIV/HBV or HBV/HDV coinfection (Table 2) [40, 41].

Hepatitis D virus (HDV)/HBV coinfection merits further comments. HDV is a small defective RNA virus that requires the surface antigen of the hepatitis B virus for its replication,

assemblage, and transmission. HDV donor screening is recommended in HBsAg positive donors, whereas the presence of HBV-DNA alone in the absence of HBsAg does not require screening for HDV virus. HDV infection is documented by a positive HDV antigen or positive anti-HDV IgM (a negative HDV IgM result does not exclude chronic HDV infection) or anti-HDV IgG with a titer >1:100. If anti-HDV is positive, follow up with HDV RNA testing is recommended to confirm active infection. Organ allocation must be done on the basis of donor and recipient screening due to the risk of HDV superinfection or recurrence (Table 3) [42]. HDV/HBV-coinfecting liver transplant recipients do not represent a risky population compared with HBV infected patients in general, but further data on use of HDV/HBV positive organs in HDV/HBV positive recipients are needed [43]. Currently there is no approved treatment for HDV after transplant due to poor Interferon efficacy and limited role of bulevirtide in the context of liver transplantation. As thing stand now, the most effective method for preventing HDV infection of transplanted liver in these patients is dependent on preventing HBV recurrence, with an indefinite combination of NAs with HB Ig. [44, 45]. HBV vaccination remains the cornerstone of prevention of HBV and HDV [46].

In conclusion the use of antiHBc and HBsAg+ grafts was is and feasible and might play an important role in expanding donor organs especially in area of high prevalence of HBV infection. However, more data are needed to study the long-term risk of DDI HBV infection and define the optimal management strategies.

## Hepatitis E

Hepatitis E virus (HEV) is an RNA virus and a major cause of acute hepatitis worldwide. HEV has 8 genotypes, but most infections are due to genotypes HEV-1 and HEV-2, that are associated with fecal-oral route via contaminated drinking water in low-income countries and to HEV-3 and HEV-4, that are related with foodborne zoonotic infections in developed regions. HEV-3 is the dominant strain in Europe and the Americas, while HEV-4 predominates in Asia (China).



TABLE 2 HBV surveillance and treatment protocol for the recipient of HBV positive donors according to recipient serological status.

| Donor                              | Recipient                                                                                                                                                                                                                                                                                                                   | Recipient                                                                                                                                                                                                                                                                                                                              | Recipient                                                                                                                                                                                                                                                                                                                                                                                |
|------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| HBcAb positive<br>HBsAg negative   | <ul style="list-style-type: none"> <li>HBsAg (–); HBsAb (–); HbcAb (+)</li> <li>HBsAg (–); HBsAb (+) ° *; HbcAb (–)</li> <li>HBsAg (–); HBsAb (–); HbcAb (–)</li> </ul>                                                                                                                                                     | <ul style="list-style-type: none"> <li>HBsAg (–); HBsAb (+) °; HbcAb (+)</li> </ul>                                                                                                                                                                                                                                                    | <ul style="list-style-type: none"> <li>HBsAg (+)</li> </ul>                                                                                                                                                                                                                                                                                                                              |
|                                    | Prophylaxis and monitoring                                                                                                                                                                                                                                                                                                  | Prophylaxis and monitoring                                                                                                                                                                                                                                                                                                             | Prophylaxis and monitoring                                                                                                                                                                                                                                                                                                                                                               |
|                                    | Liver <ul style="list-style-type: none"> <li>NA lifelong prophylaxis</li> <li>Every 3 months during the first year: liver function, HBsAg, HBV DNA</li> <li>Every 3–6 months indefinitely: liver function, HBsAg, HBV DNA, liver function</li> <li>Every 6–12 months: liver ultrasound + AFP</li> </ul>                     | Liver <ul style="list-style-type: none"> <li>NA lifelong prophylaxis or according to center policy</li> <li>Every 3 months during the first year: liver function, HBsAg, HBV DNA</li> <li>Every 3–6 months indefinitely: liver function, HBsAg, HBV DNA, liver function</li> <li>Every 6–12 months: liver ultrasound + AFP</li> </ul>  | Liver <ul style="list-style-type: none"> <li>NA lifelong ± HB Ig (short § or long-term )</li> <li>Every 3 months during the first year: liver function, HBsAg, HBV DNA</li> <li>Every 3–6 months indefinitely: liver function, HBsAg, HBV DNA, liver function</li> <li>Every 6–12 months: liver ultrasound + AFP</li> </ul>                                                              |
|                                    | Non liver organs <ul style="list-style-type: none"> <li>No prophylaxis</li> <li>Every 3 months during the first 6 months: liver function, HBsAg, HBV DNA</li> <li>Every 6 months later on after 6 months post-transplant: liver function, HBsAg, HBV DNA, liver function</li> </ul>                                         | Non liver organs <ul style="list-style-type: none"> <li>No prophylaxis or according to center policy</li> <li>Every 3 months during the first 6 months: liver function, HBsAg, HBV DNA</li> <li>Every 6 months later on after 6 months post-transplant: liver function, HBsAg, HBV DNA, liver function</li> </ul>                      | Non liver organs <ul style="list-style-type: none"> <li>NA lifelong +/- HBIG intraoperatively</li> <li>Every 3 months during the first 6 months: liver function, HBsAg, HBV DNA</li> <li>Every 6 months later on after 6 months post-transplant: liver function, HBsAg, HBV DNA, liver function</li> <li>Every 12 months: screening for HCC with abdominal ultrasound and AFP</li> </ul> |
| HBsAg positive<br>HDV-RNA negative | HBsAg (–); HBsAb (+) °; HbcAb (+)<br>HBsAg (–); HBsAb(+) ° *; HbcAb (–)                                                                                                                                                                                                                                                     | HBsAg (–); HBsAb (–); HbcAb (–)<br>HBsAg (–); HBsAb (–); HbcAb (+)                                                                                                                                                                                                                                                                     | HBsAg (+) HDV negative                                                                                                                                                                                                                                                                                                                                                                   |
|                                    | Prophylaxis and monitoring                                                                                                                                                                                                                                                                                                  | Prophylaxis and monitoring                                                                                                                                                                                                                                                                                                             | Prophylaxis and monitoring                                                                                                                                                                                                                                                                                                                                                               |
|                                    | Liver <ul style="list-style-type: none"> <li>NA lifelong + HB Ig (short = or long-term )</li> <li>Every 3 months during the first year: liver function, HBsAg, HBV DNA</li> <li>Every 3–6 months indefinitely: liver function, HBsAg, HBV DNA, liver function</li> <li>Every 6–12 months: liver ultrasound + AFP</li> </ul> | Liver <ul style="list-style-type: none"> <li>NA lifelong + HB Ig (short = or long-term )</li> <li>Every 3 months during the first year: liver function, HBsAg, HBV DNA</li> <li>Every 3–6 months indefinitely: liver function, HBsAg, HBV DNA, liver function</li> <li>For liver: Every 6–12 months: liver ultrasound + AFP</li> </ul> | Liver <ul style="list-style-type: none"> <li>NA lifelong DO + HB Ig (short = or long-term )</li> <li>Every 3 months during the first year: liver function, HBsAg, HBV DNA</li> <li>Every 3–6 months indefinitely: liver function, HBsAg, HBV DNA, liver function</li> <li>Every 6–12 months: liver ultrasound + AFP</li> </ul>                                                           |
|                                    | Non liver organs <ul style="list-style-type: none"> <li>NA 6–12 months + HB Ig (short)</li> <li>Every 3 months during the first year: liver function, HBsAg, HBV DNA</li> <li>Every 3–6 months indefinitely: liver function, HBsAg, HBV DNA, liver function</li> </ul>                                                      | Non liver organs <ul style="list-style-type: none"> <li>NA 6–12 months + HB Ig (short)</li> <li>Every 3 months during the first year: liver function, HBsAg, HBV DNA</li> <li>Every 3–6 months indefinitely: liver function, HBsAg, HBV DNA, liver function</li> </ul>                                                                 | Non liver organs <ul style="list-style-type: none"> <li>NA lifelong DO + HB Ig (short = or long-term )</li> <li>Every 3 months during the first year: liver function, HBsAg, HBV DNA</li> <li>Every 3–6 months indefinitely: liver function, HBsAg, HBV DNA, liver function</li> </ul>                                                                                                   |

Abbreviations: AFP, alpha fetoprotein; anti-HBc, Hepatitis B anti-core antibody; anti-HBs, Hepatitis B surface antibody; HBsAg, Hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; NA, nucleos(t)ide analog.

Symbols: ° (titer >10 mIU/mL); \*vaccinated; § short: 1–7 days; long term: one year-lifelong for HBV-HIV, or HBV-HDV, coinfecting patients. To maintain an HBsAb titer of 50–500 IU/mL, depending on the recipient's risk level.

In immunocompetent hosts, HEV typically causes a self-limited acute hepatitis, whereas in SOT recipients, HEV infection can cause chronic hepatitis (defined by replication beyond 3 months after infection), that can occur in around two-thirds of infected SOT. Moreover, HEV in SOT can lead to cirrhosis, graft dysfunction or rejection [47]. Persistent hepatitis E occurs primarily involving HEV-3 and 4 [48].

Transmission of HEV through solid organs is very efficient (especially for higher plasma viral load > 10<sup>3</sup> IU/mL), particularly through liver grafts and highlights the importance of surveillance and screening protocols [5]. The decision to implement universal organ donor and recipient HEV screening depends on HEV incidence, since in regions with low baseline HEV prevalence routine universal screening yields minimal impact on overall transplant safety [9, 49, 50].

TABLE 3 Organ allocation in HDV/HBV coinfecting donors and recipients.

| Donor                              | Recipient                                                                                      | Recipient                                                                                      |
|------------------------------------|------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
|                                    | <ul style="list-style-type: none"> <li>• HBsAg positive</li> <li>• HDV RNA positive</li> </ul> | <ul style="list-style-type: none"> <li>• HBsAg positive</li> <li>• HDV RNA negative</li> </ul> |
| HBsAg positive<br>HDV RNA positive | transplant allowed                                                                             | transplant of any organ not recommended:<br>Risk of fatal HDV superinfection                   |
| HBsAg positive<br>HDV RNA negative | liver transplant not recommended:<br>Risk of HDV hepatitis recurrence                          | transplant of any organ allowed                                                                |

TABLE 4 Management of recipient based on donor and recipient serological and virological Markers of Hepatitis E.

| Donor                                           | Management of the recipient                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|-------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| HEV IgM or IgG positive<br>HEV RNA negative     | <ul style="list-style-type: none"> <li>• Accept organs</li> <li>• Post-transplant surveillance of the recipient: HEV-RNA every 2–3 months for the first year after transplant</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| HEV RNA positive                                | <ul style="list-style-type: none"> <li>• Consider accepting organs</li> <li>• Post-transplant surveillance of the recipient: HEV-RNA weekly for the first month</li> <li>• If HEV-RNA positive in the recipient (after 1 month) <ul style="list-style-type: none"> <li>◦ Liver test at month 1; HEV-RNA in serum and stool at month 2 and 3</li> <li>◦ Pre-emptive ribavirin soon after transplant (starting 1–3 months after transplant depending on individual risk factors *</li> <li>◦ Change of immunosuppression, if possible</li> </ul> </li> <li>• If HEV-RNA negative in the recipient (after 1 month) <ul style="list-style-type: none"> <li>◦ HEV serology after 3 months</li> </ul> </li> </ul> |
| Recipient                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| HEV IgM or IgG positive<br>and HEV-RNA negative | <ul style="list-style-type: none"> <li>• Proceed with listing and transplantation</li> <li>• Post-transplant surveillance of the recipient: HEV-RNA every 2–3 months for the first year after transplant</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| HEV RNA positive                                | <p>With normal liver function:</p> <ul style="list-style-type: none"> <li>• Proceed with active transplant listing</li> <li>• Monitor HEV RNA</li> <li>• Plan for post-transplant ribavirin therapy if needed</li> </ul> <p>Without normal liver function</p> <ul style="list-style-type: none"> <li>• Delay transplantation if possible</li> <li>• Prioritize early ribavirin administration prior to the transplant</li> <li>• Change of immunosuppression, if possible</li> </ul>                                                                                                                                                                                                                        |

\*Risk factors to consider: liver transplant, marginal liver graft, underlying liver disease, polyclonal antibodies induction.

The screening method of choice includes both serology (HEV IgM, IgG) and PCR testing. HEV-RNA is more sensitive than serologic testing [51]. Undetectable HEV-RNA in donor serum would indicate low risk for HEV transmission, although liver grafts may harbor infectious HEV RNA even in the absence of systemic markers of infection [51]. Donor testing result for HEV infection might be done pre- or post-transplant. Detection of HEV RNA in donor plasma is not a contraindication for organ donation, but triggers improved recipient management. Managing strategies for donor derived HEV infection is based on early detection of infection (HEV-RNA weekly for the first month) and reduction of immunosuppression (especially mammalian target of rapamycin (mTOR) inhibitors and tacrolimus). In addition, for patients without spontaneous viral clearance after 1 month,

a 3-month course of ribavirin is recommended, achieving sustained virologic response in approximately 80% of cases (Table 4) [52].

Vaccination against HEV represents a promising prophylactic tool, though availability remains geographically restricted (licensed in China and Pakistan) [53].

## HIV

Overall, there is increasing and solid literature supporting transplantation in people with HIV [54]. However, opportunities exist to improve knowledge and overcome misconceptions about SOT for people with HIV. At the European level, the use of organs from HIV D+ is legally allowed only in 6 out of 35 (17%) countries

TABLE 5 Recommended monitoring and intervention for HHV-8 high risk recipient (HHV8 D+/R– and R+).

| Donor and recipient            | Monitoring                                                                                                                                                                                                                                                                                                                                                                                                                           | Treatment                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|--------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| HHV8 D+/R–<br>HHV8 D+/- and R+ | HHV8 DNA test <ul style="list-style-type: none"> <li>• Every 15 days for the first 3 months</li> <li>• Monthly between the 4th and 12th month</li> <li>• From the 12th month onwards, in anti-HHV-8 positive recipients repeat HHV-8 DNA testing during routine check-ups and during treatment for acute rejection/intensification of immunosuppression</li> <li>• Skin self-examination for detection of KS-like lesions</li> </ul> | Primary infection or reactivation with persistent positive HHV-8 DNA and symptomatic infection <ul style="list-style-type: none"> <li>• Start an antiviral (foscarnet or cidofovir preferred over ganciclovir)</li> <li>• Replacement of CNIs with mTOR inhibitors, when feasible</li> <li>• If confirmed KICS, additional rituximab</li> <li>• If confirmed KS: use Liposomal Doxorubicin</li> <li>• If confirmed MCD, PEL: follow specific treatment guidelines</li> </ul> |

Abbreviations: CNIs, calcineurin inhibitors; DNA, deoxyribo nucleic acid; KS, kaposi sarcoma; KICS, kaposi sarcoma inflammatory cytokine syndrome; mTOR, mammalian target of rapamycin; MCD, multicentric castelman disease; PEL, primary effusion lymphoma.

and there is a high willingness to participate in an HIV D+/R+ among experts in the field [55]. Moreover, awareness of the ability of patients with HIV to both receive and donate organs is limited [56]. Since the 2015, HOPE Act prospective studies have demonstrated that kidney and liver transplantation from HIV D+/R+ recipients are safe and effective, with similar graft and patient survival to HIV D–/R+ transplantation. Moreover donor-derived HIV superinfection in recipients of HIV D+/R+ transplants is rare, and the clinical ramifications appear negligible [57].

A recent retrospective small experience after heart transplantation in HIV-positive recipients using HIV-positive or HIV-negative donors (4 HIV D +/R+, 6 HIV D –/R+) found no significant differences in short-term survival, rejection, infection, heart graft function and HIV suppression between both groups at 6 months. Moreover, the lack of difference in donor-derived cell free DNA provided reassurance that use of hearts from HIV D+ did not trigger increased myocyte injury [58]. This early experience provides evidence and the inclusion of cardiothoracic patients in trials might be a necessary evolutionary step [59, 60].

Prior to 2017, transplants from HIV-positive donors to HIV-negative recipients occurred only inadvertently, because the donor's positive HIV status was discovered after the organs had been transplanted. In 2017, the first intentional liver transplantation from a living HIV-positive mother to an HIV-negative child was performed. Multidisciplinary evaluation, research trial approval and specific consent to the procedure were obtained [61]. The recipient started antiviral therapy before the transplantation to minimize the risk of HIV transmission, continued it after transplantation. No plasma or cell-associated HIV-1 DNA or RNA was detected at any stage in the recipient, although the child's HIV status was uncertain [61].

Thanks to modern highly effective antiviral therapy, the use of organs from deceased donors with HIV for HIV-negative recipients might become a medical consideration in the future, allowing to expand the donor pool. However, this approach requires strict precautions against transmission and deeply informed, voluntary patient consent to manage the remaining ethical and clinical complexities [59, 60].

## Human herpesvirus 8

Human herpesvirus 8 (HHV-8) is an emerging topic in DDI. HHV-8 is a double-stranded DNA virus, whose life cycle is similar to

that of other herpesviruses. Primary infection is usually asymptomatic in immunocompetent hosts. However, HHV-8 primary infection or reactivation in immunocompromised hosts can lead to a spectrum of diseases, ranging from malignancies (Kaposi Sarcoma- KS-, Primary Effusion Lymphoma, Diffuse Large B-cell Lymphoma and Multicentric Castleman Disease) to severe inflammatory syndromes (Kaposi Sarcoma Herpesvirus Inflammatory Cytokine Syndrome, KICS).

HHV-8 prevalence varies with geography and ethnicity. Variable ranges have been observed in North Europe (5%), United States (3%–7%), the Mediterranean (20%–30%) and Sub-Saharan Africa (> 50%). Moreover, seroprevalence is influenced by behavioral risk, being significantly increased in people living with HIV, especially in men who have sex with men (MSM) [62]. In particular a recent study performed in United States found a higher prevalence of HHV-8 among kidney donors with HIV compared with non-HIV (25.2% and 7.5%, respectively), particularly among MSM [63].

In the context of SOT, HHV-8 might cause a DDI, whose timing, clinical picture and severity depends on several factors. D+/R-serological status might favor primary infection that is usually associated with inflammatory syndromes and aggressive visceral forms of KS. Moreover, the degree of immunosuppression and lung and liver transplants tend to be associated with more severe disease forms and poorer outcome.

Implementation of universal HHV-8 screening for donors and recipients should be primarily driven on one hand by local HHV-8 incidence and resource availability and on the other hand by the disproportionate severity of donor-derived post-transplant infections. A targeted screening strategy based on donor risk factors could balance safety while avoiding the economic burden of universal screening. In non-endemic areas, HHV-8 testing could be prioritized for donors presenting specific epidemiological or behavioral risk profiles, such as a history of recent incarceration, injection drug use, high-risk sexual behavior, HIV coinfection or origin from highly endemic regions [62]. In addition, there is a lack of standardized screening since Enzyme-Linked Immunosorbent Assay (ELISA) has a poor performance and may miss antibodies that target specific latent-phase proteins, whereas Indirect Immunofluorescence Assay (IFA) is the preferred approach but is operator dependent [62].

Greater awareness of risk through pre-transplant screening could support accurate and timely diagnosis, enabling earlier therapeutic intervention and potentially reducing morbidity and mortality

associated with HHV-8 DDI. Strict clinical and molecular monitoring is strongly recommended for high-risk patients (HHV-8 mismatch D+/R- or R+). In addition, in case of persistent HHV-8 DNAemia and symptomatic infections, switch to an immunosuppressive regimen by replacing calcineurin inhibitors with mammalian target of rapamycin (mTOR) inhibitors and antiviral treatment (foscarnet or cidofovir, that are preferred over ganciclovir) are recommended, although use of antivirals is debatable in this setting. In the case of KICS, rituximab might be additionally provided (Table 5) [8].

## Respiratory viruses

Respiratory viruses have been largely unknown and underestimated as a cause of DDI. The SARS-CoV-2 pandemic and the increased use of molecular testing have raised awareness of the role of respiratory viruses in respiratory tract infections, including in the context of DDI [64–66].

In general, transplantation of non-lung organs from donors with active respiratory viral infection is considered possible and well tolerated, without transmission, except in case of organ specific contra-indications (for example myocarditis, hepatitis). Since major transplant societies offer limited guidance, the utilization of these organs is determined by individual centers, leading to significant variations in practice at both national and international levels. In contrast, lung and bowel recipients remain uniquely vulnerable due to the high likelihood of direct viral transmission in the respiratory or gastrointestinal tracts, depending on the type of virus. In general lungs should not be used from donors with proven respiratory viral infection as the primary cause of death or causing clear lower respiratory tract involvement. However, since molecular testing detects viral RNA/DNA even in the absence of active, infectious virions, this approach is associated with the risk of wasting organs that could otherwise be safely transplanted [67].

Most available literature is focused on SARS-CoV-2 [10]. As thing stand now, current strategies for donor evaluation still include universal microbiologic screening with standard SARS-CoV-2 PCR of lower respiratory tract 24–72 h before organ procurement in most countries worldwide [68, 69]. Because of the absence of the risk of transmitting SARS-CoV-2 through non-lung solid organs, some experts propose that universal, routine testing of asymptomatic, non-lung deceased donors should be re-evaluated [68]. Of interest that a recent Italian study found that molecular testing for SARS-CoV-2 RNA on 64 graft biopsies (liver and kidney) and 68 perfusion fluid samples at procurement were negative in 100% of cases, including in donors with lower respiratory tract samples with a high viral load (Ct value <30) [70]. Lung transplantation from SARS-CoV-2 is generally contraindicated, but careful consideration to proceeding might be done based on risks and benefits, generally provided that lower respiratory tract testing is negative and antivirals are administered to the recipient [67, 71].

The use of organs from donors with positivity for Rhinovirus/Enterovirus, seasonal Coronavirus, Influenza, Paramyxovirus (Parainfluenza Virus, Human Metapneumovirus-MPV-, Respiratory Syncytial Virus-RSV-), Adenovirus and Boacavirus is a challenging topic, due to limited available data [72–74]. Screening for respiratory viral pathogens other than SARS-CoV-2 at donor evaluation is not recommended routinely and is usually conducted only in symptomatic

donors. Moreover, deepening on guidelines, screening might be limited only to Influenza virus during epidemic periods or might include a complete multiplex PCR panel [75]. Therefore, the true incidence and burden of these diseases remain poorly understood. Certain viruses, namely Influenza and those from the Paramyxovirus are more likely to be associated with lower respiratory tract infections, higher hospitalization rates compared with Rhinovirus/Enterovirus and seasonal Coronavirus in lung transplant recipients [76]. Particular attention should be paid to Adenovirus-positive donors, given the virus propensity to cause systemic dissemination or end-organ damage in immunocompromised recipients depending on the virus serotype and the organ involved and to influenza A/H5N1 for the higher risk of viremia [73, 77]. However, there is limited reported evidence to support systematic viral testing of donor respiratory specimens, with a concrete risk of difficult to interpret result and discard of low-risk donors.

Most available literature on risk of DDI is based on influenza virus. There is currently no evidence demonstrating transmission of the virus through transplantation of non-lung organs, nor evidence of increased morbidity among recipients of these organs [69]. There are 2 reports of possible transmission of seasonal influenza A and B viruses by transplantation of lungs and kidneys from infected donors, but detailed molecular proof was lacking [78, 79]. Some experts recommend that recipients of organs from influenza positive donors should receive antiviral treatment for 5–10 days following transplant, unless the donor has already received a full course of treatment to reduce severity and infectivity [80]. Of note that a recent case report described a case of fatal acute respiratory distress syndrome caused by donor-derived human MPV after lung transplantation from an asymptomatic donor, confirmed after next-generation sequencing of MPV genomes [81]. Further studies are essential to precisely quantify the transmission risks of respiratory viruses during lung transplants to refine diagnostic and screening protocols.

Maintaining up-to-date vaccinations for circulating respiratory viruses in transplant candidates is crucial to mitigating risk. In general, recipients should be protected with vaccination against SARS-CoV-2, influenza and RSV [82].

## Conclusion

Balancing the transmission risk of viral DDIs against the high risk of waitlist mortality is crucial, because of the growing ethical imperative to ensure equitable transplant access. By comprehensively understanding donors at risk for viral infections and their potential clinical consequences, transplant teams can perform targeted mitigation strategies, through rigorous pretransplant and posttransplant microbiological testing and target therapies.

However, the current clinical landscape is significantly limited by a lack of standardized consensus screening and management protocols for old and emerging risk viruses. Future research priorities in DDI viral infections should focus on improving the current use of post-transplant quantitative molecular testing and on exploring the role of post-exposure prophylaxis against preemptive treatment strategies in recipients. Moreover, clinical trials are urgently required through evaluating the role new advanced diagnostics, including rapid metagenomic next-generation sequencing, and new



novel therapeutics, as well as virus-specific T-cell immunotherapies. Finally, coordinated financial and infrastructural investments are needed to strengthen epidemiological vigilance registries and communication networks to ensure rapid trace-backs investigations.

## Author contributions

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## References

- Graziano E, Peghin M, Balsamo ML, Givone F, De Martino M, Montemurro A, et al. Donors infectious risk stratification: activity of the Italian national transplant center. *Transpl Infect Dis* (2026) 28(1):e70131. doi: 10.1111/tid.70131
- Grossi PA, Wolfe C, Peghin M. Non-standard risk donors and risk of donor-derived infections: from evaluation to therapeutic management. *Transpl Int* (2024) 37:12803. doi: 10.3389/ti.2024.12803
- CDC. National Center for Health Statistics Mortality Data on CDC Wonder (2026). Available online at: <https://wonder.cdc.gov/mcd.html>.
- Eldin C, Grossi PA, Manda V, Kamar N, Lortholary O, Hirsch HH, et al. Updates on donor-derived infection in solid organ transplantation, report from the 2024 GTI (infection and transplantation group) annual meeting. *Transpl Int* (2025) 38:14237. doi: 10.3389/ti.2025.14237
- Khan J, Kozuch J, Aslam S. Hepatitis B, C, and E viruses in solid organ transplantation: donor and recipient perspectives. *JHLT Open* (2026) 12:100533. doi: 10.1016/j.jhlto.2026.100533
- Lopez-Soler RI, Joyce C, Cotiguala L, Aguirre O, Samra M, Trotter C, et al. Utilization of hepatitis B viremic donors (NAT+) leads to improved kidney transplant access for older adult recipients with little to no wait time. *Transpl Infect Dis* (2024) 26(3):e14295. doi: 10.1111/tid.14295
- Croome KP, Lee DD, Pungpapong S, Keaveny AP, Taner CB. What are the outcomes of declining a public health service increased risk liver donor for patients on the liver transplant waiting list? *Liver Transpl* (2018) 24(4):497–504. doi: 10.1002/lt.25009
- Mularoni A, Cona A, Bulati M, Busà R, Miele M, Timoneri F, et al. Serologic screening and molecular surveillance of kaposi sarcoma herpesvirus/human herpesvirus-8 infections for early recognition and effective treatment of kaposi sarcoma herpesvirus-associated inflammatory cytokine syndrome in solid organ transplant recipients. *Am J Transpl* (2025) 25(5):1070–85. doi: 10.1016/j.ajt.2024.11.013
- Ushiro-Lumb I, Forsythe J, Haywood B, Geoghegan C, Maddox V, Ijaz S, et al. Impact of hepatitis E virus screening in the UK deceased organ donor population. *Transpl Int* (2023) 36:11673. doi: 10.3389/ti.2023.11673
- Peghin M, Grossi PA. COVID-19 positive donor for solid organ transplantation. *J Hepatol* (2022) 77(4):1198–204. doi: 10.1016/j.jhep.2022.06.021
- Alcayna T, Rao VB, Lowe R. Identifying the climate sensitivity of infectious diseases: a conceptual framework. *Lancet Planet Health* (2025) 9(8):101291. doi: 10.1016/j.lanplh.2025.101291
- Tan SSX, Phoompoung P, Okamoto K, Chayakulkeeree M, Koh XX, Tan C, et al. Donor-derived infections-insights from Singapore, Japan, and Thailand. *Transpl Infect Dis* (2024) 26(Suppl. 1):e14370. doi: 10.1111/tid.14370
- Levitsky J, Formica RN, Bloom RD, Charlton M, Curry M, Friedewald J, et al. The American society of transplantation consensus conference on the use of hepatitis C viremic donors in solid organ transplantation. *Am J Transpl* (2017) 17(11):2790–802. doi: 10.1111/ajt.14381
- Bari K, Luckett K, Kaiser T, Diwan T, Cuffy M, Schoech MR, et al. Hepatitis C transmission from seropositive, nonviremic donors to non-hepatitis C liver transplant recipients. *Hepatology* (2018) 67(5):1673–82. doi: 10.1002/hep.29704
- Zingg SW, Lemon K. Donor viral hepatitis and liver transplantation. *Surg Clin North Am* (2024) 104(1):67–77. doi: 10.1016/j.suc.2023.07.002
- Rivosecchi RM, Sanchez PG, Sacha LM, Horn ET, Keebler M, Silveira FP. A single center experience of concomitant administration of sofosbuvir/velpatasvir and amiodarone after heart and lung transplantation. *JHLT Open* (2025) 9:100279. doi: 10.1016/j.jhlto.2025.100279
- IDSA. (2026). Available online at: <https://www.hcvguidelines.org/> (Accessed June 30, 2026).
- Trapianti. Ora possibile l'utilizzo di organi di donatori con epatite C su riceventi negativi: via libera all'uso tempestivo delle nuove terapie. (2026). Available online at: <https://www.trapianti.salute.gov.it/cnt-primopiano/ora-possibile-lutilizzo-di-organi-di-donatori-con-epatite-c-su-riceventi-negativi/> (Accessed September 22, 2025).
- Papanikolla J, McGowan M, Chunduru M, Winters H, Pesavento T, Smith R, et al. Real-world experience in treatment of donor-derived hepatitis C virus in kidney transplant recipients with delayed initiation, shortened course glecaprevir/pibrentasvir versus standard of care. *Transpl Infect Dis* (2024) 26(6):e14366. doi: 10.1111/tid.14366
- Buchanan-Pearl KA, Pagan J, Martin E, Turkeltaub J, Reese P, Goldberg DS. Temporal changes in the utilization of kidneys from hepatitis C virus-infected donors in the United States. *Am J Transpl* (2023) 23(6):831–8. doi: 10.1016/j.ajt.2023.03.001
- Stewart ZA, Stern J, Ali NM, Kalia HS, Khalil K, Jonchhe S, et al. Clinical and financial implications of 2 treatment strategies for donor-derived hepatitis C infections. *Transpl Direct* (2021) 7(10):e762. doi: 10.1097/TXD.0000000000001222
- Mazur M, Pisani B, Carmona Rubio A, Eisen HJ, Bhat G, Nunez M. Early outcomes in patients undergoing heart re-transplantation in the era of HCV-viremic donors. *J Cardiol* (2025) 87:340–6. doi: 10.1016/j.jcc.2025.09.005
- Addison V, Goldberg DS, Vianna R, Martin E, Garcia J. Multivisceral transplantation utilizing hepatitis C virus-viremic donors for hepatitis C virus-negative recipients. *Am J Transpl* (2025) 25(1):204–8. doi: 10.1016/j.ajt.2024.09.006
- Khan S, Mazumder R, Wang X, Wang Y, Sims OT, Budev M, et al. Hepatitis C viremic lung transplantation to aviremic recipients: comprehensive outcomes and post-transplant viremia. *Clin Transpl* (2024) 38(5):e15325. doi: 10.1111/ctr.15325
- Kwon JH, Hill MA, Patel R, Tedford RJ, Hashmi ZA, Shorbaji K, et al. Outcomes of over 1000 heart transplants using hepatitis C-Positive donors in the modern era. *Ann Thorac Surg* (2023) 115(2):493–500. doi: 10.1016/j.athoracsurg.2022.11.002
- Schlendorf KH, Zalawadiya S, Shah AS, Perri R, Wigger M, Brinkley DM, et al. Expanding heart transplant in the era of direct-acting antiviral therapy for hepatitis C. *JAMA Cardiol* (2020) 5(2):167–74. doi: 10.1001/jamacardio.2019.4748
- Pratt CG, Noriega N, Whitrock JN, Carter MM, Moore AN, Kaiser TE, et al. Follow up and safety of use of hepatitis C virus discordant liver transplants. *HPB (Oxford)* (2025) 28:524–33. doi: 10.1016/j.hpb.2025.12.027
- Nakayama T, Krist DT, Akabane M, Imaoka Y, Esquivel CO, Kwong AJ, et al. Hepatitis C-positive grafts for hepatitis C-negative recipients in liver transplantation: buried treasure or depleting resource? *Liver Transpl* (2025) 31(9):1154–64. doi: 10.1097/LVT.0000000000000557
- Paternostro K, Birs A, Aslam S, Adler E, Hong K, Wettersten N. Intermediate-term risk of cardiac allograft vasculopathy following heart transplantation from hepatitis C viremic donors in the era of direct-acting antiviral therapy. *JHLT Open* (2026) 11:100416. doi: 10.1016/j.jhlto.2025.100416
- Woolley AE, Gandhi AR, Jones ML, Kim JJ, Mallidi HR, Givertz MM, et al. The cost-effectiveness of transplanting hearts from Hepatitis C-infected donors into uninfected recipients. *Transplantation* (2023) 107(4):961–9. doi: 10.1097/TP.0000000000004378

## Conflict of interest

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31. Russo FP, Viganò M, Stock P, Ferrarese A, Pugliese N, Burra P, et al. HBV-positive and HIV-positive organs in transplantation: a clinical guide for the hepatologist. *J Hepatol* (2022) 77(2):503–15. doi: 10.1016/j.jhep.2022.03.007
32. Len O, Los-Arcos I, Aguado JM, Blanes M, Bodro M, Carratalà J, et al. Selection criteria of solid organ donors in relation to infectious diseases: a Spanish consensus. *Transpl Rev (Orlando)* (2020) 34(2):100528. doi: 10.1016/j.tre.2020.100528
33. Peel J, Tang MJ, Morrissey CO. Hepatitis B in transplant recipients: an Australian single-centre study of screening, vaccination and management. *Intern Med J* (2025) 55(11):1824–31. doi: 10.1111/imj.70202
34. Elharabi Z, Saba J, Akin H. Navigating the latest Hepatitis B virus reactivation guidelines. *Diseases* (2025) 13(11):355. doi: 10.3390/diseases13110355
35. Alvarez-Lopez P, Riveiro-Barciela M, Oleas-Vega D, Flores-Cortes C, Román A, Perelló M, et al. Anti-HBc impacts on the risk of hepatitis B reactivation but not on survival of solid-organ transplant recipients. *Medicine (Baltimore)* (2020) 99(9):e19407. doi: 10.1097/MD.00000000000019407
36. Yin S, Chen X, Li X, Zhang F, Wu J, Lin T. Was antiviral prophylaxis necessary after kidney transplantation utilizing HBcAb+ donors? A systematic review and meta-analysis. *Transpl Rev (Orlando)* (2024) 38(2):100840. doi: 10.1016/j.tre.2024.100840
37. Belga S, Wright RC, Lee SB, Durand CM, Doucette K. Hepatitis B virus donor positive to recipient negative (D+/R-) heart and lung transplantation: analysis from the organ procurement and transplantation network. *Transpl Infect Dis* (2025) 27(5):e70054. doi: 10.1111/tid.70054
38. Vinaixa C, DiMaira T, Russo FP, Goldberg D, Mazzola A, Walabh P, et al. Use of HBsAg-positive donors in liver transplantation: an ILTS-EASL-AASLD multisociety survey. *Liver Transpl* (2024) 30(11):1116–22. doi: 10.1097/LVT.0000000000000432
39. Tam PCK, Katz-Greenberg G, Wolfe CR, Lott K, Berg CL, DeVore AD, et al. Hepatitis B virus NAT positive donors in non-hepatic organ transplant: quantifying viral loads to optimize recipient risk stratification and management in the prevention of donor-derived infection. *Clin Transpl* (2025) 39(7):e70236. doi: 10.1111/ctr.70236
40. European Association for the Study of the Liver, Jaroszewicz J, Kennedy P, Lampertico P, Lemoine M, et al. EASL clinical practice guidelines on the management of hepatitis B virus infection. *J Hepatol* (2025) 83(2):502–83. doi: 10.1016/j.jhep.2025.03.018
41. Ghany MG, Pan CQ, Lok AS, Feld JJ, Lim JK, Wang SH, et al. AASLD ISDA practice guideline on treatment of chronic hepatitis B. *Hepatology* (2026) 83(4):974–97. doi: 10.1097/HEP.0000000000001549
42. Franchello A, Ghisetti V, Marzano A, Romagnoli R, Salizzoni M. Transplantation of hepatitis B surface antigen-positive livers into hepatitis B virus-positive recipients and the role of hepatitis Delta coinfection. *Liver Transpl* (2005) 11(8):922–8. doi: 10.1002/lt.20471
43. Angelico R, Trapani S, Manzia TM, Lenci I, Grossi P, Ricci A, et al. Liver transplantation for hepatitis D virus/hepatitis B virus coinfection in Italy: an intention-to-treat analysis of long-term outcomes. *Am J Transpl* (2025) 25(7):1502–14. doi: 10.1016/j.ajt.2025.03.003
44. Martini S, Tandoi F, Romagnoli R, Rizzetto M. Liver transplantation in hepatitis B/Hepatitis D (delta) virus coinfection recipients. *Transplantation* (2022) 106(10):1935–9. doi: 10.1097/TP.00000000000004138
45. Wedemeyer H, Aleman S, Brunetto MR, Blank A, Andreone P, Bogomolov P, et al. A phase 3, randomized trial of bulevirtide in chronic hepatitis D. *N Engl J Med* (2023) 389(1):22–32. doi: 10.1056/NEJMoa2213429
46. Chiu CY, Sampathkumar P, Brumble LM, Vikram HR, Watt KD, Beam E. Hepatitis B vaccine compliance, serologic response, and durability in adult thoracic organ transplant recipients. *Clin Transpl* (2024) 38(9):e15464. doi: 10.1111/ctr.15464
47. Hansrivijit P, Trongtorsk A, Puthenpura MM, Boonpheng B, Thongprayoon C, Wijarnpreecha K, et al. Hepatitis E in solid organ transplant recipients: a systematic review and meta-analysis. *World J Gastroenterol* (2021) 27(12):1240–54. doi: 10.3748/wjg.v27.i12.1240
48. Sridhar S, Cheng VCC, Wong SC, Yip CCY, Wu S, Lo AWI, et al. Donor-derived genotype 4 hepatitis E virus infection, Hong Kong, China, 2018. *Emerg Infect Dis* (2019) 25(3):425–33. doi: 10.3201/eid2503.181563
49. Binda B, Picchi G, Bruni R, Di Gasbarro A, Madonna E, Villano U, et al. The prevalence, risk factors, and outcomes of hepatitis E virus infection in solid organ transplant recipients in a highly endemic area of Italy. *Viruses* (2025) 17(4):502. doi: 10.3390/v17040502
50. Lhomme S, Bardiaux L, Abravanel F, Gallian P, Kamar N, Izopet J. Hepatitis E virus infection in solid organ transplant recipients, France. *Emerg Infect Dis* (2017) 23(2):353–6. doi: 10.3201/eid2302.161094
51. Abravanel F, Lhomme S, Marion O, Peron JM, Kamar N, Izopet J. Diagnostic and management strategies for chronic hepatitis E infection. *Expert Rev Anti Infect Ther* (2023) 21(2):143–8. doi: 10.1080/14787210.2023.2166932
52. Riveiro-Barciela M, Roade L, Martínez-Camprecios J, Vidal-González J, Rodríguez-Diez B, Perelló M, et al. mTOR inhibitors a potential predisposing factor for chronic hepatitis E: results from the prospective collaborative CHES study (chronic hepatitis E screening in patients with immune impairment and increased transaminases levels). *Gastroenterol Hepatol* (2023) 46(10):764–73. doi: 10.1016/j.gastrohep.2023.01.010
53. Huang S, Zhang X, Su Y, Zhuang C, Tang Z, Huang X, et al. Long-term efficacy of a recombinant hepatitis E vaccine in adults: 10-Year results from a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* (2024) 403(10429):813–23. doi: 10.1016/S0140-6736(23)02234-1
54. Gray ML, Tingle SJ, Elzawahry M, Hurn E, Mahendran B, Post FA, et al. The impact of recipient HIV status on kidney transplant outcomes in the United Kingdom: have previous studies overestimated risk? *Transplantation* (2026) 110(3):e670–e679. doi: 10.1097/TP.0000000000005585
55. Miro JM, Amondarain NL, Serrano L, Salmanton-García J, Manzardo C, Malano-Barletta D, et al. Current situation of solid organ transplantation from HIV-positive donors to HIV-positive recipients in Europe: the Spanish perspective. *Clin Microbiol Infect* (2026) 32(1):137–46. doi: 10.1016/j.cmi.2025.08.025
56. Griffin D, Vaz K, Basu G, Mulley W, Kotecha S, Levin K, et al. Knowledge and attitudes about solid organ transplantation for people with HIV in Australia: a cross-sectional survey of people with and without HIV. *J Acquir Immune Defic Syndr* (2026) 101(4):432–40. doi: 10.1097/QAI.0000000000003815
57. Rozek GM, Yang P, Eby Y, Benner SE, Martens C, Habteyimer F, et al. HIV superinfection in kidney transplant recipients with HIV who received organs from donors with HIV. *J Infect Dis* (2025) 232(2):359–63. doi: 10.1093/infdis/jiaf284
58. Saeed O, Hemmige V, Angelo L, Gjelij C, Puius YA, Madan S, et al. Early experience in heart transplantation utilizing donors with HIV. *J Heart Lung Transpl* (2026) 45(3):468–72. doi: 10.1016/j.healun.2025.09.011
59. Balachandran IC, Adekunle RO. A new frontier of HIV organ policy equity - expanding the transplant donor pool. *J Heart Lung Transpl* (2026) 45(3):473–4. doi: 10.1016/j.healun.2025.10.030
60. Grossi PA, Grossi AA. Solid organ transplantation from HIV-Positive donors to HIV-positive recipients in Europe: a growing opportunity. *Clin Microbiol Infect* (2026) 32(1):16–8. doi: 10.1016/j.cmi.2025.11.001
61. Botha J, Conradie F, Etheredge H, Fabian J, Duncan M, Haeri Mazanderani A, et al. Living donor liver transplant from an HIV-positive mother to her HIV-negative child: opening up new therapeutic options. *AIDS* (2018) 32(16):F13–F19. doi: 10.1097/QAD.0000000000002000
62. Mularoni A, Cona A, Mikulska M, Pecoraro F, Piazza C, De Vita E, et al. HHV-8/KSHV in solid organ transplantation: current gaps of knowledge and future directions. *Transpl Infect Dis* (2026) 28:e70179. doi: 10.1111/tid.70179
63. Nambiar P, Liang T, Labo N, Hand J, Blumberg EA, Rana MM, et al. Kaposi sarcoma-associated herpesvirus risk and disease in kidney donors and transplant recipients with HIV in the United States. *Clin Infect Dis* (2025) 82:709–19. doi: 10.1093/cid/ciaf229
64. Lombardi A. Molecular respiratory pathogen panels in lung transplantation. *Transpl Infect Dis* (2026) 28(1):e70119. doi: 10.1111/tid.70119
65. Lombardi A, Renisi G, Liparoti A, Bobbio C, Bandera A. Application of syndromic panels for respiratory tract infections in lung transplantation: a critical review on current evidence and future perspectives. *Transpl Infect Dis* (2026) 28(1):e14448. doi: 10.1111/tid.14448
66. Gardiner BJ, Ison MG. Donor-derived infections in heart and lung transplant recipients. *JHLT Open* (2025) 10:100376. doi: 10.1016/j.jhlto.2025.100376
67. Asija R, Singh R, Paneitz DC, Wolfe SB, Chukwudi C, Michel E, et al. Is transplantation with coronavirus disease 2019-Positive donor lungs safe? A US nationwide analysis. *Ann Thorac Surg* (2023) 116(5):1046–54. doi: 10.1016/j.athoracsur.2023.05.048
68. Peghin M, Graziano E, De MM, Balsamo ML, Isola M, López-Fraga M, et al. Acceptance of organs from deceased donors with resolved or active SARS-CoV-2 infection: a survey from the council of Europe. *Transpl Int* (2024) 37:13705. doi: 10.3389/ti.2024.13705
69. Cockbain AJ, Jacob M, Ecuyer C, Hostert L, Ahmad N. Transplantation of solid organs procured from influenza A H1N1 infected donors. *Transpl Int* (2011) 24(12):e107–10. doi: 10.1111/j.1432-2277.2011.01342.x
70. Petrisli E, Gabrielli L, De Cillia C, Liberatore A, Piccirilli G, Venturoli S, et al. Molecular testing in organ biopsies and perfusion fluid samples from severe acute respiratory syndrome coronavirus 2 positive donors. *Viruses* (2025) 17(12):1611. doi: 10.3390/v17121611
71. Kothadia SM, Wolfe CR, Baker AW, Young KA, Reynolds JM, Hartwig MG, et al. Outcomes of lung transplantation from SARS-CoV-2 positive donors during the omicron wave. *JHLT Open* (2025) 8:100249. doi: 10.1016/j.jhlto.2025.100249
72. Goren LR, Adeyi O, Thielen BK. Possible donor-derived infection in a pediatric liver transplant patient with granulomatous hepatitis. *Cureus* (2023) 15(11):e49136. doi: 10.7759/cureus.49136
73. Pettengill MA, Babu TM, Prasad P, Chuang S, Drage MG, Menegus M, et al. Probable donor-derived human adenovirus type 34 infection in 2 kidney transplant recipients from the same donor. *Open Forum Infect Dis* (2019) 6(3):ofy354. doi: 10.1093/ofid/ofy354
74. Kaul DR, Vece G, Blumberg E, La Hoz RM, Ison MG, Green M, et al. Ten years of donor-derived disease: a report of the disease transmission advisory committee. *Am J Transpl* (2021) 21(2):689–702. doi: 10.1111/ajt.16178

75. *Multidisciplinary expertise at the service of public health*. (2026). Available online at: <https://www.hcsp.fr> (Accessed July 18, 2024).
76. Peghin M, Hirsch HH, Len O, Codina G, Berastegui C, Sáez B, et al. Epidemiology and immediate indirect effects of respiratory viruses in lung transplant recipients: a 5-Year prospective study. *Am J Transpl* (2017) 17(5):1304–12. doi: 10.1111/ajt.14042
77. Narsana N, Ha D, Ho DY. Treating adenovirus infection in transplant populations: therapeutic options beyond cidofovir? *Viruses* (2025) 17(5):599. doi: 10.3390/v17050599
78. Meylan PR, Aubert JD, Kaiser L. Influenza transmission to recipient through lung transplantation. *Transpl Infect Dis* (2007) 9(1):55–7. doi: 10.1111/j.1399-3062.2006.00175.x
79. Le Page AK, Kainer G, Glanville AR, Tu E, Bhonagiri D, Rawlinson WD. Influenza B virus transmission in recipients of kidney and lung transplants from an infected donor. *Transplantation* (2010) 90(1):99–102. doi: 10.1097/TP.0b013e3181da1933
80. *Organ donation and seasonal influenza: guidance from SaBTO's virology review subcommittee*. Available online at: <https://www.gov.uk/government/publications/organ-donation-and-seasonal-flu/organ-donation-and-seasonal-influenza-guidance-from-sabtos-virology-review-subcommittee> (Accessed October 23, 2019).
81. Hulot S, Prunet V, Coiffard B, Zandotti C, Boschi C, D'Journo XB, et al. Fatal acute respiratory distress syndrome caused by donor-derived human metapneumovirus after lung transplantation. *Am J Transpl* (2025) 25(10):2239–43. doi: 10.1016/j.ajt.2025.05.020
82. Nellore A, Goepfert P, Tan CS, Bajema K, Belden K, Blumberg D, et al. IDSA 2025 guidelines on the use of vaccines for the prevention of seasonal COVID-19, influenza, and RSV infections in immunocompromised patients. *Clin Infect Dis* (2026) 82:i105–i110. doi: 10.1093/cid/ciag114



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# Nutritional strategies in kidney transplantation: optimising outcomes beyond surgery

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Although kidney transplantation (KTx) remains the optimal therapy for end-stage kidney disease, long-term graft survival and patient outcomes continue to be limited by cardiometabolic complications and chronic allograft dysfunction. Nutritional management has evolved from supportive care to a core component of post-transplant follow-up. The early post-transplant phase is marked by catabolism, high-dose immunosuppression, and metabolic instability, leading to increased protein requirements but also potential susceptibility to hyperfiltration injury. In the stable post-transplant phase, a moderate protein intake similar to that recommended for chronic kidney disease (CKD) may support graft longevity, although evidence in kidney transplant recipients (KTRs) remains limited and inconsistent. Plant-forward dietary models, including Mediterranean and DASH patterns, consistently improve cardiometabolic risk, inflammation, oxidative stress, and dietary acid load, with potential benefits for graft outcomes. Predominantly plant-based diets may further reduce uremic toxin generation and improve mineral metabolism, though careful monitoring for micronutrient deficiencies is required. Low-protein approaches, such as PLADO, remain largely theoretical in transplant populations, while ketogenic or very low-carbohydrate diets raise concerns regarding acid–base balance, renal hemodynamics, and long-term tolerability. Overall, personalised nutritional strategies tailored to graft function, immunosuppression, and metabolic profile are essential to optimise long-term transplant health.

### KEYWORDS

immunosuppression, ketogenic diet, kidney transplantation, mediterranean diet, metabolic complications

## Introduction

Kidney transplantation is the preferred treatment for end-stage kidney disease, offering superior survival and quality of life compared to chronic dialysis, but long-term outcomes are limited by metabolic complications, cardiovascular disease, and chronic allograft dysfunction.

[1] Nutrition, once considered ancillary, is now recognized as a pivotal determinant of graft and patient outcomes [1–3].

In the immediate post-transplant period, high-dose immunosuppression and catabolic stress necessitate adequate protein intake to maintain nitrogen balance and support lean mass. The recommended protein intake during this phase is typically 1.0–1.3 g/kg/day, as



supported by recent reviews and observational studies [4–6]. However, excessive protein intake may induce glomerular hyperfiltration and accelerate graft injury, so intake above 1.4 g/kg/day is generally not advised for prolonged periods [4, 5]. During stable phases, moderate protein restriction (0.8–1.0 g/kg/day) may be beneficial for graft longevity, though evidence in transplant recipients is limited and sometimes conflicting [4, 7, 8]. For those with chronic allograft dysfunction or reduced eGFR (<25 mL/min), lower protein targets (0.55–0.60 g/kg/day) may be considered [4].

Beyond macronutrient targets, dietary patterns such as the Mediterranean and Dietary Approaches to Stop Hypertension (DASH) diets are associated with reduced cardiovascular risk and improved graft outcomes, emphasizing plant-based foods, reduced processed meats, and lower sodium intake [1, 7, 9, 10]. These patterns may also beneficially modulate the immune system and gut microbiome [3, 11].

In summary, nutritional management in kidney transplant recipients should be individualized, with attention to phase-specific protein requirements, overall dietary patterns, and metabolic risk factors, as supported by current evidence [1–12].

## Methods

This manuscript is a narrative review summarising the current evidence on nutritional strategies for kidney transplant recipients. A non-systematic literature search was performed using the PubMed/MEDLINE, Scopus and Google Scholar databases to identify studies published up to January 2026. Search terms included combinations of the following: “kidney transplantation,” “kidney transplant recipients,” “nutrition,” “diet,” “protein intake,” “Mediterranean diet,” “DASH diet,” “plant-based diet,” “vegetarian diet,” “ketogenic diet,” “microbiota,” and “allograft function.” Priority was given to clinical practice guidelines, randomised controlled trials, cohort studies, meta-analyses and high-quality narrative reviews published in English. This review aimed to provide an overview of emerging dietary approaches and nutritional management strategies in kidney transplantation from a clinical perspective, rather than conducting a formal systematic review or meta-analysis.

## Pre-transplant and peri-transplant nutritional optimization: prehabilitation and ERAS-based approaches

Nutritional management in kidney transplantation should not only begin after surgery. The pre- and peri-transplant periods are critical for identifying malnutrition, sarcopenia, obesity, frailty, micronutrient deficiencies and metabolic risk factors, which can affect surgical recovery and long-term transplant outcomes [1–4]. In

other surgical populations, multimodal prehabilitation and Enhanced Recovery After Surgery (ERAS) protocols combining nutritional optimisation, physical exercise, anaemia correction, glycaemic control and patient education have been associated with a shorter hospital stay, fewer postoperative complications, faster functional recovery and improved survival [5–8].

However, direct evidence remains limited in kidney transplant candidates. Available studies suggest that poor nutritional status, sarcopenia, frailty and obesity prior to transplantation are associated with delayed graft function, infectious complications, prolonged hospitalisation, cardiovascular events and reduced patient and graft survival [9–14]. Therefore, pre-transplant nutritional education may be clinically relevant, particularly when integrated into multidisciplinary care involving nephrologists, nutritionists, transplant surgeons and rehabilitation specialists [1, 15]. Key objectives include optimising protein-energy status, avoiding protein-energy wasting in dialysis patients, managing weight in obese patients, correcting vitamin and mineral deficiencies, restricting sodium for blood pressure control, and counselling on food safety and metabolic risk after transplantation [1, 15–17].

Peri-transplant nutrition should aim to minimise catabolism and support wound healing, early mobilisation and recovery [6–8]. Although ERAS pathways are being adopted more widely in solid-organ transplantation, there is still insufficient standardisation of kidney-transplant-specific nutritional protocols [1, 3]. Currently, recommendations are largely extrapolated from general surgery, chronic kidney disease (CKD), dialysis, and transplant observational studies, rather than from adequately powered randomised trials in kidney transplant recipients [1, 3].

Future studies should evaluate whether structured pre-transplant nutritional education and multimodal prehabilitation programmes can improve clinically meaningful outcomes. These outcomes include postoperative complications, delayed graft function, length of hospital stay, readmissions, reversal of frailty, sarcopenia, quality of life, graft function trajectory and long-term patient survival [1, 3, 18].

## Dietary approaches for kidney transplant recipients

Over the past decade, several dietary patterns have grown in popularity due to their potential to reduce cardiovascular risk, exhibit anti-inflammatory properties, and promote weight loss [13]. Among these, plant-based diets and low-carbohydrate or ketogenic diets have received considerable attention (Supplementary Tables S1, S2).

### Plant-based diets

In earlier decades, it was generally assumed that animal-derived proteins contain all the essential amino acids that the human body cannot produce in sufficient quantities (classifying them as proteins of high biological value), and they are more effective at maintaining the body's protein pool due to their higher content of sulphur-containing amino acids [14, 15]. However, increasing interest in plant-based dietary approaches has challenged this paradigm. Diets composed primarily or exclusively of plant-derived foods are now considered feasible in populations with chronic diseases, including

**Abbreviations:** CKD, Chronic kidney disease; DDKT, Deceased donor kidney transplantation; DASH, Dietary Approaches to Stop Hypertension; KD, Ketogenic diets; KTRs, kidney transplant recipients; KTx, Kidney transplantation; PLADO, Plant-Dominant Low-Protein Diet; PLAFOND, Plant-Focused Nutrition in Diabetes; SCFA, Short-chain fatty acid; TMAO, Trimethylamine N-oxide.

CKD [16]. A recent study demonstrated that the risk of incident CKD in the general population was reduced by 4% for each additional 0.1 g/kg/day of plant protein intake [14, 17]. Within nephrology, plant-based dietary patterns uniquely allow for the implementation of very low protein regimens (0.4 g/kg body weight/day) [1]. Compared with animal-based diets, plant-based diets provide higher amounts of fiber and alkali, which contribute to the improved correction of CKD-associated metabolic acidosis and the prevention of intestinal dysbiosis [3, 14]. It is important to note that plant-based diets and veganism are not synonymous. Rather, plant-based diets represent a spectrum of dietary models in which the majority of protein intake is derived from plant sources. Examples include the Mediterranean diet, the Dietary Approaches to Stop Hypertension (DASH) diet, and the PLADO diet. Due to their favorable metabolic effects, recent clinical practice guidelines on nutrition in CKD have endorsed plant-based dietary patterns. While both sets of guidelines acknowledge the limited evidence available to recommend one specific protein source, the KDOQI guidelines do not rule out plant-based diets, and the KDIGO guidelines explicitly recommend them [1, 14, 18].

### Mediterranean diet

The Mediterranean diet is a dietary pattern that is primarily cardioprotective and anti-inflammatory. It originates from the traditional eating habits of people living in coastal regions of the Mediterranean. It is characterized by a high consumption of extra-virgin olive oil, vegetables, legumes and whole grains, as well as a moderate intake of fish and wine. Overall, it involves moderate protein intake of around 0.8 g/kg/day without being specifically designed as a low-protein regimen. [19]. Robust evidence supports its role in reducing the risk of type 2 diabetes, cardiovascular disease and all-cause mortality [20].

Adherence to the Mediterranean diet is associated with multiple metabolic and vascular benefits, including reductions in inflammation, oxidative stress, endothelial dysfunction, metabolic acidosis, hyperlipidemia, hyperglycemia and elevated blood pressure [4, 9, 10, 21, 22]. In both the general population and kidney transplant recipients (KTRs), higher Mediterranean Diet Scores correlate with improved renal outcomes [4, 23]. In a large multi-ethnic cohort of 3,298 individuals followed for 15 years, higher adherence was associated with approximately 50% lower odds of incident eGFR <60 mL/min/1.73 m<sup>2</sup> [23]. Similarly, among 632 KTRs, adherence to the Mediterranean diet was protective against kidney function decline and graft loss, with stronger effects observed in patients with proteinuria [4].

The renoprotective effects of the Mediterranean diet likely arise from the modulation of several interconnected pathways, including oxidative stress, systemic inflammation, endothelial function, dietary acid load, lipid metabolism, protein intake and glycemic control. High consumption of fruits and vegetables increases antioxidant and dietary fiber intake, while a greater proportion of omega-3 fatty acids contributes to reductions in oxidative stress and inflammation [4, 24–26]. Low meat intake combined with high fruit and vegetable consumption improves acid–base balance [4, 27], and a lower dietary acid load is associated with reduced CKD risk [28].

The largely plant-based protein profile of the Mediterranean diet may also reduce proteinuria. Plant proteins, which have lower bioavailability and acidogenic potential, may induce less

glomerular hyperfiltration and intraglomerular pressure than animal proteins, thereby reducing renal hemodynamic stress. Typical protein intake approximates 0.8 g/kg/day, mainly from non-red and non-processed sources [22]. Additionally, plant proteins are associated with a lower endogenous acid load and reduced sulphur and phosphorus amino acid content, potentially limiting tubular injury and hyperfiltration [3].

Finally, the diet's high content of monounsaturated fats and polyphenols, and low saturated fat intake, further contributes to its protective effects [29–32]. In KTRs, improvements in oxidative status, acid–base balance and glomerular hemodynamics have been documented [4, 9, 10, 21–23, 29, 33]. Overall, the Mediterranean diet emerges as a valuable strategy to support general health and potentially long-term graft survival in KTRs.

### Dietary approaches to stop hypertension (DASH) diet

The Dietary Approaches to Stop Hypertension (DASH) diet was originally developed by the US National Institutes of Health to treat or prevent hypertension. The DASH diet primarily targets sodium reduction and blood pressure control by encouraging the consumption of fruits, vegetables and low-fat dairy products, while reducing saturated fat intake and maintaining moderate protein consumption. It does not specifically promote a plant-based diet.

In the United States, the average sodium intake exceeds 3,400 mg per day, surpassing the maximum recommended intake of 2,300 mg per day set out in the 2015–2020 Dietary Guidelines [34]. Excess sodium intake has been linked to hypertension, cardiovascular events and, in experimental models, to the promotion of chronic allograft rejection [34]. Clinical evidence demonstrates that the DASH diet significantly lowers blood pressure, total cholesterol and low-density lipoprotein cholesterol levels [35]. In an 8-week controlled feeding trial, the DASH diet reduced systolic and diastolic blood pressure by 5.5 mmHg and 3.0 mmHg respectively compared to standard diets [36]. The DASH diet remains one of the most effective dietary strategies for blood pressure control [37].

Hypertension affects 50%–80% of adult KTRs, contributing to shorter graft survival and increased cardiovascular morbidity and mortality [38]. Thus, the DASH diet is a particularly relevant intervention for this population. In a prospective cohort study of 632 KTRs, greater adherence to the DASH diet was associated with a lower risk of decline in kidney function (hazard ratio: 0.95;  $P = 0.008$ ) and reduced all-cause mortality (hazard ratio: 0.95;  $P = 0.01$ ) [7]. Similar renal and cardiovascular benefits have been observed in other studies [9, 10, 39], which are likely to be mediated through favorable effects on serum lipids, blood pressure, insulin resistance, inflammation, oxidative stress, arterial stiffness and endothelial function. In summary, the DASH diet provides a robust, evidence-based approach to restricting sodium and controlling blood pressure in KTx patients.

### Vegetarian and vegan diets

Vegetarian diets, historically adopted for religious, cultural or ethical reasons, are increasingly promoted for health and disease prevention. Evidence suggests that reducing or eliminating meat intake may attenuate kidney disease progression [9, 10, 40]. Vegetarian diets include heterogeneous patterns, from lacto-ovo

vegetarian to vegan diets, and do not inherently guarantee nutritional quality, as processed foods may still be consumed [40].

In kidney transplant recipients, protein source and quantity critically influence graft survival. High protein intake, especially from animal sources, induces afferent arteriolar dilation, glomerular hyperfiltration and intraglomerular hypertension, promoting fibrosis and progressive renal injury [40–42]. Protein restriction reduces hyperfiltration and stabilizes kidney function [42]. Data from chronic kidney disease populations show that low-protein diets delay dialysis initiation and slow progression to end-stage kidney disease [43, 44], whereas high-protein and red-meat-based diets are linked to faster kidney function decline and increased microalbuminuria [45, 46].

Plant-based diets improve mineral metabolism and acid–base balance. Plant phosphorus has lower bioavailability, resulting in reduced serum phosphate and FGF23 levels associated with better graft outcomes [9, 40, 47–51]. Plant potassium generally has milder effects due to enhanced fecal excretion and alkalinisation [9, 40, 52, 53]. High fiber intake reduces uremic toxins and inflammation [54–56]. Nutritional risks include vitamin B12 and iron deficiency, requiring monitoring [57]. Overall, well-planned plant-based low-protein diets may support long-term graft preservation [12, 58].

#### Low protein intake and plant dominant low protein diet (PLADO)

There is growing evidence that protein restriction slows chronic kidney disease progression [3, 59–62], although its impact on kidney transplantation outcomes remains uncertain. Dietary protein intake drives urea and nitrogenous waste production, increasing renal excretory burden. Protein restriction activates protective mechanisms, including downregulation of the renin–angiotensin system [3, 63, 64], reduced glomerular hemodynamic stress [59, 65, 66], limited renal hypertrophy [63], decreased ammoniogenesis [63] and lower metabolic activity [67]. Conversely, high-protein diets increase metabolic demand, promote renal vasodilatation and exacerbate intraglomerular pressure in transplant kidneys, leading to hyperfiltration and structural injury [42, 59].

Plant-focused low-protein dietary approaches, including the Plant-Dominant Low-Protein Diet (PLADO) and the Plant-Focused Nutrition in Diabetes (PLAFOND), have been proposed for CKD and diabetes management. The Plant-Dominant Low-Protein Diet (PLADO) is a structured, nephroprotective dietary strategy designed to reduce glomerular hyperfiltration and metabolic burden. It involves controlled protein restriction (0.6–0.8 g/kg/day) and a diet consisting predominantly of plant-derived protein sources (generally 50%–75%) [2, 68]. The Plant-Focused Nutrition in Diabetes (PLAFOND) model further emphasises high fiber intake, low glycemic index and adequate energy provision [69]. Although these diets may slow CKD progression, evidence supporting benefits or safety in kidney transplant recipients is lacking.

Evidence on protein intake after transplantation is conflicting. Low intake may preserve graft function, whereas higher intake supports nitrogen balance, muscle mass, fatigue and quality of life [2, 4, 6, 70, 71].

#### Low-carb diets

Metabolic complications are common after kidney transplantation, and dietary strategies have been explored to limit weight gain, hypertension, dyslipidemia and insulin resistance [13]. Immunosuppressive therapies, especially glucocorticoids, promote hyperglycemia and obesity [72–76]. Adequate glycemic control is clinically relevant because it is associated with slower kidney disease progression [77–79].

Ketogenic diets have gained interest for metabolic control, improving satiety, weight reduction and insulin sensitivity, while reducing inflammation [80–82]. A ketogenic diet usually limits carbohydrates to 30–50 g/day, inducing nutritional ketosis through glycogen depletion and ketone body production [83, 84]. Ketone bodies provide energy for several organs, including kidneys, during carbohydrate restriction.

Nutritional ketosis is defined by ketone levels of 0.5–3 mmol/L [85], whereas therapeutic ketosis reaches higher concentrations [86]. Keto-adaptation involves mitochondrial and enzymatic upregulation in oxidative tissues [85, 87]. Importantly, nutritional ketosis differs from diabetic ketoacidosis, as pH and glucose remain normal.

In kidney transplant recipients, evidence on ketogenic diets is limited and controversial. Potential concerns include acid load, hyperkalemia, protein catabolism and graft injury, although early CKD data suggest preserved pH during nutritional ketosis [85, 88]. Reported adverse effects include fatty liver and insulin resistance [40, 89, 90]. Data in transplant populations are scarce, heterogeneous and insufficient to define safety or graft benefit. Short-term use under specialist supervision appears feasible for obesity, but long-term advantages resemble those of Mediterranean-style diets [91].

High reliance on processed ketogenic products may be inappropriate for transplant recipients. Moreover, long-term adherence is difficult, and carbohydrate quality may be more relevant than quantity [92, 93]. Diets rich in whole, high-fiber carbohydrates show superior cardiovascular and metabolic outcomes compared with ketogenic diets [94]. In the post-transplant setting, high-quality carbohydrates may reduce post-transplant diabetes and obesity risk [95, 96]. Therefore, balanced dietary patterns emphasizing carbohydrate quality may better address metabolic complications after transplantation.

#### Dietary protein intake: implication for kidney allograft survival and function

There is currently insufficient evidence to determine the optimal quantity or type of dietary protein for KTRs, so formal recommendations cannot yet be established. Nevertheless, certain patient- and transplant-related factors may inform the individualisation of protein intake.

The 2020 KDOQI Clinical Practice Guideline for Nutrition in CKD recommends a low-protein diet of 0.55–0.60 g/kg/day or a very low-protein diet of 0.28–0.43 g/kg/day, supplemented with keto acid or amino acid analogues, for adults without diabetes. For adults with CKD and concomitant diabetes, an intake of 0.6–0.8 g/kg/day of protein is considered appropriate. It is notable that no specific

TABLE 1 Suggested protein intake in kidney transplant recipients across different clinical phases.

| Clinical phase                                                                | Immunosuppressive status                                             | Suggested protein intake                                                                                                                             | Evidence strength                                                                                                                                                                                                                                                              |
|-------------------------------------------------------------------------------|----------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Immediate post-transplant (0-1 month, high-dose corticosteroids) [11, 12, 17] | Escalated immunosuppression, high-dose steroids                      | 1.0–1.3 g/kg/day to maintain nitrogen balance and prevent muscle wasting                                                                             | Higher intake ( $\geq 1.4$ g/kg/d; mean $\sim 1.6$ – $1.8$ ) associated with better early eGFR, hemoglobin, lipids and no increase in short-term complications or infections<br>Suggested evidence strength: Low–moderate (retrospective cohorts, observational)               |
| Early stable post-transplant (1–6 months, low or stable steroid dose) [13]    | Reduced/stable immunosuppression                                     | $\geq 0.72$ g/kg IBW/day to avoid skeletal muscle loss                                                                                               | $\geq 0.72$ g/kg IBW/day predicted to prevent net skeletal muscle loss at 12 months. Several cohorts link higher intake ( $\sim 1.0$ – $1.1$ g/kg/d) with better fatigue, QoL, and survival proxies<br>Evidence strength: Low–moderate (observational, modeling)               |
| Medium -long term post-transplant [13]                                        | Reduced/stable immunosuppression                                     | $\geq 0.72$ g/kg IBW/day to avoid skeletal muscle loss                                                                                               | Reviews suggest 0.8– $1.0$ g/kg/d for stable KTRs, with cautious trial of 0.6– $0.8$ g/kg/d low-protein, plant-dominant patterns to mitigate hyperfiltration, if nutritional status is preserved<br>Evidence strength: Low (narrative reviews, conflicting observational data) |
| Chronic allograft dysfunction/rejection [1]                                   | Variable; inflammation may be present, corticosteroids often tapered | $\geq 0.55$ – $0.60$ g/kg/day (low protein diet may improve glomerular permselectivity, but intakes $<0.55$ g/kg/day risk negative nitrogen balance) | Proposed range aims to slow progression while avoiding negative nitrogen balance; largely extrapolated from CKD LPD data and one crossover KTR nitrogen-balance study<br>Evidence strength: Low (CKD RCTs, small transplant studies, extrapolation)                            |
| Failed allograft (return to dialysis) [15]                                    | Immunosuppression generally minimized or stopped                     | 1.0– $1.2$ g/kg/day (aligned with ESKD recommendations)                                                                                              | Aligns with ESKD guidelines and hemodialysis data, where $\geq 1.0$ – $1.2$ g/kg/d is recommended to prevent protein-energy wasting<br>Evidence strength: Moderate (CKD/ESKD trials and guidelines; indirect for failed graft)                                                 |

ESKD: end stage kidney disease; IBW: ideal body weight.

dietary protein intake targets are currently provided for adult kidney transplant recipients [1].

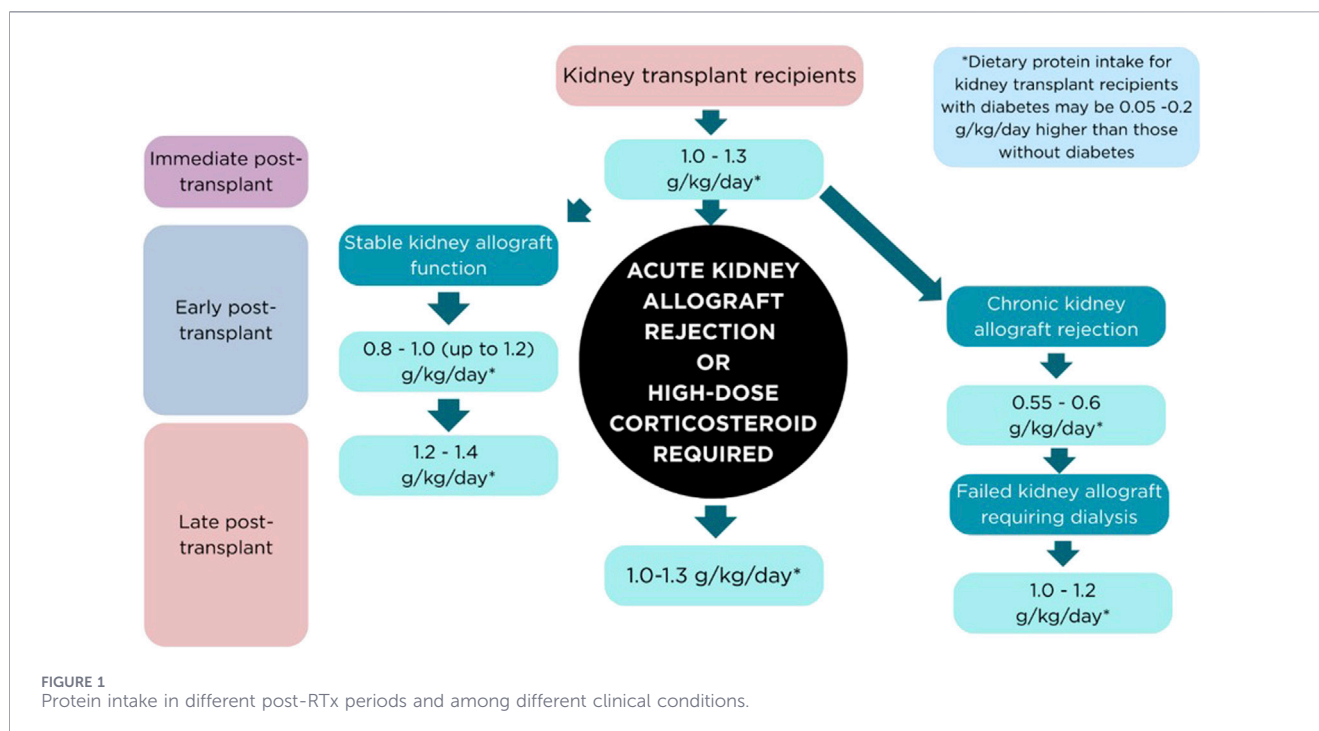
## The net state of immunosuppression and dietary protein requirements in kidney transplant recipients

The immunosuppressive status of KTRs fluctuates over time. During the immediate post-transplant period and when treating acute rejection, escalating the use of immunosuppressive agents — particularly high-dose corticosteroids — can accelerate protein catabolism and lead to muscle wasting [2, 97]. To counteract muscle loss, protein intake should be increased [2].

Evidence from two key studies has provided insights into protein needs (Table 1). A prospective cohort study of KTRs after deceased donor kidney transplantation (DDKT) found that patients consuming a low-protein diet had higher protein catabolic rates than those with higher protein intake [6]. Similarly, a randomised controlled trial involving non-diabetic KTRs found that low protein intake was associated with negative nitrogen balance and muscle mass loss, whereas higher protein intake resulted in positive nitrogen balance and muscle gain [2]. These studies suggest that an intake of at least 1.0–1.3 g/kg/day of protein may help preserve lean mass during the early post-transplant phase.

When steroid doses are tapered, the need for protein appears to be lower. A cross-sectional study indicated that a minimum intake of 0.72 g/kg/day may be sufficient 1 year after transplantation [2].





Conversely, in cases of chronic allograft dysfunction, the inflammatory environment may increase protein requirements. Data from a crossover RCT involving 14 KTRs with chronic rejection compared low (0.55 g/kg/day) and high (2.0 g/kg/day) protein diets. The low-protein group showed lower urinary protein and better glomerular permselectivity, though they only achieved a slightly positive nitrogen balance [98].

It is important to note that current nutritional recommendations for kidney transplant recipients are supported by relatively limited transplant-specific evidence and are often based on studies conducted in populations with chronic kidney disease. The unique physiological and metabolic characteristics of kidney transplant recipients, including chronic immunosuppression, altered inflammatory status, post-transplant metabolic complications and the presence of a functioning allograft, may limit the direct applicability of CKD-derived nutritional strategies. Consequently, adequately powered randomised controlled trials conducted specifically in kidney transplant populations are urgently needed to define optimal dietary patterns, protein and energy requirements, and long-term nutritional interventions capable of improving graft survival, cardiometabolic outcomes, sarcopenia and patient quality of life.

### Dietary calorie-protein intake across post-transplant periods

In addition to immunosuppression, the post-transplant period itself influences protein requirements. In a 12-year prospective cohort of 43 KTRs, a low-protein intake (0.73 g/kg/day) was associated with stable GFR, whereas higher intake (1.40 g/kg/day) was associated with a significant decline [99].

However, evidence from observational studies remains inconsistent. In a cross-sectional analysis of 204 KTRs, protein

intake >1.2 g/kg/day was associated with proteinuria [5], while a larger cohort of 625 KTRs showed no association with creatinine clearance [100]. It is important to note that excessively low protein intake may also be detrimental: in cohorts of 604 and 940 KTRs, lower protein intake was associated with an increased risk of graft loss and mortality [8, 101].

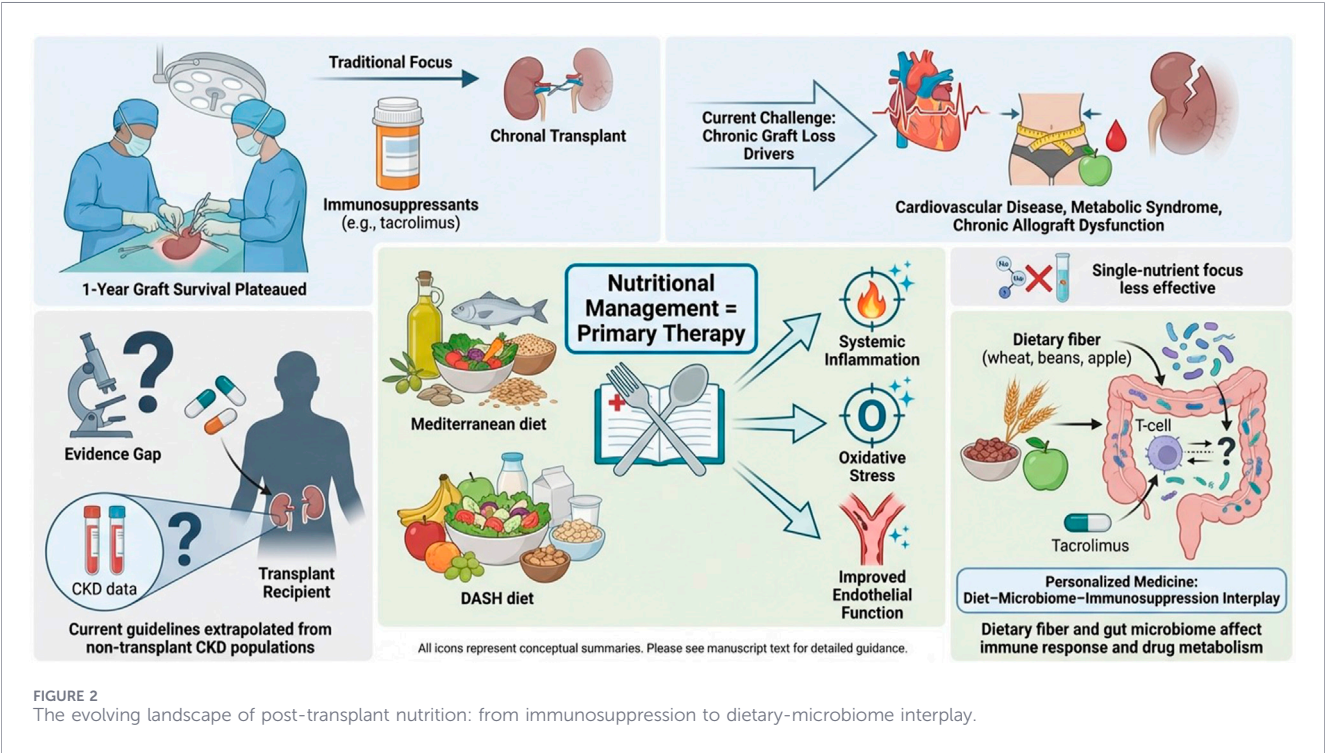
Overall, the current evidence base supports a moderate protein intake of 0.8–1.0 g/kg/day for the first three to twelve months after transplant, with the possibility of increasing this to 1.2–1.4 g/kg/day thereafter. Recommendations following graft failure are in line with the guidelines for end-stage kidney disease (ESKD) (0.55–0.6 g/kg/day) (Figure 1) [1].

Adequate energy intake is a critical component of nutritional management after kidney transplantation and should accompany protein prescriptions to maintain neutral nitrogen balance and prevent muscle catabolism [1, 2, 70]. In the immediate post-transplant period, calorie requirements generally increase due to surgical stress, exposure to corticosteroids, tissue repair and heightened metabolic demand [2, 6, 70]. Approximately 30–35 kcal/kg/day is commonly suggested as an energy intake during the early catabolic phase, whereas lower requirements (25–30 kcal/kg/day) may be appropriate during stable long-term follow-up, depending on factors such as age, physical activity, body composition, graft function, and metabolic comorbidities [1, 58]. It is important to note that inadequate caloric intake can lead to the breakdown of endogenous proteins and sarcopenia, even when protein intake appears sufficient [2, 6, 71]. Conversely, excessive calorie intake can lead to obesity, post-transplant diabetes mellitus, metabolic syndrome and cardiovascular complications. These remain major determinants of long-term graft and patient outcomes [13, 72, 95, 96].

TABLE 2 comparison of the main features of different nutrition guidelines focusig on KTRs.

| Guideline                 | Kidney transplant recipient (KTR) coverage                              | Key nutrition recommendations                                                                                                                                                                                                                                                                                                                                                           | Limitations                                                                                         |
|---------------------------|-------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| ESPEN (2021; 2024 update) | No specific guidance for stable KTR; transplant nutrition not addressed | Inpatient focus: energy/protein delivery, artificial nutrition routes, refeeding prevention                                                                                                                                                                                                                                                                                             | Gap in outpatient and post-transplant care; limited applicability beyond hospitalization            |
| KDOQI (2020)              | Explicitly includes patients with functioning kidney transplants        | <ul style="list-style-type: none"><li>- Routine nutrition screening and assessment (validated tools, body composition)</li><li>- Individualized protein/energy prescriptions by stage (higher in acute post-transplant, tailored for long-term)</li><li>- Sodium restriction for BP control</li><li>- Promotion of dietary patterns based on whole, minimally processed foods</li></ul> | Does not mandate a single protein target for all KTR; evidence for long-term outcomes still limited |
| KDIGO (2009–2022)         | Scattered transplant-related recommendations across thematic guidelines | <ul style="list-style-type: none"><li>- Lifestyle and CV risk management integrated with immunosuppression</li><li>- Pre-transplant risk factor optimization (e.g., obesity)</li><li>- Post-transplant: BP control (standardized measurement, sodium moderation), weight management, PTDM prevention</li></ul>                                                                          | No standalone nutrition CPG for KTR; lacks detailed macronutrient prescriptions                     |

KTR: kidney transplant recipients; BP: blood pressure; CV: cardiovascular; PTDM: Post-transplant Diabetes Mellitus; CPG: clinical practice guidelines.



Comparison of international guidelines (ESPEN, KDOQI, KDIGO)

Compared with older, nutrient-restriction paradigms, today’s triad of ESPEN–KDOQI–KDIGO collectively pushes toward: early anabolic support; longer-term weight, blood pressure (BP), and glycemic control; and endorsement of healthy dietary patterns rather than transplant-unique restrictions. Collectively, these guidelines reflect a paradigm shift: from nutrient restriction to holistic, patient-centered dietary strategies (Table 2).

Intestinal microbiota and nutritional regimens in kidney transplant patients

The gut microbiota is increasingly recognized as a key modulator of health and transplant outcomes. After transplantation, KTRs commonly develop dysbiosis, with reduced microbial diversity and depletion of short-chain fatty acids (SCFAs)-producing bacteria such as *Faecalibacterium prausnitzii* [102]. Immunosuppressive agents directly alter microbial composition and function [103]. This imbalance

may impair gut barrier integrity and promote systemic inflammation [104].

Dietary modulation offers an effective strategy. High-fiber diets enhance microbial fermentation and SCFA production, which support epithelial integrity and improve tacrolimus bioavailability [11]. Butyrate specifically promotes regulatory T-cell differentiation [105]. Conversely, patterns high in red meat and refined sugars increase production of harmful metabolites such as trimethylamine N-oxide [106] (Figure 2).

## Clinical recommendations and future directions

The following recommendations, derived from available and current evidences, provide a roadmap for the longitudinal care of KTRs, emphasizing a multidisciplinary, phase-specific approach.

## Macro-structural dietary recommendations

- **Prioritizing the Mediterranean Framework:** For the stable KTR, the Mediterranean diet should be the default recommendation. Its emphasis on extra-virgin olive oil, high fiber, and plant-derived proteins offers a synergistic effect that addresses the leading cause of death in this population: cardiovascular disease.
- **The DASH Alternative:** In patients where hypertension is the primary driver of allograft injury, the DASH diet provides a structured, evidence-based approach to sodium restriction while ensuring nutritional adequacy.
- **Carbohydrate Quality and Metabolic Syndrome:** Given the high incidence of post-transplant diabetes mellitus (PTDM), clinicians should move away from simple carbohydrate restriction. Instead, the focus should be on carbohydrate quality—prioritizing whole grains and low-glycemic-index foods to counteract the metabolic disturbances induced by glucocorticoids and calcineurin inhibitors.

## The precision protein prescription

Protein intake must be tailored to the “chronological age” of the transplant and the patient’s metabolic demands.

- **The Early Catabolic Window (0–3 Months):** To counteract surgical stress and steroid-induced muscle wasting, an intake of 1.0–1.3 g/kg/day is recommended.
- **The Maintenance Phase (3–12 Months):** Transition to 0.8–1.0 g/kg/day. Clinicians must be vigilant to ensure intake does not fall below 0.72 g/kg/day to avoid sarcopenia, which is independently associated with poor outcomes.
- **The Dysfunction Phase (Chronic Allograft Nephropathy):** In the setting of declining eGFR, moderate restriction to 0.55–0.60 g/kg/day may be protective, provided the patient is not in a state of chronic inflammation.

## Microbiome-targeted interventions

**Fiber as an Immunomodulator:** Clinicians should encourage high dietary fiber intake (25–30g/day) not just for gastrointestinal health, but as a strategy to produce short-chain fatty acids (SCFAs).

SCFAs, specifically butyrate, may support immune tolerance and protect the gut-vascular barrier.

To advance the field of transplant nutrition, future research must prioritize large-scale, transplant-specific randomized controlled trials to evaluate the long-term impact of plant-dominant dietary patterns, such as the PLADO diet, on eGFR slopes and graft longevity. Additionally, rigorous investigations are needed into the safety and efficacy of nutritional ketosis as a targeted intervention for refractory post-transplant obesity, specifically assessing its potential to improve metabolic markers without precipitating metabolic acidosis. There is also a compelling need for precision nutrition strategies that utilize microbial profiling to personalize fiber and protein intake based on the individual’s unique gut environment.

Ultimately, while the technical success of a kidney transplant begins in the operating room, its long-term preservation is sustained in the kitchen; transitioning to personalized, pattern-based nutritional therapy is therefore essential to ensuring that the gift of a transplant lasts a lifetime (Figure 3.).

## Conclusions: a new paradigm in transplant nephrology

The historical focus of transplant medicine has traditionally centered on the refinement of surgical techniques and the optimization of immunosuppressive regimens to prevent acute rejection. However, as 1-year graft survival rates have plateaued at high levels, the challenge has shifted toward mitigating the chronic drivers of graft loss: cardiovascular disease, metabolic syndrome, and chronic allograft dysfunction.

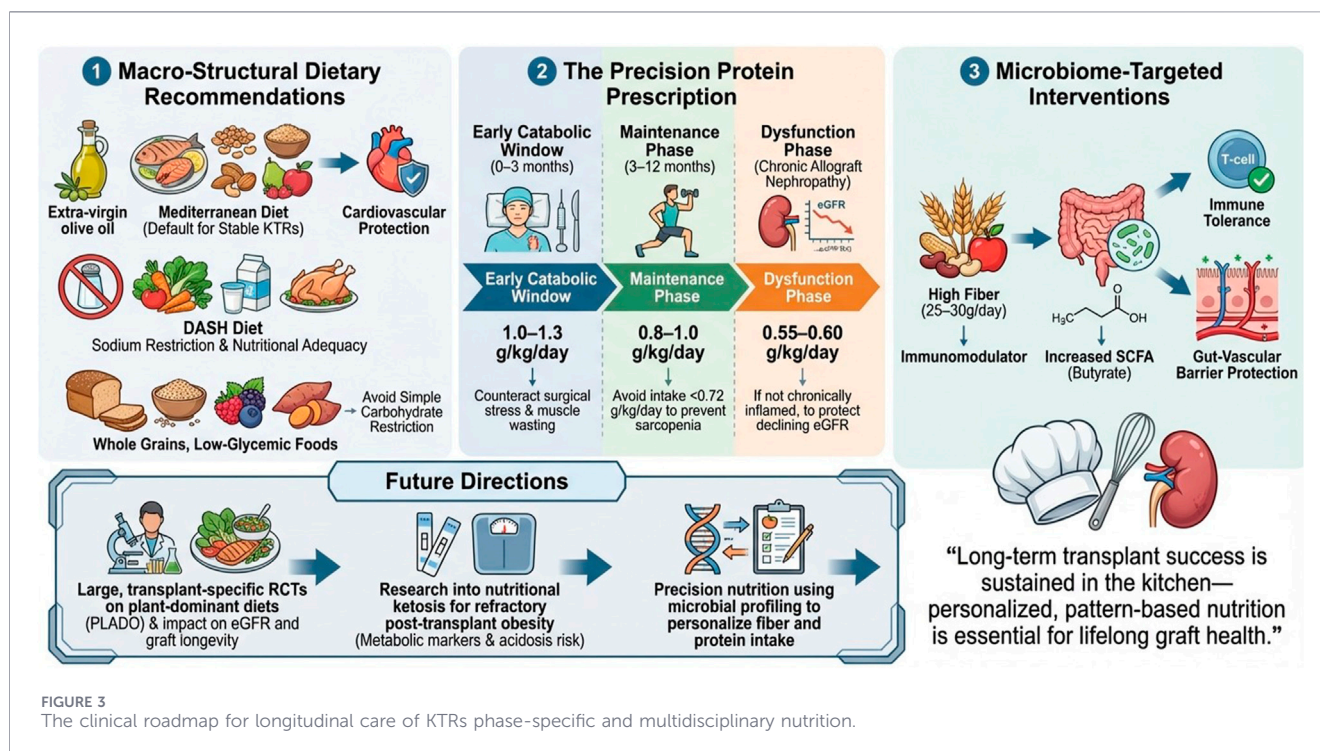
This review highlights a significant transition in clinical thought: nutritional management is no longer a secondary support mechanism but a primary therapeutic intervention.

Data from literature underscores that focusing on single nutrients (e.g., sodium or protein) in isolation is less effective than adopting comprehensive dietary patterns. The Mediterranean and DASH diets emerge as the most robust frameworks because they address the multi-faceted nature of post-transplant health—simultaneously targeting systemic inflammation, oxidative stress, and endothelial dysfunction.

While the biological rationale for plant-based and low-protein diets is strong—centering on the reduction of glomerular hyperfiltration and acid load—this review identifies a critical “evidence gap.” Much of our current practice relies on the extrapolation of data from non-transplant CKD populations. The unique physiology of the transplant recipient, characterized by pharmacological immunosuppression and a solitary functioning kidney, necessitates a dedicated body of research.

One of the most compelling frontiers identified is the interaction between nutrition and the gut microbiome. The realization that dietary fiber can modulate the immune response and potentially alter the metabolism of drugs like tacrolimus opens a new chapter in personalized transplant medicine. This suggests that the “net state of immunosuppression” is not merely a product of drug dosage, but a complex interplay between pharmacology and the intestinal environment (Figure 2).





The evidence supporting nutritional interventions in kidney transplant recipients is constrained by substantial methodological limitations. Most available studies are observational, retrospective, or conducted in relatively small cohorts, which restricts statistical power, external validity, and causal inference. Dietary intake is typically assessed using self-reported questionnaires, introducing recall and measurement bias. Residual confounding is also considerable, as healthier dietary patterns often cluster with other favourable lifestyle behaviours. In addition, transplant populations are highly heterogeneous with respect to immunosuppressive regimens, graft function, and comorbid conditions. Finally, many nutritional recommendations—particularly regarding plant-based and low-protein diets—are extrapolated from chronic kidney disease cohorts rather than derived from randomized trials in transplant recipients, highlighting the urgent need for adequately powered, transplant-specific studies.

## Author contributions

PM and LC wrote the manuscript; MG, GC, and CA were responsible for project administration; SVt, AR, SVd, MG, GC, and CA reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

SVt served as a consultant at advisory boards for Merk Sharp & Dohme, Astra Zeneca, Boehringer and Ingelheim, and held a sponsored lecture by Dr. Shär. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2026.16322/full#supplementary-material>



## References

- Ikizler TA, Burrowes JD, Byham-Gray LD, Campbell KL, Carrero JJ, Chan W, et al. KDOQI clinical practice guideline for nutrition in CKD: 2020 update. *Am J Kidney Dis* (2020) 76(3 Suppl. 1):S1–S107. doi: 10.1053/j.ajkd.2020.05.006
- Tantisattamo E, Kalantar-Zadeh K. Dietary protein intake and plant-dominant diets to mitigate risk of allograft dysfunction progression in kidney transplant recipients. *Curr Opin Nephrol Hypertens* (2024) 33(1):43–52. doi: 10.1097/MNH.0000000000000944
- Carrero JJ, González-Ortiz A, Avesani CM, Bakker SJL, Bellizzi V, Chauveau P, et al. Plant-based diets to manage the risks and complications of chronic kidney disease. *Nat Rev Nephrol* (2020) 16(9):525–42. doi: 10.1038/s41581-020-0297-2
- Gomes-Neto AW, Osté MCJ, Sotomayor CG, van den Berg E, Geleijnse JM, Berger SP, et al. Mediterranean style diet and kidney function loss in kidney transplant recipients. *Clin J Am Soc Nephrol* (2020) 15(2):238–46. doi: 10.2215/CJN.06710619
- Lau WP, Ng KP, Ganapathy SS, Tah PC, Ismail R, Jalalonmuhali M, et al. Prevalence rate of proteinuria and metabolic acidosis among kidney transplant recipients in a tertiary teaching hospital and its relationship to dietary intake. *Transpl Proc* (2022) 54(2):355–61. doi: 10.1016/j.transproceed.2021.12.019
- Cogan MG, Sargent JA, Yarbrough SG, Vincenti F, Amend WJ, Jr. Prevention of prednisone-induced negative nitrogen balance. Effect of dietary modification on urea generation rate in patients on hemodialysis receiving high-dose glucocorticoids. *Ann Intern Med* (1981) 95(2):158–61. doi: 10.7326/0003-4819-95-2-158
- Osté MCJ, Gomes-Neto AW, Corpeleijn E, Gans ROB, de Borst MH, van den Berg E, et al. Dietary approach to stop hypertension (DASH) diet and risk of renal function decline and all-cause mortality in renal transplant recipients. *Am J Transpl* (2018) 18(10):2523–33. doi: 10.1111/ajt.14707
- Said MY, Deetman PE, de Vries AP, Zelle DM, Gans ROB, Navis G, et al. Causal path analyses of the association of protein intake with risk of mortality and graft failure in renal transplant recipients. *Clin Transpl* (2015) 29(5):447–57. doi: 10.1111/ctr.12536
- Kim H, Caulfield LE, Garcia-Larsen V, Steffen LM, Grams ME, Coresh J, et al. Plant-based diets and incident CKD and kidney function. *Clin J Am Soc Nephrol* (2019) 14(5):682–91. doi: 10.2215/CJN.12391018
- Clegg DJ, Hill Gallant KM. Plant-based diets in CKD. *Clin J Am Soc Nephrol* (2019) 14(1):141–3. doi: 10.2215/CJN.08960718
- Chan S, Hawley CM, Pascoe EM, Cao C, Campbell SB, Campbell KL, et al. Prebiotic supplementation in kidney transplant recipients for preventing infections and gastrointestinal upset: a randomized controlled feasibility study. *J Ren Nutr* (2022) 32(6):718–25. doi: 10.1053/j.jrn.2022.02.006
- Joshi S, Shah S, Kalantar-Zadeh K. Adequacy of plant-based proteins in chronic kidney disease. *J Ren Nutr* (2019) 29(2):112–7. doi: 10.1053/j.jrn.2018.06.006
- Radić J, Vučković M, Gelemanović A, Kolak E, Bučan Nenadić D, Begović M, et al. Associations between advanced glycation end products, body composition and mediterranean diet adherence in kidney transplant recipients. *Int J Environ Res Public Health* (2022) 19(17):11060. doi: 10.3390/ijerph191711060
- Torreggiani M, Avesani CM, Contzen B, Cupisti A, Czaja-Stolc S, D'Alessandro C, et al. Dos and don'ts in kidney nutrition: practical considerations of a panel of experts on protein restriction and plant-based diets for patients living with chronic kidney disease. *Nutrients* (2025) 17(12):2002. doi: 10.3390/nu17122002
- Clinical practice guidelines for nutrition in chronic renal failure. K/DOQI, national kidney foundation. *Am J Kidney Dis*. (2000) 35(6):S17–S104. doi: 10.1053/ajkd.2000.v35.aajkd03517
- Barsotti G, Morelli E, Cupisti A, Meola M, Dani L, Giovannetti S. A low-nitrogen low-phosphorus vegan diet for patients with chronic renal failure. *Nephron* (1996) 74(2):390–4. doi: 10.1159/000189341
- Heo GY, Koh HB, Kim HJ, Kim KW, Jung CY, Kim HW, et al. Association of plant protein intake with risk of incident CKD: a UK biobank study. *Am J Kidney Dis* (2023) 82(6):687–97.e1. doi: 10.1053/j.ajkd.2023.05.007
- 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(4S):S117–S314. doi: 10.1016/j.kint.2023.10.018
- Stamler J. Toward a modern mediterranean diet for the 21st century. *Nutr Metab Cardiovasc Dis* (2013) 23(12):1159–62. doi: 10.1016/j.numecd.2013.08.002
- Sofi F, Abbate R, Gensini GF, Casini A. Accruing evidence on benefits of adherence to the mediterranean diet on health: an updated systematic review and meta-analysis. *Am J Clin Nutr* (2010) 92(5):1189–96. doi: 10.3945/ajcn.2010.29673
- Bach KE, Kelly JT, Palmer SC, Khalesi S, Strippoli GFM, Campbell KL. Healthy dietary patterns and incidence of CKD: a meta-analysis of cohort studies. *Clin J Am Soc Nephrol* (2019) 14(10):1441–9. doi: 10.2215/CJN.00530119
- Chauveau P, Aparicio M, Bellizzi V, Campbell K, Hong X, Johansson L, et al. Mediterranean diet as the diet of choice for patients with chronic kidney disease. *Nephrol Dial Transpl* (2018) 33(5):725–35. doi: 10.1093/ndt/gfx085
- Khatri M, Moon YP, Scarmeas N, Gu Y, Gardener H, Cheung K, et al. The association between a Mediterranean-style diet and kidney function in the northern manhattan study cohort. *Clin J Am Soc Nephrol* (2014) 9(11):1868–75. doi: 10.2215/CJN.01080114
- Fung TT, McCullough ML, Newby PK, Manson JE, Meigs JB, Rifai N, et al. Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction. *Am J Clin Nutr* (2005) 82(1):163–73. doi: 10.1093/ajcn.82.1.163
- Smidowicz A, Regula J. Effect of nutritional status and dietary patterns on human serum C-reactive protein and interleukin-6 concentrations. *Adv Nutr* (2015) 6(6):738–47. doi: 10.3945/an.115.009415
- Lin J, Fung TT, Hu FB, Curhan GC. Association of dietary patterns with albuminuria and kidney function decline in older white women: a subgroup analysis from the nurses' health study. *Am J Kidney Dis* (2011) 57(2):245–54. doi: 10.1053/j.ajkd.2010.09.027
- Siener R. Dietary treatment of metabolic acidosis in chronic kidney disease. *Nutrients* (2018) 10(4):512. doi: 10.3390/nu10040512
- Rebholz CM, Coresh J, Grams ME, Steffen LM, Anderson CAM, Appel LJ, et al. Dietary acid load and incident chronic kidney disease: results from the ARIC study. *Am J Nephrol* (2015) 42(6):427–35. doi: 10.1159/000443746
- Stachowska E, Wesolowska T, Olszewska M, Safranow K, Millo B, Domański L, et al. Elements of mediterranean diet improve oxidative status in blood of kidney graft recipients. *Br J Nutr* (2005) 93(3):345–52. doi: 10.1079/bjn20051374
- Schwingshackl L, Hoffmann G. Mediterranean dietary pattern, inflammation and endothelial function: a systematic review and meta-analysis of intervention trials. *Nutr Metab Cardiovasc Dis* (2014) 24(9):929–39. doi: 10.1016/j.numecd.2014.03.003
- Minelli P, Montinari MR. The mediterranean diet and cardioprotection: historical overview and current research. *J Multidiscip Healthc* (2019) 12:805–15. doi: 10.2147/JMDH.S219875
- Martínez-González MA, Gea A, Ruiz-Canela M. The mediterranean diet and cardiovascular health. *Circ Res* (2019) 124(5):779–98. doi: 10.1161/CIRCRESAHA.118.313348
- van Westing AC, Küpers LK, Geleijnse JM. Diet and kidney function: a literature review. *Curr Hypertens Rep* (2020) 22(2):14. doi: 10.1007/s11906-020-1020-1
- Safa K, Ohori S, Borges TJ, Uehara M, Batal I, Shimizu T, et al. Salt accelerates allograft rejection through Serum- and glucocorticoid-regulated Kinase-1-Dependent inhibition of regulatory T cells. *J Am Soc Nephrol* (2015) 26(10):2341–7. doi: 10.1681/ASN.2014090914
- Lari A, Sohoulis MH, Fatahi S, Cerqueira HS, Santos HO, Pourrajab B, et al. The effects of the dietary approaches to stop hypertension (DASH) diet on metabolic risk factors in patients with chronic disease: a systematic review and meta-analysis of randomized controlled trials. *Nutr Metab Cardiovasc Dis* (2021) 31(10):2766–78. doi: 10.1016/j.numecd.2021.05.030
- Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH collaborative research group. *N Engl J Med* (1997) 336(16):1117–24. doi: 10.1056/NEJM199704173361601
- Sacks FM, Svetkey LP, Vollmer WM, Appel LJ, Bray GA, Harsha D, et al. Effects on blood pressure of reduced dietary sodium and the dietary approaches to stop hypertension (DASH) diet. DASH-sodium collaborative research group. *N Engl J Med* (2001) 344(1):3–10. doi: 10.1056/NEJM200101043440101
- Weir MR, Burgess ED, Cooper JE, Fenves AZ, Goldsmith D, McKay D, et al. Assessment and management of hypertension in transplant patients. *J Am Soc Nephrol* (2015) 26(6):1248–60. doi: 10.1681/ASN.2014080834
- Gutiérrez OM, Muntner P, Rizk DV, McClellan WM, Warnock DG, Newby PK, et al. Dietary patterns and risk of death and progression to ESRD in individuals with CKD: a cohort study. *Am J Kidney Dis* (2014) 64(2):204–13. doi: 10.1053/j.ajkd.2014.02.013
- Cyrino LG, Galperin J, Moore L, Borgi L, Riella LV. A narrative review of dietary approaches for kidney transplant patients. *Kidney Int Rep* (2021) 6(7):1764–74. doi: 10.1016/j.ekir.2021.04.009
- Kalantar-Zadeh K, Moore LW. Does kidney longevity mean healthy vegan food and less meat or is any low-protein diet good enough? *J Ren Nutr* (2019) 29(2):79–81. doi: 10.1053/j.jrn.2019.01.008
- Kalantar-Zadeh K, Fouque D. Nutritional management of chronic kidney disease. *N Engl J Med* (2017) 377(18):1765–76. doi: 10.1056/NEJMra1700312
- Baragetti I, De Simone I, Biazzi C, Buzzi L, Ferrario F, Luise MC, et al. The low-protein diet for chronic kidney disease: 8 years of clinical experience in a nephrology ward. *Clin Kidney J* (2019) 13(2):253–60. doi: 10.1093/cjks/sfz141
- Hahn D, Hodson EM, Fouque D. Low protein diets for non-diabetic adults with chronic kidney disease. *Cochrane Database Syst Rev* (2018) 10(10):CD001892. doi: 10.1002/14651858.CD001892.pub4
- Jhee JH, Kee YK, Park S, Kim H, Park JT, Han SH, et al. High-protein diet with renal hyperfiltration is associated with rapid decline rate of renal function: a community-based prospective cohort study. *Nephrol Dial Transpl* (2020) 35(1):98–106. doi: 10.1093/ndt/gfz115

46. Lin J, Hu FB, Curhan GC. Associations of diet with albuminuria and kidney function decline. *Clin J Am Soc Nephrol* (2010) 5(5):836–43. doi: 10.2215/CJN.08001109
47. Jeon HJ, Kim YC, Park S, Kim CT, Ha J, Han DJ, et al. Association of serum phosphorus concentration with mortality and graft failure among kidney transplant recipients. *Clin J Am Soc Nephrol* (2017) 12(4):653–62. doi: 10.2215/CJN.07090716
48. van Londen M, Aarts BM, Deetman PE, van der Weijden J, Eisenga MF, Navis G, et al. Post-transplant hypophosphatemia and the risk of death-censored graft failure and mortality after kidney transplantation. *Clin J Am Soc Nephrol* (2017) 12(8):1301–10. doi: 10.2215/CJN.10270916
49. Connolly GM, Cunningham R, McNamee PT, Young IS, Maxwell AP. Elevated serum phosphate predicts mortality in renal transplant recipients. *Transplantation* (2009) 87(7):1040–4. doi: 10.1097/TP.0b013e31819cd122
50. Stevens KK, Morgan IR, Patel RK, Geddes CC, Mark PB, Jardine AG, et al. Serum phosphate predicts mortality at one year after deceased donor renal transplantation. *Clin Transpl* (2011) 25(2):E199–E204. doi: 10.1111/j.1399-0012.2011.01400.x
51. Moe SM, Zidehsari MP, Chambers MA, Jackman LA, Radcliffe JS, Trevino LL, et al. Vegetarian compared with meat dietary protein source and phosphorus homeostasis in chronic kidney disease. *Clin J Am Soc Nephrol* (2011) 6(2):257–64. doi: 10.2215/CJN.05040610
52. St-Jules DE, Goldfarb DS, Seveck MA. Nutrient non-equivalence: does restricting high-potassium plant foods help to prevent hyperkalemia in hemodialysis patients? *J Ren Nutr* (2016) 26(5):282–7. doi: 10.1053/j.jrn.2016.02.005
53. Noori N, Kalantar-Zadeh K, Kovesdy CP, Murali SB, Bross R, Nissenson AR, et al. Dietary potassium intake and mortality in long-term hemodialysis patients. *Am J Kidney Dis* (2010) 56(2):338–47. doi: 10.1053/j.ajkd.2010.03.022
54. Black AP, Anjos JS, Cardozo L, Carmo FL, Dolenga CJ, Nakao LS, et al. Does low-protein diet influence the uremic toxin serum levels from the gut microbiota in nondialysis chronic kidney disease patients? *J Ren Nutr* (2018) 28(3):208–14. doi: 10.1053/j.jrn.2017.11.007
55. Camerotto C, Cupisti A, D'Alessandro C, Muzio F, Gallieni M. Dietary fiber and gut microbiota in renal diets. *Nutrients* (2019) 11(9):2149. doi: 10.3390/nu11092149
56. Demirci BG, Tutal E, Eminsoy IO, Kulah E, Sezer S. Dietary fiber intake: its relation with glycation end products and arterial stiffness in end-stage renal disease patients. *J Ren Nutr* (2019) 29(2):136–42. doi: 10.1053/j.jrn.2018.08.007
57. Chauveau P, Combe C, Fouque D, Aparicio M. Vegetarianism: advantages and drawbacks in patients with chronic kidney diseases. *J Ren Nutr* (2013) 23(6):399–405. doi: 10.1053/j.jrn.2013.08.004
58. Nolte Fong JV, Moore LW. Nutrition trends in kidney transplant recipients: the importance of dietary monitoring and need for evidence-based recommendations. *Front Med (Lausanne)* (2018) 5:302. doi: 10.3389/fmed.2018.00302
59. Tantisattamo E, Kalantar-Zadeh K, Molnar MZ. Nutritional and dietary interventions to prolong renal allograft survival after kidney transplantation. *Curr Opin Nephrol Hypertens* (2022) 31(1):6–17. doi: 10.1097/MNH.0000000000000757
60. Rosman JB, ter Wee PM, Meijer S, Piers-Becht TP, Sluiter WJ, Donker AJ. Prospective randomised trial of early dietary protein restriction in chronic renal failure. *Lancet* (1984) 2(8415):1291–6. doi: 10.1016/s0140-6736(84)90818-3
61. Rosenberg ME, Swanson JE, Thomas BL, Hostetter TH. Glomerular and hormonal responses to dietary protein intake in human renal disease. *Am J Physiol* (1987) 253(6):F1083–F1090. doi: 10.1152/ajprenal.1987.253.6.F1083
62. Ihle BU, Becker GJ, Whitworth JA, Charlwood RA, Kincaid-Smith PS. The effect of protein restriction on the progression of renal insufficiency. *N Engl J Med* (1989) 321(26):1773–7. doi: 10.1056/NEJM198912283212601
63. Rosenberg ME, Chmielewski D, Hostetter TH. Effect of dietary protein on rat renin and angiotensinogen gene expression. *J Clin Invest* (1990) 85(4):1144–9. doi: 10.1172/JCI114546
64. Paller MS, Hostetter TH. Dietary protein increases plasma renin and reduces pressor reactivity to angiotensin II. *Am J Physiol* (1986) 251(1 Pt 2):F34–F39. doi: 10.1152/ajprenal.1986.251.1.F34
65. Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. *Am J Physiol* (1981) 241(1):F85–F93. doi: 10.1152/ajprenal.1981.241.1.F85
66. Nath KA, Kren SM, Hostetter TH. Dietary protein restriction in established renal injury in the rat. Selective role of glomerular capillary pressure in progressive glomerular dysfunction. *J Clin Invest* (1986) 78(5):1199–205. doi: 10.1172/JCI112703
67. Nath KA, Croatt AJ, Hostetter TH. Oxygen consumption and oxidant stress in surviving nephrons. *Am J Physiol* (1990) 258(5 Pt 2):F1354–F1362. doi: 10.1152/ajprenal.1990.258.5.F1354
68. Kalantar-Zadeh K, Joshi S, Schlueter R, Cooke J, Brown-Tortorici A, Donnelly M, et al. Plant-dominant low-protein diet for conservative management of chronic kidney disease. *Nutrients* (2020) 12(7):1931. doi: 10.3390/nu12071931
69. Kalantar-Zadeh K, Rhee CM, Joshi S, Brown-Tortorici A, Kramer HM. Medical nutrition therapy using plant-focused low-protein meal plans for management of chronic kidney disease in diabetes. *Curr Opin Nephrol Hypertens* (2022) 31(1):26–35. doi: 10.1097/MNH.0000000000000761
70. Whittier FC, Evans DH, Dutton S, Ross G, Luger A, Nolph KD, et al. Nutrition in renal transplantation. *Am J Kidney Dis* (1985) 6(6):405–11. doi: 10.1016/s0272-6386(85)80103-7
71. Kosoku A, Iwai T, Ishihara T, Kabei K, Nishide S, Maeda K, et al. Influence of protein intake on the changes in skeletal muscle mass after kidney transplantation. *Clin Nutr* (2022) 41(9):1881–8. doi: 10.1016/j.clnu.2022.07.028
72. Górska M, Kurnatowska I. Nutrition disturbances and metabolic complications in kidney transplant recipients: etiology, methods of assessment and Prevention-A review. *Nutrients* (2022) 14(23):4996. doi: 10.3390/nu14234996
73. Sotler R, Poljšak B, Dahmane R, Jukić T, Pavan Jukić D, Rotim C, et al. Prooxidant activities of antioxidants and their impact on health. *Acta Clin Croat* (2019) 58(4):726–36. doi: 10.20471/acc.2019.58.04.20
74. Dennis JM, Witting PK. Protective role for antioxidants in acute kidney disease. *Nutrients* (2017) 9(7):718. doi: 10.3390/nu9070718
75. Nunford EC, Riddell MC. The metabolic implications of glucocorticoids in a high-fat diet setting and the counter-effects of exercise. *Metabolites* (2016) 6(4):44. doi: 10.3390/metabo6040044
76. Geer EB, Islam J, Buettner C. Mechanisms of glucocorticoid-induced insulin resistance: focus on adipose tissue function and lipid metabolism. *Endocrinol Metab Clin North Am* (2014) 43(1):75–102. doi: 10.1016/j.ecl.2013.10.005
77. Athinarayanan SJ, Roberts CGP, Vangala C, Shetty GK, McKenzie AL, Weimbs T, et al. The case for a ketogenic diet in the management of kidney disease. *BMJ Open Diabetes Res Care* (2024) 12(2):e004101. doi: 10.1136/bmjdr-2024-004101
78. Leow ZZX, Gueflf KJ, Davis EA, Jones TW, Fournier PA. The glycaemic benefits of a very-low-carbohydrate ketogenic diet in adults with type 1 diabetes mellitus May be opposed by increased hypoglycaemia risk and dyslipidaemia. *Diabet Med* (2018). doi: 10.1111/dme.13663
79. Krebs JD, Parry SA, Cresswell P, Reynolds AN, Hanna A, Haeusler S. A randomised trial of the feasibility of a low carbohydrate diet vs standard carbohydrate counting in adults with type 1 diabetes taking body weight into account. *Asia Pac J Clin Nutr* (2016) 25(1):78–84. doi: 10.6133/apjcn.2016.25.1.11
80. Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, et al. Ketosis and appetite-mediating nutrients and hormones after weight loss. *Eur J Clin Nutr* (2013) 67(7):759–64. doi: 10.1038/ejcn.2013.90
81. Veldhorst M, Smeets A, Soenen S, Hochstenbach-Waelen A, Hursel R, Diepvens K, et al. Protein-induced satiety: effects and mechanisms of different proteins. *Physiol Behav* (2008) 94(2):300–7. doi: 10.1016/j.physbeh.2008.01.003
82. Heinemann L. Variability of insulin absorption and insulin action. *Diabetes Technol Ther* (2002) 4(5):673–82. doi: 10.1089/152091502320798312
83. D'Alessandro C, Giannese D, Piccoli GB, Panichi V, Cupisti A. Ketogenic diets in chronic kidney disease patients: a review for skeptics by skeptics. *J Nephrol* (2025) 38(6):1541–56. doi: 10.1007/s40620-025-02285-7
84. Williams MS, Turos E. The chemistry of the ketogenic diet: updates and opportunities in organic synthesis. *Int J Mol Sci* (2021) 22(10):5230. doi: 10.3390/ijms22105230
85. Gershuni VM, Yan SL, Medici V. Nutritional ketosis for weight management and reversal of metabolic syndrome. *Curr Nutr Rep* (2018) 7(3):97–106. doi: 10.1007/s13668-018-0235-0
86. Zhu H, Bi D, Zhang Y, Kong C, Du J, Wu X, et al. Ketogenic diet for human diseases: the underlying mechanisms and potential for clinical implementations. *Signal Transduct Target Ther* (2022) 7(1):11. doi: 10.1038/s41392-021-00831-w
87. Cahill GF Jr, Aoki TT, Ruderman NB. Ketosis. *Trans Am Clin Climatol Assoc* (1973) 84:184–202.
88. Fukao T, Lopaschuk GD, Mitchell GA. Pathways and control of ketone body metabolism: on the fringe of lipid biochemistry. *Prostaglandins Leukot Essent Fatty Acids* (2004) 70(3):243–51. doi: 10.1016/j.plefa.2003.11.001
89. Ellenbroek JH, van Dijk L, Töns HA, Rabelink TJ, Carlotti F, Ballieux BEPB, et al. Long-term ketogenic diet causes glucose intolerance and reduced  $\beta$ - and  $\alpha$ -cell mass but no weight loss in mice. *Am J Physiol Endocrinol Metab* (2014) 306(5):E552–E558. doi: 10.1152/ajpendo.00453.2013
90. Seidelmann SB, Claggett B, Cheng S, Henglin M, Shah A, Steffen LM, et al. Dietary carbohydrate intake and mortality: a prospective cohort study and meta-analysis. *Lancet Public Health* (2018) 3(9):e419–e428. doi: 10.1016/S2468-2667(18)30135-X
91. Bravata DM, Sanders L, Huang J, Krumholz HM, Olkin I, Gardner CD, et al. Efficacy and safety of low-carbohydrate diets: a systematic review. *JAMA* (2003) 289(14):1837–50. doi: 10.1001/jama.289.14.1837
92. Ludwig DS, Hu FB, Tappy L, Brand-Miller J. Dietary carbohydrates: role of quality and quantity in chronic disease. *BMJ* (2018) 361:k2340. doi: 10.1136/bmj.k2340
93. Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet* (2019) 393(10170):434–45. doi: 10.1016/S0140-6736(18)31809-9
94. Giugliano D, Maiorino MI, Bellastella G, Esposito K. More sugar? No, thank you! the elusive nature of low carbohydrate diets. *Endocrine* (2018) 61(3):383–7. doi: 10.1007/s12020-018-1580-x

95. Sharif A, Moore R, Baboolal K. Influence of lifestyle modification in renal transplant recipients with postprandial hyperglycemia. *Transplantation* (2008) 85(3):353–8. doi: 10.1097/TP.0b013e3181605ebf
96. Wissing KM, Pipeleers L. Obesity, metabolic syndrome and diabetes mellitus after renal transplantation: prevention and treatment. *Transpl Rev (Orlando)* (2014) 28(2): 37–46. doi: 10.1016/j.trre.2013.12.004
97. Gruessner RW. Tacrolimus in pancreas transplantation: a multicenter analysis. Tacrolimus pancreas transplant study group. *Clin Transpl* (1997) 11(4):299–312.
98. Salahudeen AK, Hostetter TH, Raatz SK, Rosenberg ME. Effects of dietary protein in patients with chronic renal transplant rejection. *Kidney Int* (1992) 41(1):183–90. doi: 10.1038/ki.1992.25
99. Bernardi A, Biasia F, Pati T, Piva M, D'Angelo A, Bucciante G. Long-term protein intake control in kidney transplant recipients: effect in kidney graft function and in nutritional status. *Am J Kidney Dis* (2003) 41(3 Suppl. 1):S146–S152. doi: 10.1053/ajkd.2003.50105
100. van den Berg E, Engberink MF, Brink EJ, van Baak MA, Gans ROB, Navis G, et al. Dietary protein, blood pressure and renal function in renal transplant recipients. *Br J Nutr* (2013) 109(8):1463–70. doi: 10.1017/S0007114512003455
101. Deetman PE, Said MY, Kromhout D, Dullaart RPF, Kootstra-Ros JE, Sanders JSF, et al. Urinary urea excretion and long-term outcome after renal transplantation. *Transplantation* (2015) 99(5):1009–15. doi: 10.1097/TP.0000000000000464
102. Lee JR, Muthukumar T, Dadhania D, Toussaint NC, Ling L, Pamer E, et al. Gut microbial community structure and complications after kidney transplantation: a pilot study. *Transplantation* (2014) 98(7):697–705. doi: 10.1097/TP.0000000000000370
103. Hobby GP, Karaduta O, Dusio GF, Singh M, Zybailov BL, Arthur JM. Chronic kidney disease and the gut microbiome. *Am J Physiol Ren Physiol* (2019) 316(6): F1211–F1217. doi: 10.1152/ajprenal.00298.2018
104. Winichakoon P, Chaiwarith R, Chattipakorn N, Chattipakorn SC. Impact of gut microbiota on kidney transplantation. *Transpl Rev (Orlando)* (2022) 36(1):100668. doi: 10.1016/j.trre.2021.100668
105. Meijers BK, De Loo H, Bammens B, Verbeke K, Vanrenterghem Y, Evenepoel P. p-Cresyl sulfate and indoxyl sulfate in hemodialysis patients. *Clin J Am Soc Nephrol* (2009) 4(12):1932–8. doi: 10.2215/CJN.02940509
106. Canyelles M, Borràs C, Rotllan N, Tondo M, Escolà-Gil JC, Blanco-Vaca F. Gut microbiota-derived TMAO: a causal factor promoting atherosclerotic cardiovascular disease? *Int J Mol Sci* (2023) 24(3):1940. doi: 10.3390/ijms24031940



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# Normothermic regional perfusion in controlled donation after circulatory death liver transplantation: an updated systematic review and meta-analysis versus non-NRP and brain-death donors

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Normothermic regional perfusion (NRP) may mitigate ischemic injury in controlled donation after circulatory death (cDCD) liver transplantation. We updated the most recent meta-analysis (search closed June 2023) by incorporating subsequent evidence and comparing NRP with non-NRP cDCD and with donation after brain death (DBD). We performed a systematic review of five databases and random-effects meta-analyses; dichotomous outcomes were pooled as risk ratios, the Mantel-Haenszel estimator was preferred for rare events, pooled incidences used a logit-binomial model, risk of bias was assessed with ROBINS-I and certainty with GRADE. Twenty observational studies were included (1776 NRP-cDCD liver recipients). Versus non-NRP cDCD, NRP reduced ischemic cholangiopathy (risk ratio 0.16, 95% confidence interval 0.09–0.28; number needed to treat 9), graft loss (0.41, 0.32–0.54), recipient death (0.46, 0.34–0.62), hepatic artery thrombosis (0.51, 0.30–0.85), other biliary complications (0.49, 0.32–0.73) and primary non-function (0.51, 0.28–0.94); these reductions persisted after excluding grafts managed with *ex situ* machine perfusion. Versus DBD, no outcome differed significantly. Pooled ischemic cholangiopathy incidence was 1.8%, 12.7% and 1.2% with NRP, non-NRP and DBD. NRP markedly reduces ischemic cholangiopathy and improves other outcomes versus non-NRP cDCD, approaching DBD; the evidence remains observational, with moderate-to-serious risk of bias.

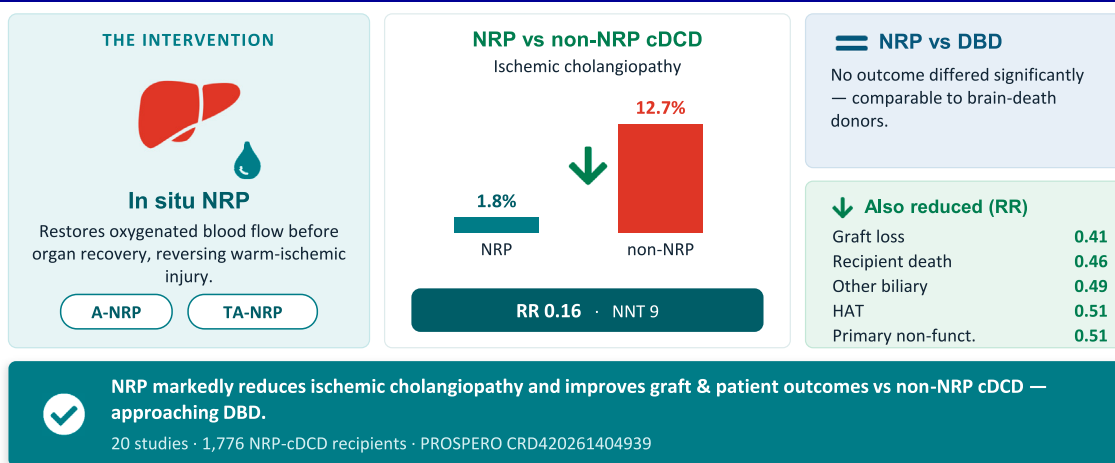
**Systematic Review Registration:** [https://www.crd.york.ac.uk/prospero/display\\_record.php?ID=CRD420261404939](https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD420261404939), identifier PROSPERO CRD420261404939.

### KEYWORDS

normothermic regional perfusion, donation after circulatory death, liver transplantation, ischemic cholangiopathy, graft survival, biliary complications



## Normothermic Regional Perfusion in Controlled Donation After Circulatory Death Liver Transplantation: An Updated Systematic Review and Meta-Analysis Versus Non-NRP and Brain-Death Donors



Ramírez-Esteban, et al. · *Transpl. Int.* 2026



GRAPHICAL ABSTRACT

## Introduction

Liver transplantation from controlled donation after circulatory death (cDCD) is now an established means of enlarging the donor pool, but the mandatory interval of warm ischemia that follows withdrawal of life-sustaining treatment and circulatory arrest predisposes to ischemic cholangiopathy (IC), early allograft dysfunction (EAD) and biliary and vascular complications at rates exceeding those of donation after brain death (DBD) [1–3]. IC—non-anastomotic biliary strictures arising in a graft with a patent hepatic artery—is the most feared of these, frequently irreversible, and a leading cause of graft loss and retransplantation in cDCD.

Normothermic regional perfusion (NRP) addresses this injury at its origin. By restoring oxygenated, normothermic blood flow to the abdominal organs *in situ* before procurement, NRP reverses the warm-ischemic insult, replenishes cellular energy substrates and permits functional assessment of the graft before acceptance [3, 4]. The technique may be confined to the abdomen (abdominal NRP, A-NRP) or extended to the thorax for combined cardi thoracic recovery (thoracoabdominal NRP, TA-NRP). It is distinct from *ex situ* machine perfusion, in which the explanted organ is perfused on a device; the two can also be combined sequentially.

*situ* machine perfusion, in which the explanted organ is perfused on a device; the two can also be combined sequentially.

NRP programs matured first in Europe—Spain [1, 2, 5–8], the United Kingdom [3, 9], France [10, 11], the Netherlands [12] and Sweden [13]—and have since expanded across the United States, where thoracoabdominal recovery for simultaneous heart procurement has driven uptake [14–18]. Single-center and registry series have consistently reported lower IC and improved graft survival with NRP, and several matched analyses suggested that NRP-cDCD outcomes approach those of DBD [5, 6, 10]. Yet the evidence is fragmented: comparators differ (super-rapid recovery with static cold storage [SCS] or *ex situ* normothermic machine perfusion [NMP]), cohorts overlap (national registries encompassing single-center series; clustered registry data), outcome definitions are inconsistent, and most series are small and single-center.

The most recent systematic review and meta-analysis pooled 11 studies (search closed 9 June 2023) and found that, versus non-NRP cDCD, NRP reduced IC, primary non-function (PNF), graft loss and recipient death, with no difference versus DBD [19]. A subsequent broader systematic review and meta-analysis compared hypothermic, normothermic and regional machine-perfusion strategies and reached concordant conclusions on ischemic cholangiopathy [20], but it evaluated NRP as one of three perfusion techniques and reported only two NRP outcomes, rather than focusing specifically on NRP in cDCD liver transplantation across the full outcome set of the reference review that we update [19]. Since that search the evidence base has nearly doubled, propelled chiefly by large North-American cohorts and registry analyses [15–18]. We therefore performed

**Abbreviations:** A-NRP, abdominal normothermic regional perfusion; CI, confidence interval; cDCD, controlled donation after circulatory death; DBD, donation after brain death; EAD, early allograft dysfunction; GRADE, Grading of Recommendations Assessment, Development and Evaluation; HAT, hepatic artery thrombosis; IC, ischemic cholangiopathy; NMP, normothermic machine perfusion; NNT, number needed to treat; NRP, normothermic regional perfusion; PNF, primary non-function; ROBINS-I, Risk Of Bias In Non-randomised Studies of Interventions; RR, risk ratio; SCS, static cold storage; SRR, super-rapid recovery; TA-NRP, thoracoabdominal normothermic regional perfusion.

an updated systematic review and meta-analysis incorporating all subsequent evidence, applying explicit anti-duplication rules for overlapping cohorts and contemporary statistical methods, to deliver the most current and robust estimate of the effect of NRP in cDCD liver transplantation, and to clarify outcomes that remained uncertain in the previous synthesis.

## Materials and methods

### Protocol, eligibility, and search

The review was conducted and reported in accordance with the PRISMA 2020 statement [21] and updated the protocol of the reference meta-analysis [19]; it was prospectively registered (PROSPERO CRD420261404939) and additionally followed the MOOSE recommendations for meta-analyses of observational studies. The PICO framework comprised adult ( $\geq 18$  years) recipients of a liver graft from Maastricht-III controlled donation after circulatory death (population); graft recovery by *in situ* NRP, whether abdominal (A-NRP) or thoracoabdominal (TA-NRP) (intervention); and two comparators analyzed separately—non-NRP cDCD procurement (super-rapid recovery with static cold storage, with or without *ex situ* HOPE or NMP) and donation after brain death (comparators). Primary outcomes were ischemic cholangiopathy, primary non-function and recipient death; secondary outcomes were graft loss, early allograft dysfunction (Olthoff criteria [22]), hepatic artery thrombosis, other biliary complications (anastomotic strictures and leaks), length of stay and graft utilization.

Eligible studies were randomized trials or comparative cohort studies (prospective or retrospective) reporting at least one primary outcome for NRP-cDCD versus non-NRP cDCD and/or DBD, in adults, published in English or Spanish, with no start-date restriction. We excluded uncontrolled (uDCD) donation without disaggregable cDCD data, studies without a comparator, purely technical descriptions, animal studies, case reports, and conference abstracts with insufficient data, and—consistent with the reference review—grafts from jurisdictions imposing a mandatory no-touch (stand-off) period exceeding 5 min (for example, Italy, 20 min). When cohorts overlapped, the most complete or most recent publication was retained and the decision documented.

Five databases were searched: MEDLINE (via PubMed), Embase, Scopus, the Cochrane Library (CENTRAL) and the Web of Science Core Collection, complemented by hand-searching the reference lists of recent reviews and of the included studies. The strategy combined three concept blocks with the Boolean AND—(i) normothermic regional perfusion, (ii) circulatory-death donation, and (iii) liver and transplantation—deliberately omitting comparator and outcome blocks to preserve sensitivity, with both resolved at screening; the term “ECMO” was admitted only when co-occurring with circulatory-death or donation terms, to retrieve the foundational NRP literature without the noise of cardiorespiratory-support ECMO. Records were managed in Rayyan, de-duplicated, and screened in duplicate at the title/

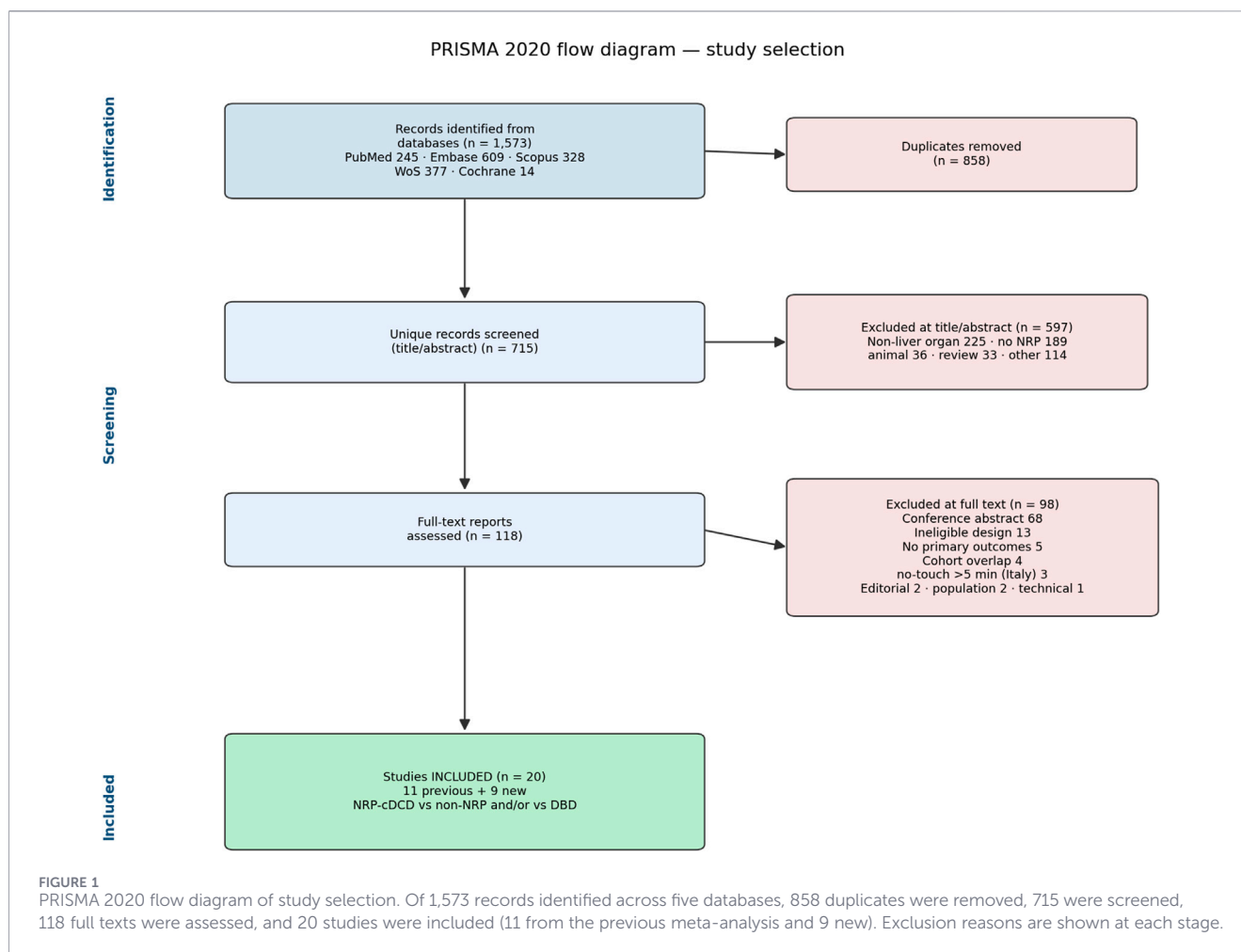
abstract and full-text levels, with disagreements resolved by an arbiter. The full search string for each database and the per-database record counts are provided in the [Supplementary Material](#).

### Data extraction and risk of bias

Two reviewers extracted data independently using a standardized, piloted form that captured study characteristics (design, period, center or registry, country), donor data (age, NRP type and duration, ischemia times), recipient data (age, MELD), and every predefined outcome with its original definition. Extraction was subjected to multichannel verification—triangulation of figures, tables and text; verification of column order against reported percentages (several primary studies list the comparator or DBD arm in the first column); recomputation of every percentage from raw counts; 1:1 reconciliation of sample sizes against the previous meta-analysis; and a second independent extraction. Ischemic cholangiopathy was defined as non-anastomotic biliary strictures in a graft with a patent hepatic artery; “other biliary complications” as anastomotic strictures plus leaks, excluding ischemic cholangiopathy and hepatic artery thrombosis. Risk of bias was assessed independently by two reviewers with ROBINS-I [23], with disagreements resolved by an arbiter and the results displayed as a traffic-light plot; no randomized trial was identified. The certainty of evidence for each outcome was rated with GRADE [24]. As a final quality-control step, an expert clinician performed a final clinical reading of the extracted dataset and of the complete manuscript to confirm the clinical plausibility and internal consistency of all outcomes and conclusions.

### Statistical analysis

The two comparisons were analyzed independently, and abdominal and thoracoabdominal NRP were grouped as “NRP,” as in the reference review. Dichotomous outcomes were pooled as risk ratios (RR) using DerSimonian-Laird random-effects models [25], cross-checked with REML and with Hartung-Knapp; a 0.5 continuity correction was applied only to studies with a zero cell. For rare-event outcomes the Mantel-Haenszel estimator without continuity correction was preferred, with the Peto odds ratio in support, per Cochrane guidance [26]; this mattered for ischemic cholangiopathy versus DBD, where the inverse-variance estimate (RR 1.21) was unstable because of multiple double-zero studies, whereas the Mantel-Haenszel (0.70), Peto (0.70), crude (0.79) and drop-zero (0.91) estimates were concordant and non-significant. Recipient death and graft loss were pooled as RR from counts, too few hazard ratios being available to pool, with individual hazard ratios described narratively. Pooled incidences used a logit-binomial random-effects model, which is more robust than the Freeman-Tukey transformation under high heterogeneity [27]; the latter is reported only as a cross-check. Heterogeneity was summarized with  $I^2$ ,  $\tau^2$  and 95% prediction intervals computed with the t-distribution ( $k-2$  degrees of freedom) [28], and continuous outcomes were converted from medians to means by the method of Wan et al. [29]. Prespecified sensitivity (fixed versus random effects, Mantel-Haenszel and Peto, drop-zero, and



exclusion of the salvage study and of higher-risk studies) and subgroup (comparator type, region, and use of *ex situ* machine perfusion) analyses were performed, complemented by leave-one-out and Baujat influence diagnostics. To address potential confounding by *ex situ* machine perfusion (normothermic machine perfusion [NMP] or hypothermic oxygenated perfusion [HOPE]), an additional analysis restricted the NRP versus non-NRP comparison to grafts managed without *ex situ* perfusion in either arm; on full-text re-review, one study whose comparator was entirely NMP [11] and one in which dual HOPE was used in both arms [12] were excluded, and a three-arm study contributed through its static-cold-storage arm only, with its NMP arm removed [9]. Publication bias was not formally tested because every analysis included fewer than 10 studies [26]; contour-enhanced funnel plots are provided for completeness (Supplementary Figure S2). Overlapping cohorts were resolved with predefined hierarchical rules applied to each outcome-by-comparison pair: each study competed only within its comparison; national registries served as anchors; the 11 previously included studies were retained; and the new anti-duplication was applied chiefly to the 9 added studies. For GRADE, observational studies began at low certainty and were downgraded for risk of bias, inconsistency ( $I^2 > 60\%$ ) or imprecision (a confidence interval crossing 1), and upgraded for a large ( $RR \leq$

0.55) or very large ( $RR \leq 0.20$ ) effect, applied cautiously given possible residual confounding. All analyses were reproduced independently in R/metafor and in Python with exact agreement; the complete statistical and anti-duplication decisions, and the per-study data (Supplementary Table S2), are reported in the Supplementary Material.

## Results

### Study selection and characteristics

Of 1,573 records identified, 858 duplicates were removed, 715 were screened, 118 full texts were assessed and 20 studies were included (11 from the previous meta-analysis by Mastrovangelis et al. [19] and 9 published subsequently); 98 reports were excluded with documented reasons (Figure 1) [19]. The included studies comprised 1776 NRP-cDCD liver recipients across the 20 included studies (1,512 in the 16 studies contributing to the meta-analysis) and their non-NRP and DBD comparators across Europe (predominantly Spain) and North America, and ranged from single-center series to national registry cohorts (Table 1). All 20 studies contributed to the qualitative synthesis, whereas 16 contributed to the quantitative synthesis

TABLE 1 Characteristics of the 20 included studies.

| Study                      | Country     | Design                  | Comparison                              | NRP/<br>comparator (n) | Pooled                |
|----------------------------|-------------|-------------------------|-----------------------------------------|------------------------|-----------------------|
| Hessheimer 2022 [1]        | Spain       | National registry       | vs. cDCD-SRR/SCS                        | 545/258                | Yes                   |
| Gaurav 2022 [9]            | UK          | Single-center           | vs. cDCD-SCS and vs. cDCD-NMP (3-arm)   | 69/97 (SCS) + 67 (NMP) | Yes                   |
| Mohkam 2022 [11]           | France/UK   | Multicenter, PSM        | vs. cDCD-NMP                            | 68/34                  | Yes                   |
| Schurink 2022 [12]         | Netherlands | Single-center (salvage) | vs. cDCD-SCS and vs. DBD (3-arm, DHOPE) | 20/49/81               | Yes*§                 |
| Rodríguez-Sanjuán 2019 [5] | Spain       | Single-center           | vs. DBD                                 | 11/51                  | Yes                   |
| Miñambres 2020 [2]         | Spain       | Multicenter             | vs. DBD                                 | 16/29                  | Yes                   |
| Savvier 2020 [10]          | France      | Multicenter             | vs. DBD                                 | 50/100                 | Yes                   |
| Ruiz 2021 [6]              | Spain       | Single-center, matched  | vs. DBD                                 | 100/200                | Yes                   |
| Viguera 2021 [7]           | Spain       | Multicenter             | vs. DBD                                 | 144/447                | Yes                   |
| Fernández 2022 [30]        | Spain       | Single-center, PSM†     | vs. DBD                                 | 22/51                  | Yes                   |
| Rodríguez 2022 [8]         | Spain       | Single-center           | vs. DBD                                 | 39/78                  | Yes                   |
| Brubaker 2024 [16]         | USA         | Multicenter             | vs. cDCD-SRR/SCS                        | 106/136                | Yes                   |
| Croome 2025 [17]           | USA         | Multicenter             | vs. cDCD-SRR/SCS                        | 62/297                 | Yes                   |
| Campo-Cañaveral 2023 [31]  | Spain       | National (lung + liver) | vs. DBD                                 | 145/1,162              | Yes                   |
| Bluhme 2024 [13]           | Sweden      | National pilot, matched | vs. DBD                                 | 18/28                  | Yes                   |
| Bababekov 2025 [18]        | USA         | Single-center           | vs. cDCD-SRR/SCS                        | 97/79‡                 | Yes                   |
| Bekki 2023 [14]            | USA         | Registry (UNOS)         | vs. non-NRP cDCD (registry)             | 24/1,267               | No (incidence NR)     |
| Wisel 2023 [15]            | USA         | Registry (UNOS)         | vs. non-NRP cDCD (registry)             | 133/1,219              | No (NRP not isolated) |
| Secanella 2023 [32]        | Spain       | Multicenter, matched    | TA-NRP vs. A-NRP                        | 6/12                   | No (technique)        |
| Puttappa 2025 [29]         | UK          | Single-center           | vs. cDCD-SCS or -NMP                    | 101/137                | No (overlaps Gaurav)  |

PSM, propensity-score matching; SCS, static cold storage; NMP, normothermic machine perfusion; SRR, super-rapid recovery; NR, not reported. \*Salvage study, included with caution (sensitivity analysis). †NRP, subgroup (n = 22) of a mixed cDCD, cohort. ‡Numbers are recipients transplanted; 86 NRP, and 74 comparator recipients reached 6-month follow-up (the denominator for ischemic cholangiopathy). §Dual HOPE, was used in 25% of NRP, and 40% of comparator grafts; this study was excluded from the *ex situ* perfusion-free sensitivity analysis, in which the three-arm SCS/NRP/NMP, study contributed through its static-cold-storage arm only. Comparator groups: cDCD-SRR/SCS, conventional controlled DCD, pathway (super-rapid recovery followed by static cold storage); cDCD-NMP, *ex situ* normothermic machine perfusion; DHOPE, dual hypothermic oxygenated perfusion; DBD, donation after brain death. In three-arm studies (Gaurav; Schurink) NRP, was compared with each comparator arm separately, with arm sizes shown. In registry studies (Bekki; Wisel) non-NRP cDCD, denotes any non-NRP, procurement, not separable by preservation method.

(meta-analysis); the remaining four were retained descriptively but excluded from pooling—two US registry analyses in which NRP could not be isolated or incidence was unreported [14, 15], one Cambridge cohort overlapping an included study [32], and one technique-comparison study [31]. Throughout, non-NRP cDCD comparators comprised the conventional pathway of super-rapid recovery followed by static cold storage (SRR/SCS) unless a study used *ex situ* machine perfusion—normothermic (NMP) or hypothermic oxygenated (HOPE)—which is specified in Table 1.

## Risk of bias

On ROBINS-I, no study was at low risk of bias; 7 were at moderate and 13 at serious risk, driven chiefly by confounding (12/

20 serious—inherent to non-randomized designs) and selective reporting (9/20). The two reviewers reached concordant overall judgements (Figure 2). A domain-level summary is provided in Supplementary Figure S1.

## NRP versus Non-NRP cDCD

Between four and seven studies contributed to each pooled comparison—all fewer than ten, which precluded formal small-study (publication-bias) testing [26]. Findings are presented by endpoint (a risk ratio below 1 favours NRP); the pooled estimates are summarized in Figure 3, per-study forest plots appear in Supplementary Figure S4, GRADE summaries in Table 2 and pooled incidences in Table 3.





Ischemic cholangiopathy

NRP markedly reduced ischemic cholangiopathy (RR 0.16, 95% CI 0.09–0.28; 6 studies;  $I^2 = 0\%$ ), lowering the pooled incidence from 12.7% without NRP to 1.8% (NNT 9); this non-NRP incidence reproduces the 13.2% of the reference review, an external validation of our dataset. GRADE: moderate certainty.

Graft loss

Reduced with NRP (RR 0.41, 0.32–0.54; 5 studies;  $I^2 = 0\%$ ), from a pooled incidence of 9.6%–3.4% (NNT 18). GRADE: low certainty.

Recipient death

Reduced (RR 0.46, 0.34–0.62; 4 studies;  $I^2 = 0\%$ ), from 7.0% to 4.0% (NNT 27). GRADE: low certainty.

Hepatic artery thrombosis

Reduced (RR 0.51, 0.30–0.85; 6 studies;  $I^2 = 0\%$ ), from 5.1% to 3.4% (NNT 40). GRADE: low certainty.

Other biliary complications

Reduced (RR 0.49, 0.32–0.73; 5 studies;  $I^2 = 45\%$ , moderate heterogeneity), from 20.1% to 9.5% (NNT 10). GRADE: low certainty.

Primary non-function

Reduced (RR 0.51, 0.28–0.94; 6 studies;  $I^2 = 0\%$ ; NNT 67). The marginal pooled incidence was similar between groups (3.0% vs. 3.0%) because the incidence and relative-effect pools draw on different sets of studies (see Table 3 footnote); within the comparative pool the crude rates were 2.0% versus 3.1%. GRADE: low certainty.

Early allograft dysfunction

The only heterogeneous endpoint (RR 0.65, 0.45–0.94; 7 studies;  $I^2 = 74\%$ ), with pooled incidence falling from 35.9% without NRP to 22.4%. A prespecified subgroup analysis by comparator type (Supplementary Figure S6) showed the reduction was confined to cold-storage or super-rapid-

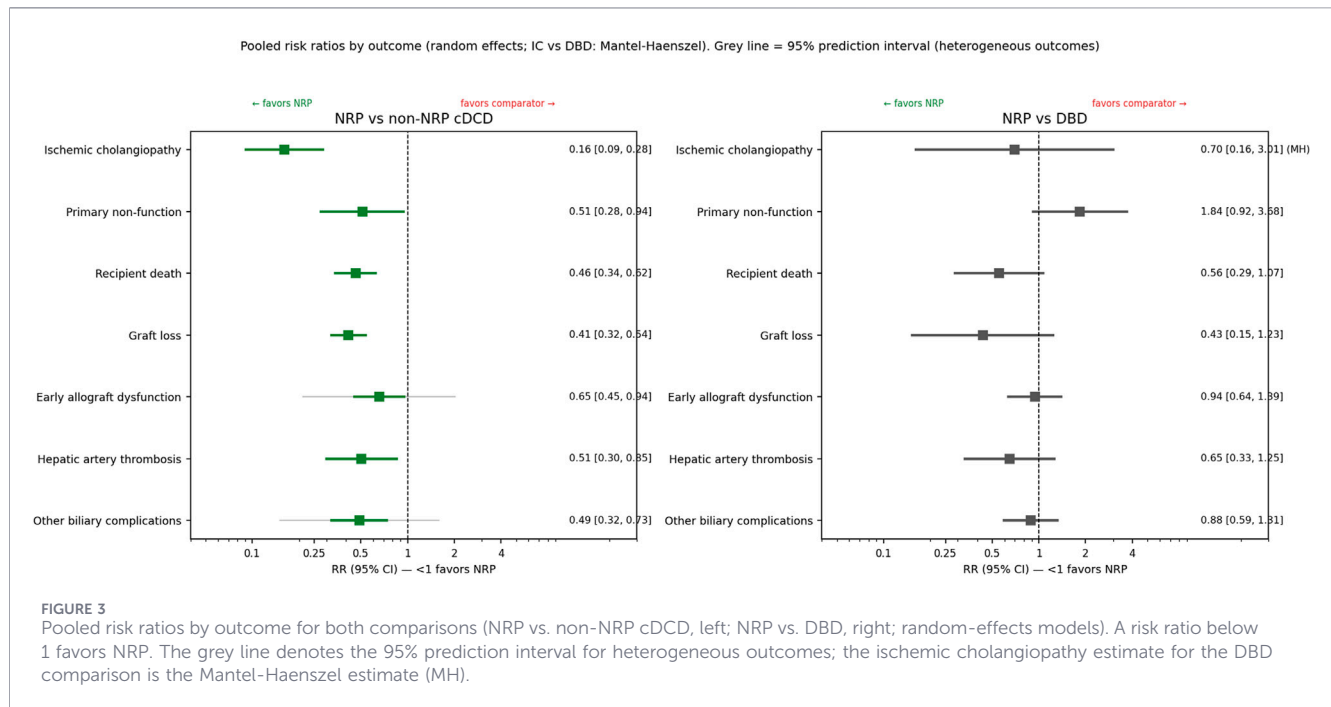


TABLE 2 Summary of findings (GRADE).

| NRP vs. non-NRP cDCD        |            |                   |                |           |
|-----------------------------|------------|-------------------|----------------|-----------|
| Outcome                     | Studies; n | RR (95% CI)       | Absolute/1,000 | Certainty |
| Ischemic cholangiopathy     | 6; 1878    | 0.16 (0.09–0.28)  | 107 fewer      | Moderate  |
| Recipient death             | 4; 1,380   | 0.46 (0.34–0.62)  | 38 fewer       | Low       |
| Graft loss                  | 5; 1,556   | 0.41 (0.32–0.54)  | 56 fewer       | Low       |
| Other biliary complications | 5; 1,542   | 0.49 (0.32–0.73)  | 103 fewer      | Low       |
| Hepatic artery thrombosis   | 6; 1734    | 0.51 (0.30–0.85)  | 25 fewer       | Low       |
| Primary non-function        | 6; 1882    | 0.51 (0.28–0.94)  | 15 fewer       | Low       |
| Early allograft dysfunction | 7; 1924    | 0.65 (0.45–0.94)  | Heterogeneous  | Very low  |
| NRP vs. DBD                 |            |                   |                |           |
| Outcome                     | Studies; n | RR (95% CI)       | Effect         | Certainty |
| Ischemic cholangiopathy     | 7; 2055    | 0.70 (0.16–3.01)‡ | No difference  | Very low  |
| Primary non-function        | 8; 2051    | 1.84 (0.92–3.68)  | No difference  | Very low  |
| Recipient death             | 6; 1,266   | 0.56 (0.29–1.07)  | No difference  | Very low  |
| Graft loss                  | 4; 613     | 0.43 (0.15–1.23)  | No difference  | Very low  |
| Early allograft dysfunction | 5; 713     | 0.94 (0.64–1.39)  | No difference  | Very low  |
| Hepatic artery thrombosis   | 8; 2,155   | 0.65 (0.33–1.25)  | No difference  | Very low  |
| Other biliary complications | 6; 1938    | 0.88 (0.59–1.31)  | No difference  | Very low  |

CI, confidence interval; RR, risk ratio. ‡Mantel-Haenszel estimate (preferred for rare events). Absolute effect = events per 1,000 fewer with NRP, relative to the pooled comparator risk.

TABLE 3 Pooled incidences of each outcome by donor and procurement group.

| Outcome                     | NRP, % (95% CI); k   | Non-NRP cDCD, % (95% CI); k | DBD, % (95% CI); k  |
|-----------------------------|----------------------|-----------------------------|---------------------|
| Ischemic cholangiopathy     | 1.8 (1.2–2.9); 14    | 12.7 (8.8–18.1); 6          | 1.2 (0.5–2.8); 7    |
| Recipient death             | 4.0 (2.1–7.5); 12    | 7.0 (1.7–24.1); 4           | 6.4 (3.9–10.5); 6   |
| Graft loss                  | 3.4 (1.4–7.9); 9     | 9.6 (3.2–25.4); 5           | 6.0 (3.8–9.4); 4    |
| Other biliary complications | 9.5 (7.5–11.9); 11   | 20.1 (14.6–27.0); 5         | 10.7 (6.4–17.3); 6  |
| Hepatic artery thrombosis   | 3.4 (2.4–4.6); 13    | 5.1 (3.3–7.8); 6            | 5.1 (3.3–7.8); 8    |
| Primary non-function        | 3.0 (2.2–4.3); 14    | 3.0 (1.7–5.2); 6            | 2.1 (1.5–2.9); 8    |
| Early allograft dysfunction | 22.4 (16.0–30.5); 11 | 35.9 (21.2–53.9); 7         | 24.7 (19.3–31.0); 5 |

Values are random-effects logit-binomial pooled incidences (%) with 95% confidence intervals; k, number of contributing studies. These are observed pooled event rates within each group and are not adjusted for between-group differences in case mix. Incidences are pooled over all studies reporting each outcome, whereas relative effects (Table 2) are pooled only over the comparative studies contributing to each comparison; because these study sets differ, the marginal incidences do not reproduce the relative effects—for example, primary non-function shows near-identical marginal incidences (3.0% vs. 3.0%) yet a significant relative effect (RR, 0.51), because within the comparative pool the crude rates are 2.0% versus 3.1%. CI, confidence interval; cDCD, controlled donation after circulatory death; DBD, donation after brain death; NRP, normothermic regional perfusion.

recovery comparators (RR 0.58, 0.39–0.86; 5 studies) and was absent versus *ex situ* normothermic machine perfusion (RR 1.68, 0.82–3.45; 2 studies—Mohkam et al. and the isolated NMP arm of Gaurav et al., 101 NMP recipients) and versus a single hypothermic-oxygenated-perfusion study (RR 0.56, 0.25–1.26). Because the three-arm cohort of Gaurav et al. contributes to two subgroups through its separate comparator arms, the subgroup study counts (2 and 1,5) sum to eight although seven studies enter the overall pool. This subgroup is exploratory and must be read with caution: EAD definitions based on early post-transplant transaminase release (Olthoff, MEAF) are susceptible to biomarker washout during *ex situ* perfusion, which our data cannot separate from a genuine functional effect. GRADE: very low certainty.

### Length of stay

Intensive-care and hospital stays did not differ significantly (mean differences of about –0.5 to –1.2 days favouring NRP; all confidence intervals crossing zero), with high heterogeneity reflecting differing health systems and reporting.

### NRP versus DBD

Four to eight studies contributed to each endpoint—again fewer than ten, precluding small-study testing—and heterogeneity was low ( $I^2 \leq 4\%$ ) for every endpoint except early allograft dysfunction ( $I^2 = 34\%$ ); no outcome differed significantly and every estimate was of very low certainty by GRADE (Table 2; per-study forest plots in Supplementary Figure S5). Incidences are given as NRP versus DBD.

### Ischemic cholangiopathy

RR 0.70 (Mantel-Haenszel 0.16–3.01; 7 studies); the inverse-variance estimate (1.21) was unstable because most studies recorded no events. Incidence 1.8% versus 1.2%.

### Primary non-function

RR 1.84 (0.92–3.68; 8 studies). Incidence 3.0% versus 2.1%.

### Recipient death

RR 0.56 (0.29–1.07; 6 studies). Incidence 4.0% versus 6.4%.

### Graft loss

RR 0.43 (0.15–1.23; 4 studies). Incidence 3.4% versus 6.0%.

### Early allograft dysfunction

RR 0.94 (0.64–1.39; 5 studies;  $I^2 = 34\%$ ). Incidence 22.4% versus 24.7%.

### Hepatic artery thrombosis

RR 0.65 (0.33–1.25; 8 studies). Incidence 3.4% versus 5.1%.

### Other biliary complications

RR 0.88 (0.59–1.31; 6 studies). Incidence 9.5% versus 10.7%.

Overall, NRP recipients had outcomes statistically indistinguishable from DBD, although wide confidence intervals and very low certainty preclude formal equivalence claims.

### Sensitivity analysis excluding *ex situ* machine perfusion

The NRP-versus-non-NRP comparison was repeated after excluding all grafts managed with *ex situ* perfusion: Mohkam et al. (comparator entirely NMP) and Schurink et al. (dual HOPE in both arms) were removed, and the three-arm cohort of Gaurav et al. contributed through its static-cold-storage arm only. Every effect persisted in the same direction and remained statistically significant, by endpoint: ischemic cholangiopathy RR 0.14 (0.08–0.26; 5 studies;  $I^2 = 0\%$ ); graft loss 0.41 (0.32–0.53; 4 studies;  $I^2 = 0\%$ ); recipient death 0.47 (0.35–0.64; 3 studies;  $I^2 = 0\%$ ); other biliary complications 0.54 (0.37–0.79; 4 studies;  $I^2 = 30\%$ ); hepatic artery thrombosis 0.48 (0.28–0.83; 4 studies;  $I^2 = 0\%$ ); primary non-function 0.49 (0.27–0.92; 5 studies;  $I^2 = 0\%$ ); and early allograft dysfunction 0.58 (0.39–0.86; 5 studies;

$I^2 = 78\%$ ) (Supplementary Table S3; Supplementary Figure S3). Heterogeneity was therefore unchanged by the exclusion, and the pooled incidences and GRADE certainty reported above apply. The NRP-versus-DBD comparison was inherently free of *ex situ* perfusion. Estimates were also robust to alternative estimators (fixed versus random effects, Mantel-Haenszel, Peto) and to exclusion of the salvage study and of higher-risk studies (Supplementary Table S1). All estimates were reproduced in R/metafor and in Python with exact agreement.

## Discussion

This updated meta-analysis, which expands the evidence base of the most recent NRP-specific synthesis [19], is consistent with and reinforces its central message: in cDCD liver transplantation, NRP substantially reduces ischemic cholangiopathy—the principal liability of cDCD—and is associated with less graft loss, mortality, hepatic artery thrombosis and biliary morbidity than non-NRP procurement. The estimates are remarkably concordant with the previous review (PNF identical at 0.51; recipient death 0.46 vs. a hazard ratio of 0.50), while the larger number of events yields greater precision and, for IC, a somewhat stronger effect (RR 0.16 vs. 0.23) [19]. That two independent datasets, methods and analytic teams converge on the same conclusion is itself reassuring.

Our update offers several contributions. First, it incorporates the emerging North-American experience—registry and multicenter cohorts [15–18]—extending generalizability beyond the predominantly European evidence of the prior review and reproducing the protective signal across health systems and across the A-NRP and TA-NRP techniques. Second, we applied explicit anti-duplication rules to overlapping cohorts that threaten naïve syntheses: Spanish single-center series nested within the national registry [1, 5–8, 30], and a clustered US registry signal [14–18], were prevented from double-counting patients, and a Cambridge cohort overlapping an included study was excluded from pooling [9, 32]. Third, during verification we detected and corrected genuine extraction pitfalls in the primary literature—reversed column order with the comparator listed first [2], evaluable rather than enrolled denominators for EAD [16, 17], a mixed cohort in which only a subgroup received NRP [33], and—prompted by peer review—a salvage cohort in which dual HOPE had been used in both arms [12], which we reclassified accordingly; these errors, left unchecked, would have biased the pooled estimates, and their correction is a methodological contribution in its own right. Reassuringly, restricting the analysis to grafts free of *ex situ* machine perfusion preserved every effect (Supplementary Table S3). Fourth, we used contemporary methods: a logit-binomial model for incidences (avoiding the documented artefacts of the Freeman-Tukey transformation [27]), the Mantel-Haenszel estimator for rare events (avoiding the continuity-correction bias that destabilized the inverse-variance IC-versus-DBD estimate), and t-based prediction intervals [28]. Finally, we report numbers needed to treat for clinical translation and reproduced every estimate in two independent engines.

The heterogeneity of EAD merits emphasis, as it was unresolved (and non-significant) in the previous review [19]. In an exploratory

subgroup analysis by comparator type (Supplementary Figure S6), the EAD benefit of NRP was apparent relative to cold storage and super-rapid recovery (RR 0.58, 0.39–0.86) but not relative to *ex situ* normothermic machine perfusion (RR 1.68, 0.82–3.45), the latter based on the two studies that provide isolated NMP comparators (Mohkam et al. and the NMP arm of Gaurav et al.; 101 NMP recipients). Because this rests on only two studies it should be regarded as hypothesis-generating. Importantly, it should not be read as evidence of a biological effect: EAD definitions that incorporate early post-transplant transaminase release (Olthoff, MEAF) are vulnerable to biomarker washout during *ex situ* perfusion, a confounder that our analysis cannot disentangle from any true functional benefit [9, 11]. EAD is therefore comparator-dependent rather than inconsistent—a clinically meaningful distinction that also cautions against pooling perfusion-based and cold-storage comparators indiscriminately. Beyond outcomes, several included studies underline a parallel benefit of NRP on organ utilization, allowing transplantation of grafts that would otherwise be discarded [14, 15]; utilization and quality gains together strengthen the case for NRP at a programmatic level.

Our findings are concordant with, and extend, previous syntheses. An earlier meta-analysis of regional perfusion across DCD solid-organ transplantation [34] and a systematic review of machine-perfusion strategies by donor type [35] reported directionally similar benefits, and a recent broader systematic review and meta-analysis of hypothermic, normothermic and regional perfusion likewise found a marked reduction in ischemic cholangiopathy with NRP versus super-rapid recovery (RR 0.10) [20]. Our contribution is specific rather than duplicative: we focus exclusively on NRP in cDCD liver transplantation with the original three-arm PICO, substantially increase the number of NRP-cDCD recipients relative to the reference NRP-specific review [19], incorporate the emerging North-American registry experience, apply explicit anti-duplication rules, correct extraction errors in the primary literature, use contemporary statistical methods and GRADE, and demonstrate that the findings persist after excluding *ex situ* machine perfusion. Convergence across independent datasets and methods strengthens confidence in the estimates while underscoring their observational nature.

The limitations are intrinsic to the evidence. All studies are observational and at moderate-to-serious risk of bias, dominated by confounding by indication: NRP may be applied to more favorable donor-recipient pairs, which could inflate its apparent benefit. Cohort overlap, although mitigated, cannot be fully excluded without individual-patient data. Events are sparse for several outcomes; fewer than 10 studies contributed to every analysis, precluding formal assessment of publication bias; and death and graft loss were pooled as risk ratios rather than time-to-event hazard ratios. Outcome definitions—particularly of IC and of EAD (Olthoff criteria vs. the MEAF score)—were not uniform [22]. Consequently certainty is moderate at best (for IC) and low to very low otherwise, and the apparent equivalence with DBD reflects absence of evidence of a difference rather than demonstrated equivalence; for IC specifically the DBD comparison was statistically unstable and should be read with caution. Although the sensitivity analysis excluding *ex situ* machine perfusion addresses one important source of confounding, residual confounding by indication



cannot be excluded in observational data, and our estimates should be interpreted as associations rather than causal effects.

These caveats notwithstanding, the consistency, magnitude and graded nature of the IC reduction (a number needed to treat of about 9, with incidence falling from 12.7% to DBD-like levels of <2%) are clinically compelling and biologically coherent. Future priorities include randomized and registry-embedded pragmatic trials, a harmonized definition of ischemic cholangiopathy, individual-patient-data meta-analysis to definitively resolve cohort overlap and to compare A-NRP with TA-NRP, and longer follow-up to confirm both the durability of the benefit and the approximation to DBD outcomes.

## Conclusion

In controlled DCD liver transplantation, NRP is associated with a marked reduction in ischemic cholangiopathy (moderate certainty) and with improvements in mortality, graft loss and vascular and biliary complications (low certainty), with results that approach those of DBD (very low certainty). This updated synthesis—building on prior work with a larger and more geographically diverse evidence base—reinforces the role of NRP in expanding the cDCD donor pool, while underscoring the observational nature of the evidence and the need for higher-certainty studies.

## Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: Available from the corresponding author on reasonable request. Requests to access these datasets should be directed to Alberto Ruiz Pacheco, albertoruizp96@gmail.com.

## Author contributions

RB conceived and supervised the study. JR-E designed the study and the statistical analysis plan. NR-G, ES, JR-E and AC performed study screening, data extraction and risk-of-bias assessment in

duplicate and independently. AR-P arbitrated screening, extraction and risk-of-bias discrepancies. JR-E performed the statistical analysis and drafted the manuscript. All authors contributed to data interpretation, critically revised the manuscript for important intellectual content, and approved the submitted version. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

RB is a coauthor of a previous study in this field (Campo-Cañaveral de la Cruz et al., *Am J Transplant* 2023).

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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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2026.17154/full#supplementary-material>

## References

- Hessheimer AJ, de la Rosa G, Gastaca M, Ruiz P, Otero A, Gómez M, et al. Abdominal normothermic regional perfusion in controlled donation after circulatory determination of death liver transplantation: outcomes and risk factors for graft loss. *Am J Transpl* (2022) 22:1169–81. doi: 10.1111/ajt.16899
- Miñambres E, Rubio JJ, Coll E, Domínguez-Gil B. Combined lung and liver procurement in controlled donation after circulatory death using normothermic abdominal perfusion. *Am J Transpl* (2020) 20:231–40. doi: 10.1111/ajt.15520
- Watson CJE, Hunt F, Messer S, Currie I, Large S, Sutherland A, et al. *In situ* normothermic perfusion of livers in controlled circulatory death donation may prevent ischemic cholangiopathy and improve graft survival. *Am J Transpl* (2019) 19:1745–58. doi: 10.1111/ajt.15241
- Hessheimer AJ, Coll E, Torres F, Ruiz P, Gastaca M, Rivas JJ, et al. Normothermic regional perfusion vs. super-rapid recovery in controlled donation after circulatory death liver transplantation. *J Hepatol* (2019) 70:658–65. doi: 10.1016/j.jhep.2018.12.013
- Rodríguez-Sanjuán JC, Ruiz N, Miñambres E, Toledo E, González-Noriega M, Fernández-Santiago R, et al. Liver transplant from controlled cardiac death donors using normothermic regional perfusion: comparison with liver transplants from brain dead donors. *Transpl Proc* (2019) 51:12–9. doi: 10.1016/j.transproceed.2018.04.067
- Ruiz P, Valdivieso A, Palomares I, Prieto M, Ventoso A, Salvador P, et al. Similar results in liver transplantation from controlled donation after circulatory death donors with normothermic regional perfusion and donation after brain death donors: a case-matched single-center study. *Liver Transpl* (2021) 27:1747–57. doi: 10.1002/lt.26281
- Viguera L, Blasi A, Reverter E, Arjona B, Caballero M, Chocron I, et al. Liver transplant with controlled donors after circulatory death with normothermic regional perfusion and brain dead donors: a multicenter cohort study of transfusion, one-year graft survival and mortality. *Int J Surg* (2021) 96:106169. doi: 10.1016/j.ijsu.2021.106169
- Pérez Rodríguez R, Sánchez Pérez B, Pérez Daga JA, Díaz FJL, Aguilar JLF, Muñoz MAS, et al. Outcome of liver transplants using donors after cardiac death with normothermic regional perfusion. *Transpl Proc* (2022) 54:37–40. doi: 10.1016/j.transproceed.2021.10.007
- Gaurav R, Butler AJ, Kosmoliaptis 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) 275:1156–64. doi: 10.1097/SLA.0000000000005428
- Savie E, Lim C, Rayar M, Orlando F, Boudjema K, Mohkam K, et al. Favorable outcomes of liver transplantation from controlled circulatory death donors using

- normothermic regional perfusion compared to brain death donors. *Transplantation* (2020) 104:1943–51. doi: 10.1097/TP.0000000000003372
11. Mohkam K, Nasralla D, Mergental H, Muller X, Butler A, Jassem W, et al. *In situ* normothermic regional perfusion versus *ex situ* normothermic machine perfusion in liver transplantation from donation after circulatory death. *Liver Transpl* (2022) 28:1716–25. doi: 10.1002/lt.26522
12. Schurink IJ, de Goeij FHC, Habets LJM, van de Leemkolk FEM, van Dun CAA, Oniscu GC, et al. Salvage of declined extended-criteria DCD livers using *in situ* normothermic regional perfusion. *Ann Surg* (2022) 276:e223–e230. doi: 10.1097/SLA.00000000000005611
13. Bluhme E, Gabel M, Martinez de la Maza L, Nilsén V, Hildebrand K, Jarsäter J, et al. Normothermic regional perfusion in controlled DCD liver procurement: outcomes of the Swedish national implementation protocol. *Liver Transpl* (2024) 30:1132–44. doi: 10.1097/LVT.0000000000000434
14. 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:e1450. doi: 10.1097/TXD.0000000000001450
15. Wisel SA, Steggerda JA, Thiessen C, Roll GR, Chen Q, Thomas J, et al. Preserved 2-y liver transplant outcomes following simultaneous thoracoabdominal DCD organ procurement despite effects on liver utilization rate. *Transpl Direct* (2023) 9:e1528. doi: 10.1097/TXD.0000000000001528
16. Brubaker AL, Sellers MT, Abt PL, Croome KP, Merani S, Wall A, et al. US liver transplant outcomes after normothermic regional perfusion vs standard super rapid recovery. *JAMA Surg* (2024) 159:677–85. doi: 10.1001/jamasurg.2024.0520
17. Croome KP, Subramanian V, Mathur AK, Aql B, Mao SA, Clendenon JN, et al. Outcomes of DCD liver transplant using sequential normothermic regional perfusion and normothermic machine perfusion or NRP alone versus static cold storage. *Transplantation* (2025) 109:1184–93. doi: 10.1097/TP.0000000000005301
18. Bababekov YJ, Hartman E, Chapman WC, Goncalves C, Choudhury R, Shinsako J, et al. Thoracoabdominal normothermic regional perfusion: real-world experience and outcomes of DCD liver transplantation. *Transpl Direct* (2025) 11:e1767. doi: 10.1097/TXD.0000000000001767
19. 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
20. Patrono D, Del Prete L, Eden J, Dutkowski P, Guarrera JV, Quintini C, et al. Machine perfusion of liver grafts: hypothermic versus normothermic versus normothermic regional perfusion. *Int J Surg* (2025) 111:5768–82. doi: 10.1097/JS9.0000000000002648
21. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* (2021) 372:n71. doi: 10.1136/bmj.n71
22. Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transpl* (2010) 16:943–9. doi: 10.1002/lt.22091
23. Sterne JAC, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* (2016) 355:i4919. doi: 10.1136/bmj.i4919
24. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* (2008) 336:924–6. doi: 10.1136/bmj.39489.470347.AD
25. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* (1986) 7:177–88. doi: 10.1016/0197-2456(86)90046-2
26. Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ *Cochrane Handbook for Systematic Reviews of Interventions*. London: Cochrane (2023). doi: 10.1002/9781119536604
27. Schwarzer G, Chemaitelly H, Abu-Raddad LJ, Rücker G. Seriously misleading results using inverse of freeman-tukey double arcsine transformation in meta-analysis of single proportions. *Res Synth Methods* (2019) 10:476–83. doi: 10.1002/jrsm.1348
28. Int'Hout J, Ioannidis JPA, Rothwell PM, Glasziou P. Plea for routinely presenting prediction intervals in meta-analysis. *BMJ Open* (2016) 6:e010247. doi: 10.1136/bmjopen-2015-010247
29. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol* (2014) 14:135. doi: 10.1186/1471-2288-14-135
30. Campo-Cañaveral de la Cruz JL, Miñambres E, Coll E, Padilla M, Sánchez-Antolín G, de la Rosa G, et al. Outcomes of lung and liver transplantation after simultaneous recovery using abdominal normothermic regional perfusion in donors after the circulatory determination of death versus donors after brain death. *Am J Transpl* (2023) 23:1059–71. doi: 10.1016/j.ajt.2023.04.016
31. Secanella L, Alconchel F, López-Monclús J, Toledo-Martínez E, Barrios O, Ramírez P, et al. Outcomes of liver transplantation with thoracoabdominal normothermic regional perfusion: a matched-controlled initial experience in Spain. *Front Transpl* (2023) 2:1280454. doi: 10.3389/frtra.2023.1280454
32. Puttappa A, Gaurav R, Kakhandki V, Swift L, Fear C, Webster R, et al. Normothermic regional and *ex situ* perfusion reduces postreperfusion syndrome in donation after circulatory death liver transplantation: a retrospective comparative study. *Am J Transpl* (2025) 25:1296–305. doi: 10.1016/j.ajt.2025.01.007
33. Fernández-de la Varga M, del Pozo-del Valle P, Béjar-Serrano S, López-Andújar R, Berenguer M, Prieto M, et al. Good post-transplant outcomes using liver donors after circulatory death when applying strict selection criteria: a propensity-score matched-cohort study. *Ann Hepatol* (2022) 27:100724. doi: 10.1016/j.aohep.2022.100724
34. De Beule J, Vandendriessche K, Pengel LHM, Bellini MI, Dark JH, Hessheimer AJ, et al. A systematic review and meta-analyses of regional perfusion in donation after circulatory death solid organ transplantation. *Transpl Int* (2021) 34:2046–60. doi: 10.1111/tri.14121
35. Liang A, Cheng W, Cao P, Cai S, Zhang L, Zhong K, et al. Effects of machine perfusion strategies on different donor types in liver transplantation: a systematic review and meta-analysis. *Int J Surg* (2023) 109:3617–30. doi: 10.1097/JS9.0000000000000661



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# Menopause influences lung inflammatory response and miRNA expression in brain death donors

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Lungs are highly affected by brain death, with females showing a higher inflammatory response, linked sex hormones acute reduction. With the aging of world population, the number of older donors is increasing. So, the study of menopause associated changes gain importance. Here we investigated menopause's effects in female brain death rats, previously subjected to transitional follicular depletion and aging. Female Wistar rats were divided in young and menopause groups. After follicular depletion, rats aged for 10 weeks. The animals were submitted to brain death and Sham operated rats served as controls. White blood cell counts, bronchoalveolar lavage were analyzed and inflammatory mediators were quantified. Lung tissue was evaluated for myeloperoxidase, intercellular adhesion molecules, miRNA expression, and protein and gene expression of estradiol receptors. In menopause group, there was increase in systemic and tissue leukocyte infiltration, myeloperoxidase expression, inducible nitric oxide synthase, intercellular adhesion molecule-1, lung edema, and loss of IL-10 regulation. Additionally, we found alterations in estradiol receptors along with changes in the expression of miRNAs associated to inflammation, vascular function and senescence. Menopause increases lung inflammation after brain death, by higher leukocyte infiltration and reduction of IL-10 and may impact the outcome of lung transplantation.

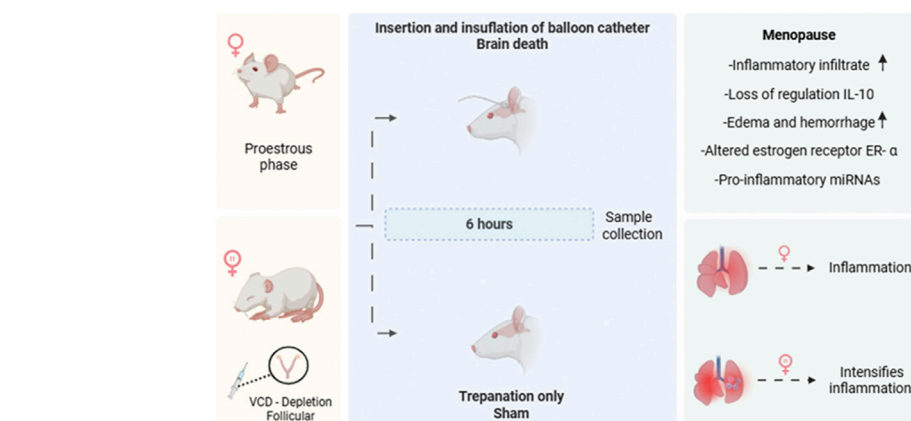
### KEYWORDS

brain death, donor, inflammation, lung, menopause, rats

## Introduction

Organ transplantation is a fundamental approach for patients with advanced stages of various diseases, offering the possibility of prolonging and improving quality of life. However, current data highlight that the shortage of organs available for transplantation remains a global and growing challenge, aggravated by the aging population and the increasing demand of patients on waiting lists [1, 2]. According to the Global Observatory on Donation and Transplantation (GODT, 2025) out of

# MENOPAUSE INFLUENCES LUNG INFLAMMATORY RESPONSE AND miRNA EXPRESSION IN BRAIN DEATH DONORS



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GRAPHICAL ABSTRACT

47,000 organ donors, 30% were over 60 years old and the average age of organ donors has been increasing over the years [3]. To respond to the high demand, the inclusion criteria were expanded, allowing the use of marginal organs, including those from older donors.

Brain death (BD), the main source of organ donation, triggers unregulated immunological and inflammatory changes that compromise the quality of organs intended for transplantation [1, 2, 4]. Among these, significant hormonal changes result from pituitary failure, with the lung being one of the organs most vulnerable to their effects and which may be associated with pulmonary edema after autonomic storm, such alterations may become a challenge for the success of lung transplantation [5, 6]. In the case of lung transplants, donors over 65 years of age are associated with shorter graft survival [7, 8].

Experimental studies, comparing males and females, confirm different responses to brain death. One such study demonstrated that female rats suffer greater lung injury after BD when compared to male rats [9]. Ferreira et al. highlighted important differences between the sexes to BD [10], mainly greater inflammation in females, but without impact on microcirculatory perfusion, an impact that was only documented in male animals [11, 12]. Such studies point to the importance of the acute reduction of female sex hormones after BD, after the loss of the hypothalamic-pituitary-ovarian axis resulting in greater inflammation in females.

Therefore, given the expansion of the criteria to include older donors and knowing that the donor's sex and hormones influence the lung status, we investigated the effects of menopause and aging in rats subjected to BD, analyzing the release of inflammatory mediators and the influx of leukocytes into the lungs.

## Materials and methods

This study was approved by the Animal Research Ethics Committee of the Faculty of Medicine of the University of São Paulo under number (SDC) no 5635 23 037. All animals were handled in accordance with “Principles of Laboratory Animal Care” written by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” published by the Institute of Laboratory Animal Resources from the National Institute of Health (NIH Publication No 86–23, revised 1996). Female Wistar rats ( $n = 48$ ) were randomized into 4 groups: Menopause rats (Menopause BD;  $n = 8$ ) and young rats (Young BD;  $n = 8$ ) with both groups subjected to the BD procedure, and as controls, sham operation were used for both groups as well, the menopause (Menopause sham;  $n = 8$ ) and young (Young sham;  $n = 8$ ).

## Menopause model and aging

The chemical follicular depletion model was based on Mayer et al. (2002) [13]. Briefly, the rats (8 weeks old) received an intraperitoneal injection of 4-vinylcyclohexene diepoxide (VCD, 80 mg/kg, Merck, USA) diluted in corn oil for 6 weeks. After treatment, they were kept aging in the rodent vivarium for 10 weeks. At the end of the last week (170–180 days old), blood samples were collected for hormonal dosages and menopause confirmation. In addition, they were monitored weekly for weight and estrous cycle.

## Identification of the estrous cycle phases

The estrous cycle phase identification was performed by means of a vaginal smear using a Pasteur pipette with saline solution. The



samples were placed on a slide and stained with 5  $\mu$ L of crystal violet dye (5%), for identification by optical microscopy.

## Determination of serum hormone levels

Blood was collected from the animal's tail vein and circulating hormone concentrations (estradiol, progesterone, corticosterone and follicle stimulating hormone (FSH)) were determined using Cayman kits (Cayman Chemical, USA) and MyBiosource (MyBiosource, USA). The protocols recommended by the manufacturers were used.

## Brain death model

The BD induction model was based on Breithaupt-Faloppa et al. [9]. Briefly, after intubation, anesthesia and vessel cannulation, a *Fogarty*® 4F catheter (Baxter Healthcare, USA) was inserted in the intracranial space for rapid infusion of 400  $\mu$ L of 0.9% NaCl. BD was confirmed by clinical criteria such as hypertensive peak pressure, bilateral mydriasis, apnea and absence of brainstem reflexes. Once confirmed, the use of isoflurane was discontinued. Animals in the sham group were subjected to surgical procedures without BD induction and remained anesthetized throughout the period.

## Determination of total and differential circulating leukocyte and platelet counts

At the beginning, at the third hour and at the end of the experiment, samples (20  $\mu$ L of blood) were obtained, homogenized in diluent and subjected to automatic cell counting on a hematologic analyzer (BC-2800vet, Mindray, China).

## Concentration of IL-1 $\beta$ and IL-10 in serum and lung culture media

The determination of inflammatory mediators was performed using commercial Kits (Duo Set, R&D System®, USA). Serum samples were obtained 6 h after BD and lung tissue culture was established as previously described in Breithaupt-Faloppa et al. [9]. After 24 h media samples were collected. The mediator's concentrations were expressed in pg/mL (serum) and pg/mL/mg of protein (media). Assays were conducted following manufacture specifications, and optical density was obtained using a spectrophotometer (SpectraMax® PLUS Microplate Reader, Molecular Devices, USA).

## Histopathological analysis of the lung

Lung fragments were fixed in formaldehyde solution (10%) for 24 h. The tissues were processed, embedded in paraffin, cut (4  $\mu$ m) and the sections stained (hematoxylin/eosin). Conventional morphometric analyses were performed by two examiners blinded to the groups in order to evaluate hemorrhage and tissue edema. The sections were captured by a DS-Ri1 digital camera (Nikon, Japan), coupled to a microscope (Nikon). Five areas per image were extracted and exported in a format compatible with the NIS Elements Software (NIS-elements, Nikon, Tokyo, Japan).

## Bronchoalveolar lavage and bone marrow cell counts

After euthanasia, bronchoalveolar lavage (BAL) fluid was collected through the trachea with a syringe containing 5 mL of Phosphate Buffered Saline (PBS) injected into the airways. The BAL sample was centrifuged and the cell pellet was resuspended in 1 mL of PBS. The samples were subjected to automatic total and differential cell counting (BC-2800vet, Mindray, China). The bone marrow, the left femur was removed and a needle connected to a syringe containing 10 mL of PBS was introduced into the medullary canal. The sample were centrifuged and the cell pellet was resuspended in 1 mL of PBS. The samples were subjected to automatic total and differential cell counting (BC-2800vet, Mindray, China).

## MPO activity in lung tissue

The lung tissue samples were homogenized with 3 mL/g of hexadecyltrimethylammonium bromide (HTAB) and centrifuged and the ortho-dianisidine and H<sub>2</sub>O<sub>2</sub> solutions were added. A sodium azide solution was added to stop the reaction. Using a spectrophotometer (Spectramax®, Molecular Devices, USA), absorbance was determined at 450 nm.

## Immunohistochemical investigation of adhesion molecules (ICAM-1), inducible nitric oxide synthase (iNOS), myeloperoxidase (MPO) and estrogen receptors (ER- $\alpha$ , ER- $\beta$ and GPER)

Lung fragments were removed and inflated with a solution of OCT (optimal cutting temperature) solution, then immersed in hexane and frozen in liquid nitrogen. Serial sections (8  $\mu$ m thick) were placed on silanized slides (StarFrost®, Knittelglass, Germany) and fixed in ice-cold acetone for 10 min. The slides were washed with TRIS-saline-tween-20 buffer solution (TBS-T) followed by blocking of endogenous peroxidase with H<sub>2</sub>O<sub>2</sub> solution (2%) for 15 min at room temperature, and blocking of nonspecific sites with a protein buffer consisting of TBST and 2% bovine serum albumin (BSA) for 1 h at 37 °C. For the immunodetection of ICAM-1 and MPO (BosterBio, USA), iNOS (Abcam, USA), and estrogen receptors (Novus Biologicals, USA), primary antibodies diluted (1:100 and 1:200 for the estrogen receptors) in TBS-T/BSA were used. The sections were incubated overnight at 4 °C. The slides were washed with TBS-T and incubated with a 1:200 solution of anti-rabbit IgG secondary antibody associated with horseradish peroxidase (HRP) (BosterBio, USA) for 2 h at 37 °C. After another wash with TBS-T, the slides were incubated with 3-amino-9-ethylcarbazole (AEC) substrate solution for 30 min and counterstained with hematoxylin. The staining present in the tissue sections was recorded using an image acquisition system with a DS-Ri1 digital camera (Nikon, Japan) and analyzed using the NIS-Elements-BR software (Nikon). For the negative control, the samples were incubated with PBS instead of primary antibodies. The results analysis was expressed as stained area/vessel area (ICAM-1) and stained area/total area (MPO, iNOS, ER- $\alpha$ , ER- $\beta$  and GPER).

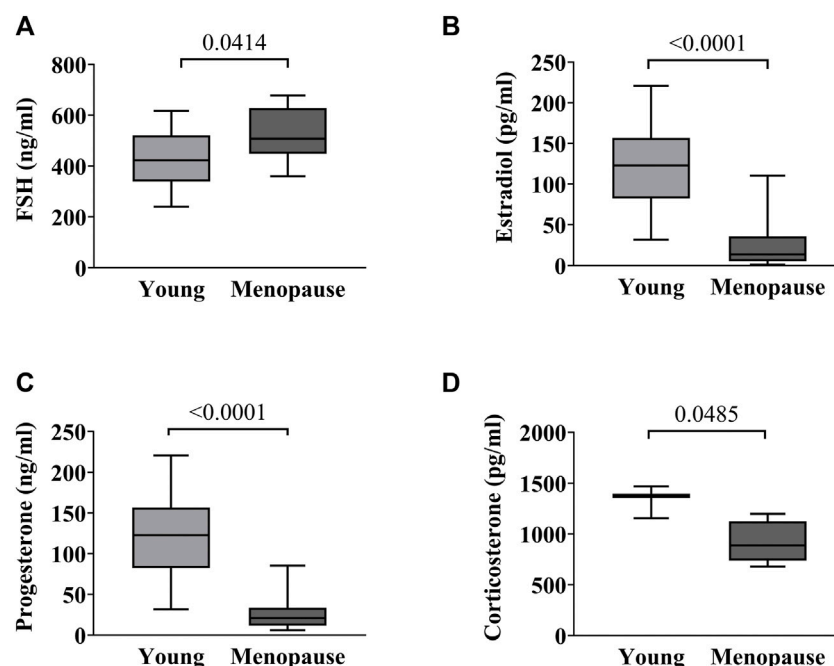


**FIGURE 1**  
Characteristics of the female rats after menopause induction. (A) FSH serum concentrations; (B) Estradiol serum concentrations (C) Progesterone serum concentrations and (D) Corticosterone serum concentrations. Data are expressed as media $\pm$ SEM from 16 animals.

## Real time PCR analysis of estradiol receptors and miRNA expression in lung tissue

RNA and miRNA extraction were conducted using a commercial kit (Mirvana, Applied Biosystems, CA, USA). Complementary DNA for the RNA and miRNA genes was synthesized using commercial kits: High-Capacity cDNA Reverse Transcription (Applied Biosystems, CA, USA) and TaqMan miRNA Reverse Transcription kit (Thermo Fisher, MA, USA), respectively. Real-time PCR (StepOnePlus; Applied Biosystems) for the estradiol receptor gene expression analysis was performed using the TaqMan primers (Applied Biosystems): GAPDH (RN01775763\_g1), ER- $\alpha$  (RN01640372\_m1), ER- $\beta$  (RN00562610\_m1), and GPER (RN00592091\_s1). For miRNA analysis, the following TaqMan primers (Applied Biosystems) were used: U6 snRNA (001973), 27a-3p (000408), 21a-3p (002493), 145-5p (002278), 155 (002571), 34c (000428), and 202-3p (462472). The targets were amplified following the following cycling conditions: 2 min at 50 °C, 10 min at 95 °C, 40 cycles of 15 s at 95 °C, and 1 min at 60 °C.

## Analysis of results

Data relating to hormones were subjected to the non-parametric Mean-Whitney test and the leukogram/platelets and blood pressure were analyzed of the three-way analysis of variance (ANOVA). The remaining results were analyzed using a two-way ANOVA, followed by the Benjamini, Kriger, and Yekutieli tests. Statistical analyses were performed using GraphPad Prism software version 10, and the results are expressed as mean  $\pm$  standard error of the mean (SEM).

## Results

### Validation of the menopause model

Hormonal concentrations of FSH, progesterone, estradiol and corticosterone were measured in serum collected 1 week before the BD experiments. Rats in the menopause group showed an increase in FSH concentration and a significant decrease in progesterone, estradiol and corticosterone concentrations compared to young females (Figure 1).

### Analysis of hemodynamic parameters

All the animals presented the hypertensive peak characteristic of the BD model, followed by a period of arterial hypotension of approximately 1 h, and by normalization of the blood pressure thereafter. The data show differences between the BD groups and their respective sham groups. BD rats showed a significant increase in mean arterial pressure (MAP) in the first minute after catheter inflation, compared to the sham group, and differences were observed between the young BD group and the menopause BD group. Menopause rats showed significantly lower PAM peak values compared to young animals, as shown in Figure 2.

### Determination of total and differential leukocyte counts

Total and differential leukocyte counts were analyzed, and a significant increase was observed in the total number of

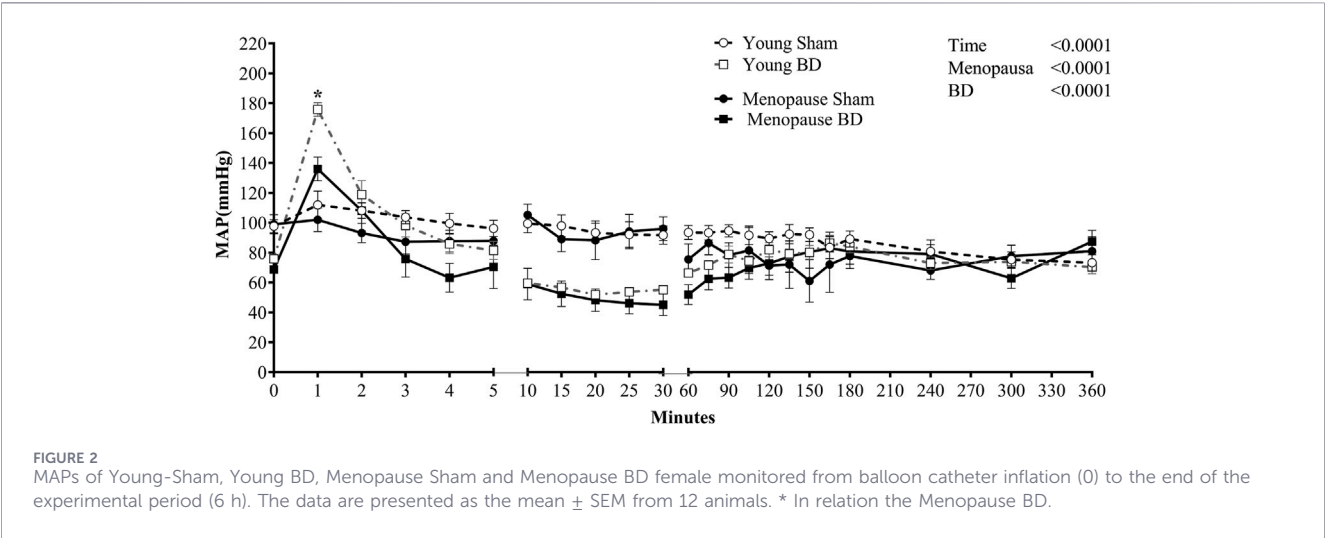


TABLE 1 Total and differential number of circulating leukocytes of rats from the young and menopausal group submitted or not (Sham) to brain death. Data represent the mean  $\pm$  SEM.

|                                       |     | WBC (cells/mm <sup>3</sup> )       |                                  |                                  |                                  | PLT ( $\times 10^9$ /L)       |
|---------------------------------------|-----|------------------------------------|----------------------------------|----------------------------------|----------------------------------|-------------------------------|
| Groups                                |     | Total                              | Lymphocytes                      | Monocytes                        | Granulocytes                     | Platelets                     |
| Young Sham                            | 0 h | 9928.6 $\pm$ 1643.1                | 6785.7 $\pm$ 1274.4              | 400 $\pm$ 72.4                   | 2742.9 $\pm$ 401.1               | 1069.4 $\pm$ 59               |
|                                       | 3 h | 17471.4 $\pm$ 1960.3*              | 4014.3 $\pm$ 987.4               | 1014.3 $\pm$ 290.7*              | 12442.9 $\pm$ 1239.6*            | 708.1 $\pm$ 165.1*            |
|                                       | 6 h | 19042.9 $\pm$ 3250.1*              | 6057.1 $\pm$ 1391.1              | 742.9 $\pm$ 84.1                 | 12242.9 $\pm$ 3583.1*            | 709.6 $\pm$ 124*              |
| Young BD                              | 0 h | 12328.6 $\pm$ 1190                 | 7585.7 $\pm$ 833.9               | 628.6 $\pm$ 119                  | 4114.3 $\pm$ 496.9               | 1211.7 $\pm$ 91.5             |
|                                       | 3 h | 11500 $\pm$ 2058.2                 | 4000 $\pm$ 901.3                 | 342.9 $\pm$ 78.2 <sup>μ</sup>    | 7157.1 $\pm$ 1683.9 <sup>μ</sup> | 687.9 $\pm$ 83.2*             |
|                                       | 6 h | 13085.7 $\pm$ 2172.3               | 2985.7 $\pm$ 689.2               | 414.3 $\pm$ 50.8                 | 9685.7 $\pm$ 1616.1*             | 654.7 $\pm$ 32.2*             |
| Menopause Sham                        | 0 h | 7377.8 $\pm$ 504.1                 | 4833.3 $\pm$ 285.8               | 377.8 $\pm$ 49.4                 | 2166.7 $\pm$ 266.7               | 817.6 $\pm$ 90 <sup>α</sup>   |
|                                       | 3 h | 13133.3 $\pm$ 2137.6               | 4833.3 $\pm$ 968.7               | 755.6 $\pm$ 157.3                | 5644.4 $\pm$ 1620.7              | 695.8 $\pm$ 95                |
|                                       | 6 h | 23311.14184.9*                     | 15833.3 $\pm$ 4432* <sup>α</sup> | 1288.9 $\pm$ 217.6* <sup>α</sup> | 6200 $\pm$ 1629.7 <sup>α</sup>   | 819.2 $\pm$ 79.4              |
| Menopause BD                          | 0 h | 8357.1 $\pm$ 737.7                 | 5557.1 $\pm$ 528.7               | 585.7 $\pm$ 113.3                | 2321.4 $\pm$ 231.9               | 913.6 $\pm$ 52.9 <sup>α</sup> |
|                                       | 3 h | 11171.4 $\pm$ 1740.8               | 5807.1 $\pm$ 1072.1              | 642.9 $\pm$ 83.7                 | 5171.4 $\pm$ 1480.2 <sup>α</sup> | 726.2 $\pm$ 60.3              |
|                                       | 6 h | 15492.9 $\pm$ 1425.2* <sup>μ</sup> | 6807.1 $\pm$ 1064.6 <sup>μ</sup> | 1092.9 $\pm$ 150.3 <sup>α</sup>  | 7592.9 $\pm$ 1535.5*             | 726.4 $\pm$ 47.6              |
| P time                                |     | <0.0001                            | 0.0211                           | 0.0015                           | <0.0001                          | <0.0001                       |
| P BD                                  |     | 0.5393                             | 0.0322                           | 0.0198                           | 0.0006                           | 0.2389                        |
| P Menopause                           |     | 0.0140                             | 0.0912                           | 0.0885                           | 0.3221                           | 0.7281                        |
| P time $\times$ BD                    |     | 0.0624                             | 0.0010                           | 0.0035                           | 0.2848                           | 0.0064                        |
| P Time $\times$ Menopause             |     | 0.0169                             | 0.0049                           | 0.0100                           | 0.2553                           | 0.2635                        |
| P BD $\times$ Menopause               |     | 0.9211                             | 0.3731                           | 0.1889                           | 0.1672                           | 0.9081                        |
| P Time $\times$ Menopause $\times$ BD |     | 0.5521                             | 0.2717                           | 0.3531                           | 0.3421                           | 0.9016                        |

\* In relation the respective Basal; <sup>α</sup> in relation a young; <sup>μ</sup> in relation the respective sham.

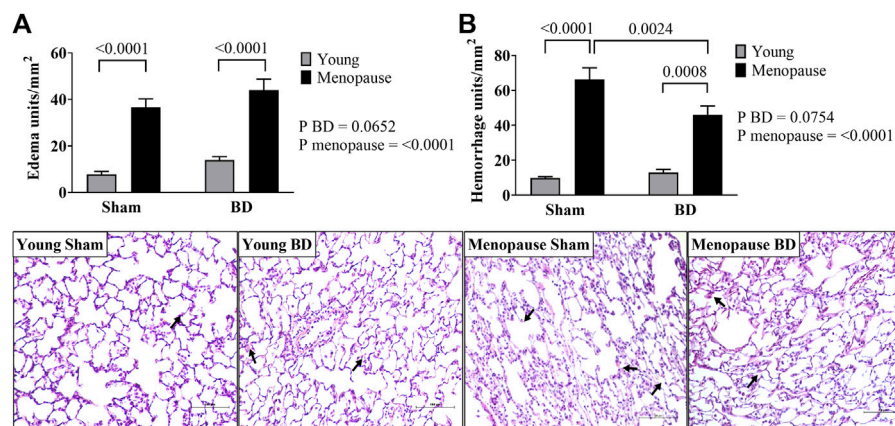


**FIGURE 3**  
Histological analysis of lung sections (HE). **(A)** edema and **(B)** hemorrhage from young or menopause rats subjected or not (Sham) to BD. Data represents the mean  $\pm$  SEM from 8 animals. Arrows show higher intensity of pulmonary hemorrhage in menopause sham animals compared to other groups.

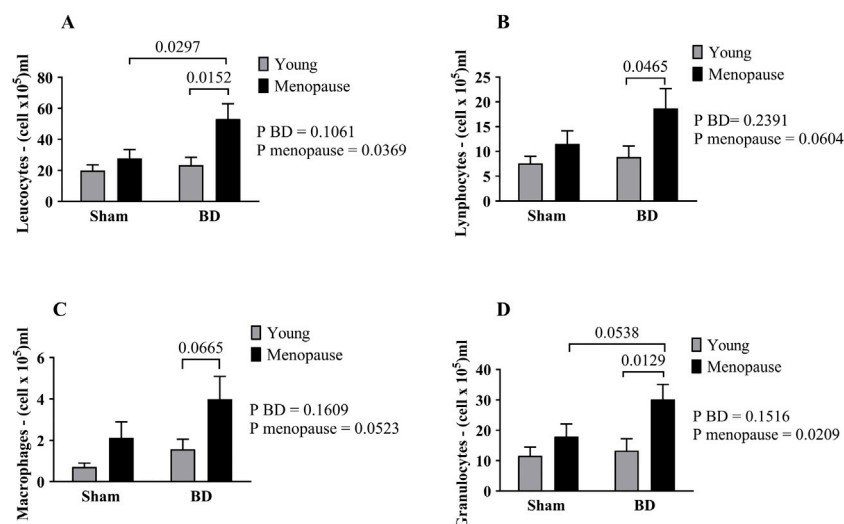


**FIGURE 4**  
Total number of cells **(A)** leucocytes; **(B)** lymphocytes, **(C)** macrophages and **(D)** granulocytes from rats from the young and menopause group submitted or not (Sham) to BD. Values represent the mean  $\pm$  SEM from 8 animals.

circulating leukocytes after BD surgical manipulation. The menopause sham group showed a significant increase, mainly in lymphocytes, at 6 h, compared to basal and the young sham group. In the young group, there was a predominantly granulocytes and monocytes increases just after 3 h, while in the menopause group, this increase only occurred after 6 h. In the 3-way ANOVA comparisons, there was a difference in relation to *time* in total leukocytes and in the cell types evaluated. In relation to *menopause*, there was a difference only in the total number of leukocytes. Regarding *BD*, there was a difference in all cell types but not in total leukocytes. In platelet analyses, a decrease in the number of platelets was observed after surgical manipulation (Table 1).

## Analysis of the hemorrhagic profile and edema in the lungs

As observed in Figure 3, menopause rats, independent of BD, presents greater edema and hemorrhage compared to young rats (Sham and BD).

## Cell quantification in bronchoalveolar lavage fluid and bone marrow

The data show a significant increase in all cell types in bronchoalveolar lavage fluid in menopause BD rats compared with young BD. In addition, there was a higher number of lymphocytes and granulocytes in the menopause BD than their



TABLE 2 Total number of bone marrow cells from young and menopause rats subjected or not (sham) to brain death. Values represent the mean  $\pm$  SEM.

| Bone marrow ( $10^6$ /mL) |                     |
|---------------------------|---------------------|
| Young sham                | 69.071 $\pm$ 8.981  |
| Young BD                  | 68.071 $\pm$ 10.261 |
| Menopause sham            | 60.044 $\pm$ 19.222 |
| Menopause BD              | 60.679 $\pm$ 19.677 |
| <i>P</i> BD               | 0.9745              |
| <i>P</i> menopause        | 0.1580              |

respective sham, as shown in Figure 4. The results of bone marrow counts do not indicate any differences between groups as shown at the Table 2.

## Concentration of serum and lung culture (explant) inflammatory mediators

In Table 3 the quantification the inflammatory mediators in the serum and lung explant is presented. There was a significant increase in serum IL-10 concentration in the young BD compared to the respective sham group, however this response was not observed in menopause BD.

## Evaluation of the presence of leukocytes in lung tissue

No significant differences were observed in the analysis of MPO activity (Figure 5B). Additionally, quantification of lung MPO positive stained cells was performed and the results showed an increase in these cells in menopause rats, in both the sham and BD groups (Figure 5A).

## Quantification of ICAM-1 and iNOS

The data indicate an increase in ICAM-1 expression in menopause rats, independent of BD (Sham and BD) (Figure 6A),

and a higher number of iNOS positive cells were also observed in the menopause group compared to young rats (Figure 6B).

## Quantification of estradiol receptors in lung tissue

Estradiol receptors expression analyses in lung tissue were also performed, as shown in Figure 7. A significant increase in ER- $\alpha$  expression was observed in menopause rats (BD and Sham) when compared to young rats. In addition, the ER- $\alpha$  gene expression is upregulated in menopause sham rats compared and young sham rats, being reduced by BD. The data also indicates a reduction in GPER expression in menopause rats (Sham and BD) and in young rats subjected to BD. The same was not observed when evaluating the gene expression of this receptor. Differently, in the analysis of ER- $\beta$  expression, there was an increase in young BD rats compared to their respective sham, with a lower expression of this receptor in menopause BD rats.

## miRNA receptors in lung tissue

miRNAs expression were analyzed in lung tissue, and an upregulation of miRNAs 27a, 21a, 145, 155, and 34c was observed in menopause sham rats compared to young sham. The miRNAs 145, 155, and 34c were upregulated in young BD rats compared to their respective sham. In addition, a downregulation of miRNA 34c was observed after BD (Figure 8).

## Discussion

Donor inclusion criteria have been expanded across organ transplantation to address organs shortage and increase the transplant opportunities. This expansion includes accepting organs from older donors, and, when the donor is female, it is important to consider menopause. Menopause is accompanied by several physiological changes, including those in the immune system. In order to understand the effects of menopause on lung donors, we submitted menopause rats to BD, comparing them with young animals. Most studies still focus on changes occurring in male

TABLE 3 Concentration of serum mediators (pg/mL) and explant (pg/mg) of rats from the young and menopause group submitted or not (Sham) to brain death. Serum 6 h and lung culture explant 24 h. Data presented as the mean  $\pm$  SEM.

| Groups             | Inflammatory mediators |                                     |                     |                                      |
|--------------------|------------------------|-------------------------------------|---------------------|--------------------------------------|
|                    | Serum                  |                                     | Explant             |                                      |
|                    | IL-1                   | IL-10                               | IL-1                | IL-10                                |
| Young sham         | 3.03 $\pm$ 0.18        | 3,137.11 $\pm$ 1,639.59             | 88.38 $\pm$ 100.53  | 4,197.70 $\pm$ 2,306.19              |
| Young BD           | 3.16 $\pm$ 0.72        | 8,687.55 $\pm$ 2,515.96*            | 239.05 $\pm$ 209.40 | 2,877.78 $\pm$ 2,400.87              |
| Menopause sham     | 4.04 $\pm$ 0.60        | 2,172.52 $\pm$ 1,539.55             | 46.02 $\pm$ 63.80   | 1,071.33 $\pm$ 375.90 <sup>a</sup>   |
| Menopause BD       | 3.96 $\pm$ 0.65        | 2,161.83 $\pm$ 2047.88 <sup>a</sup> | 180.64 $\pm$ 243.93 | 1,418.66 $\pm$ 1,314.57 <sup>a</sup> |
| <i>P</i> BD        | 0.9762                 | 0.1724                              | 0.0613              | 0.4138                               |
| <i>P</i> menopause | 0.3086                 | 0.0693                              | 0.4972              | 0.0005                               |

\*in relation to Young sham; <sup>a</sup> in relation to Young BD.

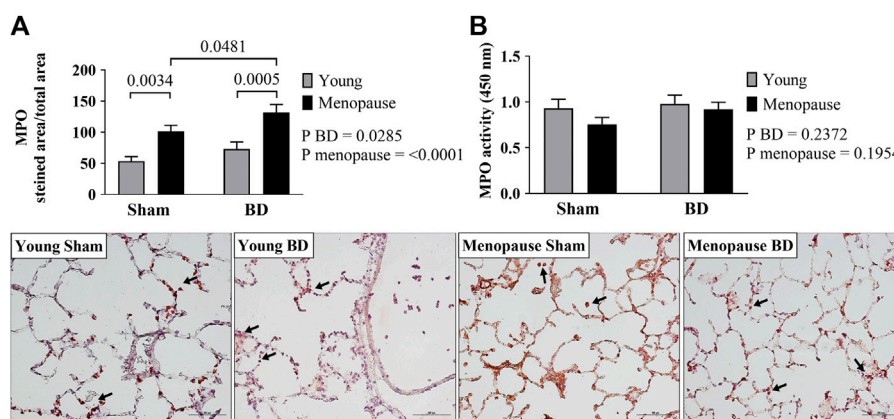


FIGURE 5

(A) Number of cells stained for MPO in tissue sections from 6 animals and (B) Myeloperoxidase (MPO) activity from 8 animals in lung tissue samples from young or menopause rats submitted or not (Sham) to BD. Data represents the mean  $\pm$  SEM. Arrows show higher number of cell stained for MPO in menopause group in relation to young.

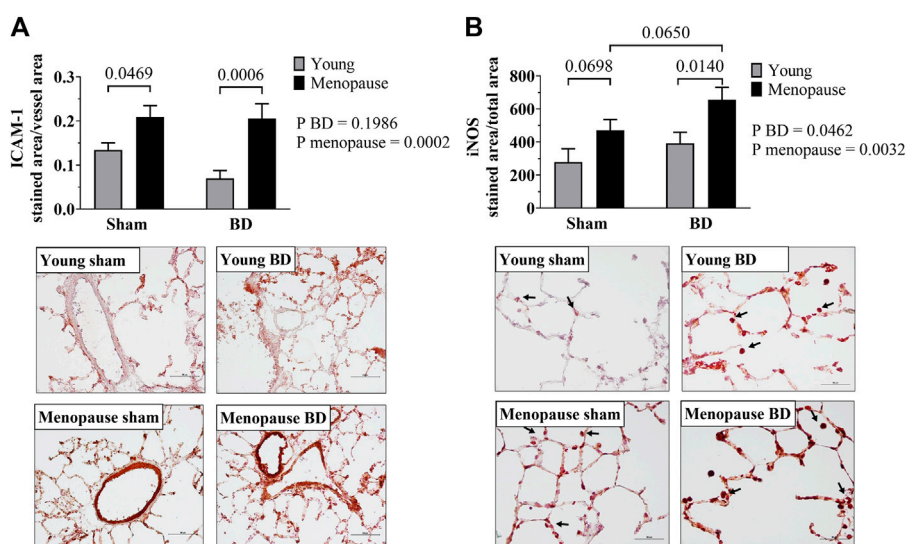


FIGURE 6

(A) Expression of intercellular adhesion molecule (ICAM)-1 and (B) inducible nitric oxide synthase (iNOS) in lung sections from young or menopause rats subjected or not (Sham) to BD. Representative photomicrographs of the immunohistochemical reaction. Data represents the mean  $\pm$  SEM from 5 animals.

donors; therefore, it is important to emphasize that this study specifically investigated the effects of menopause in organ donors, a group that has been understudied but growing steadily over the years. In this context, we chose to use an experimental model of menopause that better reflects the hormonal fluctuations observed clinically in women [13]. The association of BD with menopause exacerbated lung inflammation, reinforcing the importance of considering the woman's age and cycle stage when evaluating lung donors.

Previous clinical and experimental studies identified the acute reduction in female sex hormones following BD [9, 14]. This reduction is related to increased systemic and lung inflammation in young female rats, and treatment with estradiol is able to reduce

leukocyte infiltration and edema formation [15]. Here, menopause led to even higher leukocyte infiltration and edema, accompanied by an increase in all cell types in the BAL and increased MPO expression. In addition, in the menopause group, the leukocyte response was more intense and late compared to young animals. These results emphasize the greater mobilization of leukocytes to the lung parenchyma and suggest a compromise in the regulatory mechanisms of inflammation. In this sense, clinical studies have demonstrated that the menopause period is characterized by a pro-inflammatory state, induced mainly by the reduction of female sex hormones, and that the estrogen deficiency marked by menopause can impact the leukocyte subpopulation [16–18].

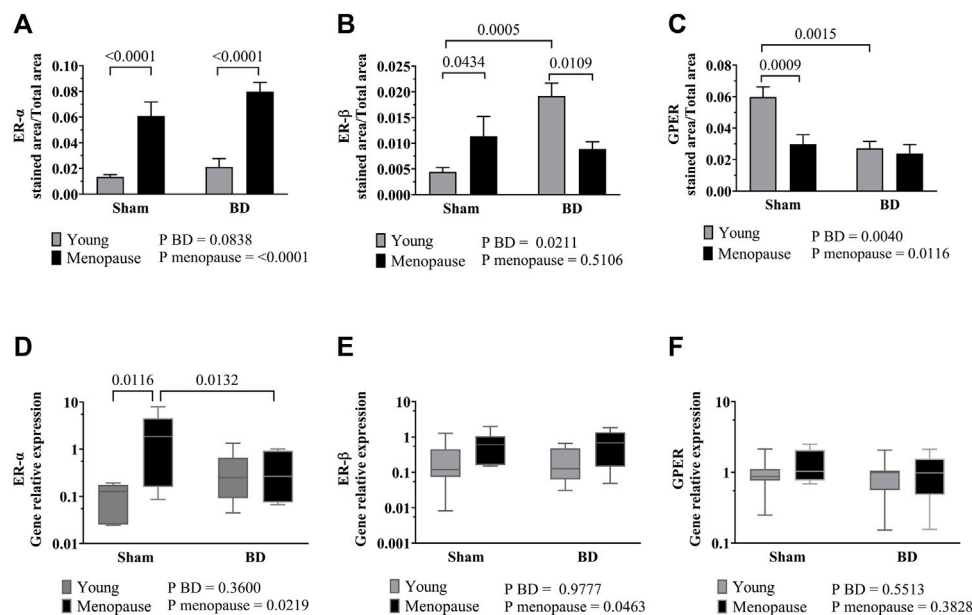


FIGURE 7

Protein and gene expression of the (A,D) estradiol receptor  $\alpha$  (ER- $\alpha$ ), (B,E) estradiol receptor  $\beta$  (ER- $\beta$ ) and (C,F) protein G associated estradiol receptor (GPER) in lung sections from young or menopause rats subjected or not (Sham) to BD. In (A–C), data represents the mean  $\pm$  SEM and in (D–F), data are expressed as median and 95% percentile interval ( $n = 8$  animals).

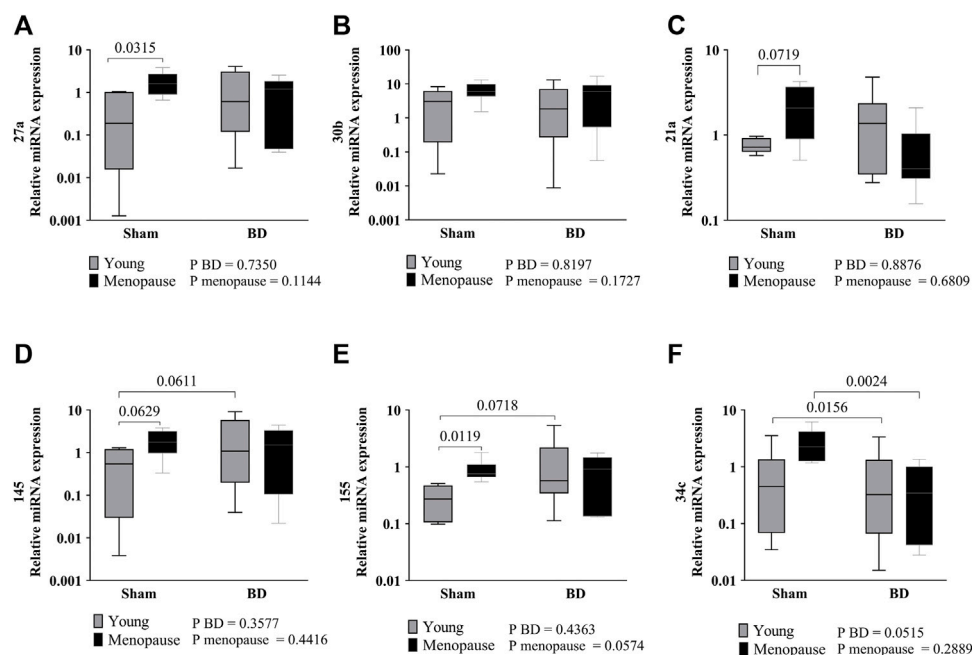


FIGURE 8

Analysis of miRNA expression in lung tissue from young or menopause rats subjected or not (Sham) to BD. (A) miRNA 27a; (B) miRNA 30b; (C) miRNA 21a; (D) miRNA 145; (E) miRNA 155 and (F) miRNA 34c. Data expressed as median and 95% percentile interval from 7 animals. Relative gene expression analysis and miRNA were normalized using endogenous control, and relative expression was determined using naive control (non-manipulated rats).

Our data indicate an increase in iNOS protein expression in lung tissue in the menopause group, as well as an increase in ICAM-1 expression, which may lead to greater leukocyte adhesion and migration to lung tissue. iNOS may contribute

to an imbalance in nitric oxide (NO) production, which can intensify the inflammatory process and favor increased vascular permeability [19]. In addition, higher lung hemorrhage was observed in the menopause sham group. These animals have

reduced hormone concentrations due to the induction of menopause and were maintained mechanically ventilated. One study showed that the reduction of sex hormones leads to the formation of new vessels and the loss of vascular protection conferred by estrogen [20]. These new vessels could, therefore, be more prone to injury and rupture, mainly to hemorrhage. This indicates that the menopause period itself can compromise vascular integrity. Previous study by the group using ovariectomized young rats [21] differ from this, specially by the time of hormonal reduction; here, the aging factor was added to the protocol, once menopause is a condition associated with higher age.

Since estrogen deficiency is associated with mechanisms that regulate inflammation, we investigated serum inflammatory mediators. IL-10 is a cytokine that helps to regulate the inflammatory response [22]. In our study, we observed increased IL-10 production in young rats, an effect not observed in the menopause group. Furthermore, we performed cytokine quantifications in lung explant culture medium (24 h), which reinforced the idea that there is a loss of regulation in the production of this cytokine. Clinical studies suggest that during menopause, women have higher production of pro-inflammatory cytokines and lower production of anti-inflammatory cytokines [22–24].

Considering the modulating role of estradiol in the inflammatory response, we performed analyses of estradiol alpha, beta, and GPER receptors. Genetic and protein alterations were observed with increased expression of ER- $\alpha$  in menopause rats, possibly a compensatory mechanism for the loss of the hormone. This receptor is strongly associated with reproductive function, vascular protection, and multiple mechanisms [25]. Regarding ER- $\beta$  and GPER receptors. ER- $\beta$  is associated with the modulation of the immune response and shows high expression in the lungs, while GPER, being a membrane receptor with rapid and non-genomic signaling, may not show detectable changes at the gene level [26–28].

To better understand the mechanisms involved in the inflammatory response observed primarily in menopause rats, we performed a miRNA screening based on their association with inflammatory processes, oxidative stress, apoptosis, senescence, and vascular function. We observed that miRNA 27a-3p, already upregulated in the menopause sham group, is associated with modulation of macrophage function and may suppress the anti-inflammatory cytokine IL-10 via TGF- $\beta$ , being largely studied as a potential therapeutic target for the treatment of inflammatory conditions [29, 30]. The miRNAs 21a, 145 and 155 were also found upregulated in the menopause sham group in comparison to young sham indicating their modulation by menopause process. The miRNA 21-5p is highly expressed in monocytes/macrophages and takes an important role in cardiovascular diseases, cancer, and immune response, in addition to its role in the process of chronic inflammation associated with aging (inflammaging) [31]. It can modulate the inflammatory response through direct and indirect activities in the NF- $\kappa$ B and NLRP3 pathways, depending on the context [32, 33]. Like miRNA 21a, miRNA 155 is strongly associated with inflammaging, and due to its central role in the innate and adaptive immune response, it has also been altered

during menopause, reinforcing its pro-inflammatory context [34, 35]. miRNA 145, is associated with vascular changes and leukocyte adhesion, in addition to its pro-inflammatory role [36]. This may contribute to the increased ICAM-1 observed in our results. In vascular smooth muscle cells from patients with diabetes type 2, the secretion of pro-inflammatory cytokines has been associated with elevated miRNA levels, suggesting a role in vascular senescence [37]. The miRNA 34c, associated with apoptosis and senescence processes [25], showed upregulation in the menopause group compared to young rats and controls. Studies show that miRNAs are related to menopause, since the hormonal decline alters post-translational regulatory mechanisms of genes involved in the regulation of the immune and inflammatory response. Our findings highlight the role of miRNAs in the context of BD induced lung inflammation in menopause females.

It is important to highlight that, from a clinical point of view, menopause in humans occurs alongside aging, which makes this model translationally relevant. The follicular depletion model using VCD represents one of the closest models to the human menopause transition. However, a limitation of the present study is that it does not allow for a complete differentiation between the effects of menopause and aging, since, after follicular depletion, the animals continue to age naturally. Therefore, some of the observed inflammatory effects may be related not only to the loss of ovarian hormones but also to processes associated with inflammaging. Additionally, the animals used do not correspond to such an advanced stage of aging, suggesting that senescence processes may still be in their initial phases. Future studies including the evaluation of hormonal rescue with estradiol or estrogen receptor modulators may help to further elucidate the mechanisms related to menopause and aging.

Taken together, our study reinforces the idea that female sex hormones have a significant influence on modulating the inflammatory state generated by BD. In addition, the results show that menopause intensifies lung inflammation, characterized by increased inflammatory infiltrate, alterations in nitric oxide synthase and adhesion molecules, and, most importantly, by the loss of IL-10 regulation. Considering the increasing number of female donors in menopause, our findings reinforce the relevance of investigating therapeutic strategies targeted at this group.

## 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 animal study was approved by Animal Research Ethics Committee of the Faculty of Medicine of the University of São Paulo under number (SDC) no 5635 23 037. The study was conducted in accordance with the local legislation and institutional requirements.



## Author contributions

EM: Participated in research design, animal operations, laboratory analyses, data analyses and writing the manuscript. NPAT, FY, PF, and MV-d-S: Participated in performing animal operations and laboratory analysis, data analyses and revision of the manuscript. LM: Participated laboratory analyses and revision of the manuscript. HL, LFPM, and CC: Participated in analyzing data and revising the manuscript. AB-F: Participated in research design and supervised the study and writing 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

The author(s) declared that generative AI was not used in the creation of this manuscript.

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## References

- Avlonitis VS, Fisher AJ, Kirby JA, Dark JH. Pulmonary transplantation: the role of brain death in donor lung injury. *Transplantation* (2003) 75(12):1928–33. doi: 10.1097/01.TP.0000066351.87480.9E
- Domínguez-Roldán JM, García-Alfaro C, Jimenez-González PI, Hernández-Hazañas F, Gascón Castillo ML, Egea Guerrero JJ. Muerte encefálica: repercusión sobre órganos y tejidos. *Med Intensiva* (2009) 33(9):434–41. doi: 10.1016/j.medin.2009.03.008
- Global Observatory on Donation and Transplantation (GODT). *International Report on Organ Donation and Transplantation Activities 2024* (2025). Report.
- Skrabal CA, Thompson LO, Potapov EV, Southard RE, Joyce DL, Youker KA, et al. Organ-specific regulation of pro-inflammatory molecules in heart, lung, and kidney following brain death. *J Surg Res* (2005) 123(1):118–25. doi: 10.1016/j.jss.2004.07.245
- Avlonitis VS, Wigfield CH, Kirby JA, Dark JH. The hemodynamic mechanisms of lung injury and systemic inflammatory response following brain death in the transplant donor. *Am J Transplant* (2005) 5(4):684–93. doi: 10.1111/j.1600-6143.2005.00755.x
- Barklin A. Systemic inflammation in the brain-dead organ donor. *Acta Anaesthesiol Scand* (2009) 53(4):425–35. doi: 10.1111/j.1399-6576.2008.01879.x
- Baldwin MR, Peterson ER, Easthausen I, Quintanilla I, Colago E, Sonett JR, et al. Donor age and early graft failure after lung transplantation: a cohort study. *Am J Transplant* (2013) 13(10):2685–95. doi: 10.1111/ajt.12428
- Bittle GJ, Sanchez PG, Kon ZN, Claire Watkins A, Rajagopal K, Pierson RN, et al. The use of lung donors older than 55 years: a review of the united network of organ sharing database. *The J Heart Lung Transplant* (2013) 32(8):760–8. doi: 10.1016/j.healun.2013.04.012
- Breithaupt-Faloppa AC, Ferreira SG, Kudo GK, Armstrong R, Tavares-de-Lima W, da Silva LFF, et al. Sex-related differences in lung inflammation after brain death. *J Surg Res* (2016) 200(2):714–21. doi: 10.1016/j.jss.2015.09.018
- Ferreira SG, Armstrong-Jr R, Kudo GK, de Jesus Correia C, dos Reis ST, Sannomiya P, et al. Differential effects of brain death on rat microcirculation and intestinal inflammation: female versus male. *Inflammation* (2018) 41(4):1488–97. doi: 10.1007/s10753-018-0794-7
- Correia Cde J, Ricardo da Silva FY, Armstrong R, Vidal dos Santos M, Anunciação LF, Sobral MLP, et al. Sex differences in the coagulation process and microvascular perfusion induced by brain death in rats. *Transpl Int* (2020) 33(11):1541–50. doi: 10.1111/tri.13731
- Simas R, Ferreira SG, Menegat L, Zanoni FL, Correia CJ, Silva IA, et al. Mesenteric hypoperfusion and inflammation induced by brain death are not affected by inhibition of the autonomic storm in rats. *Clinics (Sao Paulo)* (2015) 70(6):446–52. doi: 10.6061/clinics/2015(06)11
- Mayer LP, Pearsall NA, Christian PJ, Devine PJ, Payne CM, McCuskey MK, et al. Long-term effects of ovarian follicular depletion in rats by 4-vinylcyclohexene diepoxide. *Reprod Toxicol* (2002) 16(6):775–81. doi: 10.1016/S0890-6238(02)00048-5
- Armstrong Jr R, Ricardo-da-Silva FY, Correia CJ, Vidal-dos-Santos M, Anunciação LF, Coutinho e SRS, et al. Treatment with 17 $\beta$ -estradiol protects donor heart against brain death effects in female rat. *Transpl Int* (2020) 33(10):1312–21. doi: 10.1111/tri.13687
- Ricardo-da-Silva FY, Armstrong R, Vidal-dos-Santos M, Correia Cde J, Coutinho e SRS, da Anunciação LF, et al. 17 $\beta$ -Estradiol treatment protects lungs against brain death effects in female rat donor. *Transplantation* (2021) 105(4):775–84. doi: 10.1097/TP.0000000000003467
- Mathur S, Mathur RS, Goust JM, Williamson HO, Fudenberg HH. Cyclic variations in white cell subpopulations in the human menstrual cycle: correlations with progesterone and estradiol. *Clin Immunol Immunopathol* (1979) 13(3):246–53. doi: 10.1016/0090-1229(79)90069-2
- McCarthy M, Raval AP. The peri-menopause in a woman's life: a systemic inflammatory phase that enables later neurodegenerative disease. *J Neuroinflammation* (2020) 17(1):317. doi: 10.1186/s12974-020-01998-9
- Ryczkowska K, Adach W, Janikowski K, Banach M, Bielecka-Dabrowa A. Menopause and women's cardiovascular health: is it really an obvious relationship? *Arch Med Sci* (2023) 19(2):458–66. doi: 10.5114/aoms/157308
- Speyer CL, Neff TA, Warner RL, Guo RF, Sarma JV, Riedemann NC, et al. Regulatory effects of iNOS on acute lung inflammatory responses in mice. *Am J Pathol* (2003). 163(6):2319–28. doi: 10.1016/S0002-9440(10)63588-2
- Glinksi OV, Abraha TW, Turk JR, Rubin LJ, Huxley VH, Glinksi VV, et al. Microvascular network remodeling in dura mater of ovariectomized pigs: role for angiotensin-1 in estrogen-dependent control of vascular stability. *Am J Physiol Heart Circ Physiol* (2007) 293(2):1131–7. doi: 10.1152/ajpheart.01156.2006
- Bonnano Abib ALde O, Correia Cde J, Armstrong-Jr R, Ricardo-da-Silva FY, Ferreira SG, Vidal-Dos-Santos M, et al. The influence of female sex hormones on lung inflammation after brain death - an experimental study. *Transpl Int* (2020) 33(3):279–87. doi: 10.1111/tri.13550
- Gameiro CM, Romão F, Castelo-Branco C. Menopause and aging: changes in the immune system - a review. *Maturitas* (2010) 67:316–20. doi: 10.1016/j.maturitas.2010.08.003
- Malutan AM, Dan M, Nicolae C, Carmen M. Proinflammatory and anti-inflammatory cytokine changes related to menopause. *Menopausal Rev* (2014) 3:162–8. doi: 10.5114/pm.2014.43818
- Pfeilschifter J, Köditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. *Endocr Rev* (2002) 23(1):90–119. doi: 10.1210/edrv.23.1.0456
- Lafferty-Whyte K, Cairney CJ, Jamieson NB, Oien KA, Keith WN. Pathway analysis of senescence-associated miRNA targets reveals common processes to different senescence induction mechanisms. *Biochim Biophys Acta (Bba) - Mol Basis Dis* (2009) 1792(4):341–52. doi: 10.1016/j.bbdis.2009.02.003
- Villa A, Vegeto E, Poletti A, Maggi A. Estrogens, neuroinflammation, and neurodegeneration. *Endocr Rev* (2016) 37(4):372–402. doi: 10.1210/er.2016-1007
- Chakraborty B, Byemerwa J, Krebs T, Lim F, Chang CY, McDonnell DP. Estrogen receptor signaling in the immune system. *Endocr Rev* (2023) 44(1):117–41. doi: 10.1210/edrv/bnac017

28. Patrone C, Cassel TN, Pettersson K, Piao YS, Cheng G, Ciana P, et al. Regulation of postnatal lung development and homeostasis by estrogen receptor  $\beta$ . *Mol Cell Biol* (2003) 23(23):8542–52. doi: 10.1128/MCB.23.23.8542-8552.2003
29. Xie N, Cui H, Banerjee S, Tan Z, Salomao R, Fu M, et al. miR-27a regulates inflammatory response of macrophages by targeting IL-10. *The J Immunol* (2014) 193(1):327–34. doi: 10.4049/jimmunol.1400203
30. Li W, Qiu X, Liu J, Han Y, Wei D, Ji G, et al. miR-27a protects against acute lung injury in LPS-treated mice by inhibiting NF- $\kappa$ B-mediated inflammatory response. *Int J Clin Exp Pathol* (2018) 11(6):2980–9.
31. Olivieri F, Prattichizzo F, Giuliani A, Matacchione G, Rippon MR, Sabbatinelli J, et al. miR-21 and miR-146a: the microRNAs of inflammaging and age-related diseases. *Ageing Res Rev* (2021) 70:101374. doi: 10.1016/j.arr.2021.101374
32. Fujita S, Ito T, Mizutani T, Minoguchi S, Yamamichi N, Sakurai K, et al. miR-21 gene expression triggered by AP-1 is sustained through a double-negative feedback mechanism. *J Mol Biol* (2008) 378(3):492–504. doi: 10.1016/j.jmb.2008.03.015
33. Jenike AE, Halushka MK. miR-21: a non-specific biomarker of all maladies. *Biomark Res* (2021) 9(1):18. doi: 10.1186/s40364-021-00272-1
34. Mahesh G, Biswas R. MicroRNA-155: a master regulator of inflammation. *J Interferon Cytokine Res* (2019) 39(6):321–30. doi: 10.1089/jir.2018.0155
35. Ekiz HA, Ramstead AG, Lee SH, Nelson MC, Bauer KM, Wallace JA, et al. T cell-expressed microRNA-155 reduces lifespan in a mouse model of age-related chronic inflammation. *J Immunol* (2020) 204(8):2064–75. doi: 10.4049/jimmunol.1901484
36. Rahman MS, Ghorai S, Panda K, Santiago MJ, Aggarwal S, Wang T, et al. Dr. Jekyll or mr. Hyde: the multifaceted roles of miR-145-5p in human health and disease. *Noncoding RNA Res* (2025) 11:22–37. doi: 10.1016/j.ncrna.2024.11.001
37. Jimenez-Lucena R, Alcala-Diaz JF, Roncero-Ramos I, Lopez-Moreno J, Camargo A, Gomez-Delgado F, et al. MiRNAs profile as biomarkers of nutritional therapy for the prevention of type 2 diabetes mellitus: from the CORDIOPREV study. *Clin Nutr* (2021) 40(3):1028–38. doi: 10.1016/j.clnu.2020.06.035



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# Five years after introduction of an opt-out consent system for organ donation; lessons learned from England and the Netherlands

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Five years after changing from opt-in to opt-out consent systems in England and the Netherlands, we examine the impact on donor registrations, consent rates, donor numbers, and reflect on lessons learned. Opt-out legislation was implemented in May 2020 in England and July 2020 in the Netherlands. In England, 44% of the population is now registered; 40% recording an opt-in decision and 4% an opt-out decision. In the Netherlands, mandated donor registration resulted in the entire adult population having their decision recorded: “Yes, I want to be a donor” (34%), “No objection” (24%), or “No, I don’t want to be a donor” (31%), while 11% leave the “Decision to next of kin”. Despite these structural changes, the consent categories created by opt-out legislation (“deemed” consent in England and “No objection” in the Netherlands) remain challenging in practice, with fewer than 50% of families agreeing to donation. In England, the family consent rate declined from 69% to 57% between 2020 and 2025 and the donor numbers remain below pre-pandemic levels. In contrast, the Netherlands experienced a modest increase in family consent rates and donor numbers compared to the opt-in consent system. Opt-out legislation provides an important policy framework, but sustained system-level commitment remains essential.

### KEYWORDS

donor register, England and Netherlands, lessons learned, opt-out consent system, organ donation policy

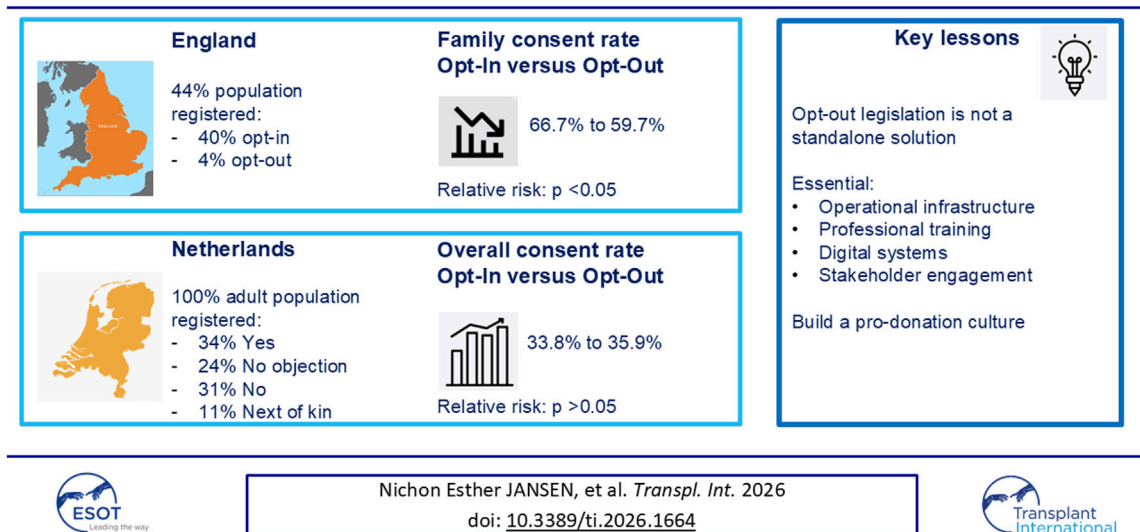
## Introduction

In May and July 2020, England and the Netherlands shifted their consent systems for organ donation from Opt-In to Opt-Out. The primary aim was to increase consent for organ donation and, ultimately, increase the number of organ donors.

In England, all adults aged 18 and over are considered potential organ and tissue donors unless they have made a decision not to donate or are part of a small number of protected groups. Under the legislation this is referred to as deemed consent. Within the United Kingdom, Wales commenced deemed consent in 2015, England 2020, Scotland in 2021 and Northern Ireland in 2023. The legislation across the four UK nations, and the UK’s Crown Dependencies, is broadly similar [1].

While there is no legal requirement to record a donation decision, individuals may choose to register an opt-in or opt-out decision on the National Health Service (NHS) Organ

## Five years after introduction of an Opt-Out consent system for organ donation; lessons learned from England and the Netherlands



GRAPHICAL ABSTRACT

Donor Register (ODR) or express their wishes verbally to family members, who can relay this information during donation discussions.

The primary route for opt-in registrations is through driving licence applications or renewals, accounting for approximately 89% of registrations [2]. In comparison, around 7% occur via the website and 4% via the NHS App. Opt-out decisions can only be recorded through the website or NHS App. In the Netherlands, an “active donor registration” process was implemented, fundamentally changing the structure of the Donor Register into a mandated choice system. By July 2021, the entire population aged 18 and older had a recorded decision. Individuals could register “Yes,” “No,” or delegate the decision to next of kin or a specific person. Those who did not respond were registered as having “No objection,” ensuring a recorded preference for all adults. Individuals can change their decision at any time. In 2022, several of the current authors (NEJ, CW, DG) published an early comparative analysis of the introduction of opt-out legislation in England and the Netherlands [3]. Full details of implementation and the consent systems are outlined in that paper. We concluded that legislation alone is unlikely to increase donation and that successful implementation requires established operational infrastructure, adoption of digital infrastructure, clinical codes of practice, professional training, sustained public awareness campaigns and engagement across society, including with faith and community groups.

We also observed that legislative reform can enable wider system investment, stimulating funding for donation infrastructure and public promotion, and opening dialogue with communities about organ donation. At the time of publication, however, it was too early to determine the impact of opt-out legislation on consent rates or donor numbers, and any early analysis would be confounded by the COVID-19 pandemic.

Now, 5 years after the introduction of opt-out legislation, this study evaluates its impact on donor registrations, consent rates and donor numbers in England and the Netherlands, and provides further insights into the lessons learned.

## Materials and methods

### England

Data on donor registrations, consent rates and numbers of organ donors were obtained from NHS Blood and Transplant (NHSBT), which maintains the ODR, the Potential Donor Audit (PDA) and the UK Transplant Registry.

The PDA captures all deaths under the age of 81 occurring in UK intensive care units or emergency departments and identifies potential organ donors. Data are entered by Specialist Nurses in Organ Donation (SNODs). A key metric derived from the PDA is the organ donation consent rate, defined as the proportion of eligible families approached for a donation decision in whom consent for donation is obtained; in England this includes those donors who have registered an opt-out decision. For comparative purposes in this paper, this is referred to as family consent. Population estimates for England were obtained from the Office for National Statistics [4].

### The Netherlands

In the Netherlands, donor registration data were obtained from the national Donor Register [5]. In addition, medical records of patients who died in intensive care units were reviewed retrospectively. Potential donors were defined as donors who are medically suitable for donation, regardless of their registration in the



Donor Register, including those with a “No” registration. In the Netherlands the key measure of consent is the overall consent rate, defined as the proportion of all potential donors in whom consent for donation is obtained; this includes those who have registered a “No” decision.

These data are recorded in NovaNORD, a database maintained by the Dutch Transplant Foundation, and entered by (organ) donor coordinators [6].

## Comparative analysis

For comparative purposes, donor register data for both countries were summarised for the period before and after the introduction of opt-out legislation (2019–2025). Organ donation consent rates were analysed for 2017–2025 and deceased donor numbers for 2010–2025 to provide longer-term contextual trends.

Relative Risk and corresponding 95% confidence intervals (CI's) were calculated to test the hypothesis that opt-out legislation would increase the overall consent rate for organ donation. The “Risk in exposed group” is analysed for the years *during* the opt-out legislation (2022–2025) and the “Risk in unexposed group” for the years *before* implementation of the legislation (2017–2019). The years 2020 and 2021 were excluded due to potential confounding effects of the COVID-19 pandemic. Statistical significance was assessed using P-values corresponding to values of the standard normal distribution; a P-value <0.05 was considered statistically significant. All Sankey diagrams created using SankeyMATIC.

## Ethics statement

This study, using data from Dutch and English organ donation data registers, was conducted in accordance with the principles of the World Medical Association Declaration of Helsinki. Both the Netherlands and England operate under ethical frameworks consistent with the Declaration of Istanbul on Organ Donation and Transplantation. No interventions or procedures involving human participants were performed, and only anonymized, registry-based data were used.

## Results

### Organ donor registrations

#### England

In January 2026, 44% of the 57.7 million residents in England were registered on the ODR, comprising 40% with an opt-in decision and 4% with an opt-out decision. This compares to 35% opt-in and 2% opt-out of the population in January 2020, immediately prior to the implementation of deemed consent legislation. Only 136 individuals had nominated a representative to make a donation decision on their behalf. Trends in donor registration between 2019 and 2026 are shown in Figure 1.

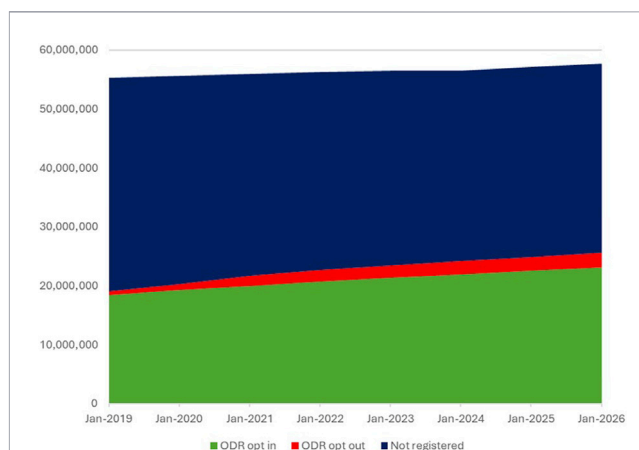


**FIGURE 1**  
Number of donor registrations in England on the NHS Organ Donor Register 1<sup>st</sup> of January 2019–2026\*. \* Only 136 individuals have nominated a representative to make a donation decision on their behalf, so this is not displayed in the figure.

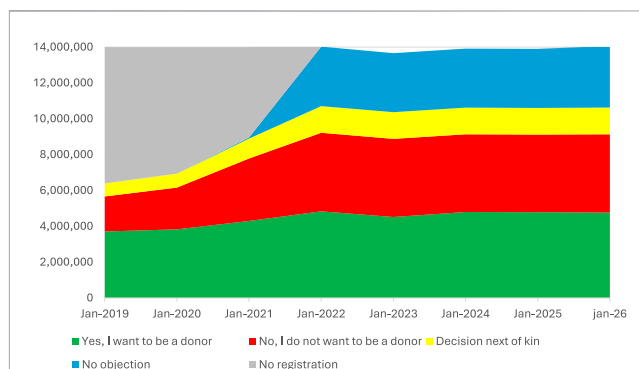


**FIGURE 2**  
Number of donor registrations in the Netherlands on the 1st of January 2019–2026.

### The Netherlands

Following the active donor registration process, the entire Dutch population aged 18 years and older was recorded in the Donor Register by July 2021. In January 2026, 34% of residents had registered “Yes, I want to be a donor,” 24% “No objection,” and 31% “No, I do not want to be a donor,” while 11% delegated the “Decision to next of kin” or “Specific person.” Overall, 58% of the population had registrations permitting organ donation. Registration proportions have remained largely unchanged since July 2021 (Figure 2).

### Family consent rates for organ donation

#### England

The annual family consent rate for organ donation following approaches to donor families in England declined from 69% in 2020 to 57% in 2025, the lowest level recorded in more than a decade (Table 1). See Supplementary Table 2 for the distinction in family

TABLE 1 Annual consent rate for organ donation following approaches of donor families in England and the Netherlands January 2017 – December 2025.

| Calendar year | England                          |                              | Netherlands                      |                              |
|---------------|----------------------------------|------------------------------|----------------------------------|------------------------------|
|               | Opt-out implemented May 20, 2020 |                              | Opt-out implemented July 1, 2020 |                              |
|               | Annual family consent rate (%)   | Monthly range (min %, max %) | Annual family consent rate (%)   | Monthly range (min %, max %) |
| 2017          | 65                               | (62, 69)                     | 45                               | Not available                |
| 2018          | 67                               | (61, 71)                     | 44                               | Not available                |
| 2019          | 68                               | (64, 74)                     | 42                               | Not available                |
| 2020          | 69                               | (65, 74)                     | 48                               | Not available                |
| 2021          | 66                               | (58, 72)                     | 60                               | (52, 71)                     |
| 2022          | 62                               | (56, 70)                     | 60                               | (51, 65)                     |
| 2023          | 61                               | (57, 70)                     | 61                               | (49, 72)                     |
| 2024          | 59                               | (52, 62)                     | 59                               | (48, 73)                     |
| 2025          | 57                               | (54, 61)                     | 56                               | (48, 61)                     |

In the Netherlands, the family consent rate includes all potential donor families approached for donation. Following the implementation of the legislation, many individuals registered as ‘No’ were no longer approached because their donation decision was already known. In England, the family consent rate includes all eligible donor families approached for donation, including those of donors who had opted out.

consent rates between donation after brain death (DBD) and donation after circulatory death (DCD) donors.

## The Netherlands

In the Netherlands, the annual family consent rate for organ donation following approaches to donor families declined from 60% in 2021 to 56% in 2025. However, this is still higher than the family consent rate under the opt-in system, which peaked at 48% in 2020 (Table 1).

## Donor registration, family approach, and overall consent rate

### England

Changes to the PDA data collection system in September 2020 enabled more accurate recording of donor registration status. Consequently, the proportion of unknown registrations declined from 71% in 2017 to 12% in 2025 (Table 2). Since September 2020, the potential donors with known registration status, the proportion registered as opt-in increased steadily to 37% by 2025, while opt-out registrations remained low at approximately 3%.

The consent group of identified eligible organ donors included in the PDA in England 2017 – 2025 is provided in Supplementary Table 1. The number of families approached for organ donation, based on the consent group, and family consent rate in England 2017 – 2025 is given in Supplementary Table 3. The Sankey visuals compare the period before and after implementation of opt-out legislation, see Figure 3.

The inferential statistical analysis, using Relative Risk, showed that the overall consent rate in England is significantly lower following the implementation of opt-out legislation compared to the period before, see Table 3.

## The Netherlands

Analysis of potential organ donors between 2017 and 2025 shows an increase in “Yes” registrations from 24% to 29% and the emergence of the “No objection” category to approximately 20% of potential donors (Table 4). Over the same period, the proportion of potential donors registered as “No, I do not want to be a donor” increased from 16% to 39%. The proportion without a recorded registration declined from 52% to 6% following implementation of active donor registration.

Family support varied by registration category. Families generally supported donation when the donor had registered “Yes,” whereas in cases of “No objection” families declined donation in more than half of cases (Supplementary Table 4). When the decision was delegated to next of kin, consent rates ranged between 28% and 52%. Considering both donor registration and family decision-making, the overall consent rate across all potential donors remained approximately 35% between 2017 and 2025.

The Sankey visuals compare the period before and after implementation of opt-out legislation, see Figure 4.

The inferential statistical analysis, using Relative Risk, showed that the overall consent rate in the Netherlands is not significantly higher following opt-out legislation compared to the period before, see Table 3.

## Organ donor numbers

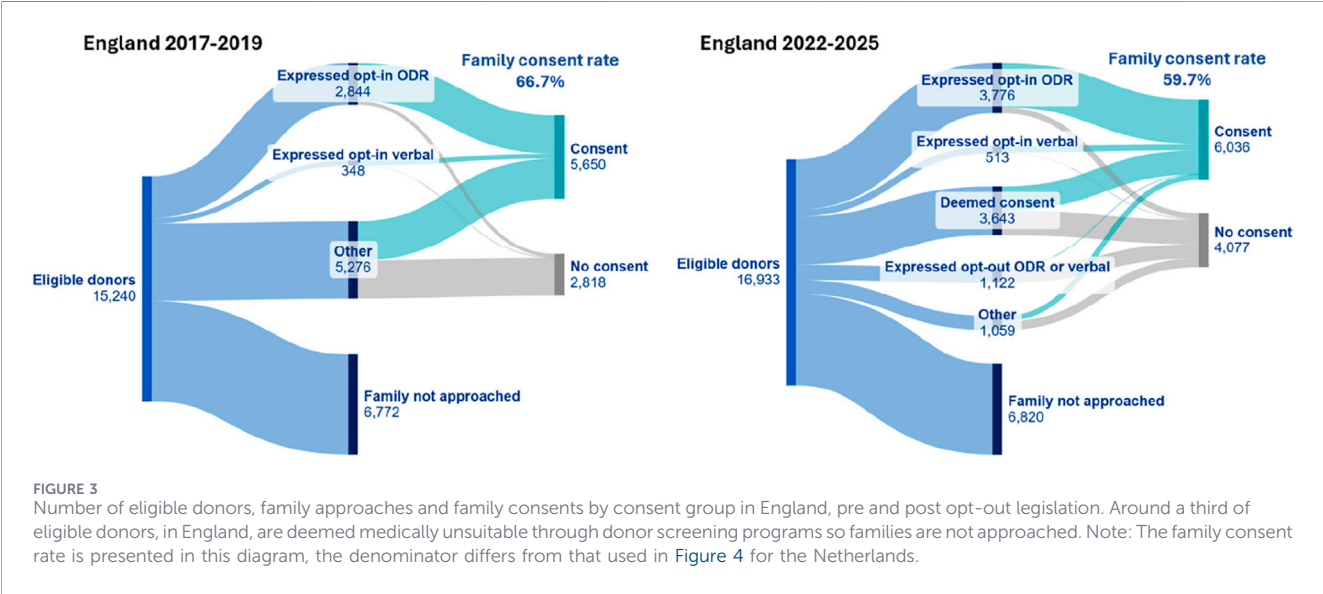
### England

Deceased donor numbers declined sharply in 2020, coinciding with the introduction of opt-out legislation and the COVID-19 pandemic. Donor numbers fell from a peak of 1,432 in 2019 to 1,043 in 2020. Numbers have since increased gradually to 1,207 in 2025, although they remain below pre-pandemic levels (Figure 5). In

TABLE 2 Registration on the Organ Donor Register (where known) of identified potential organ donors\* in England included in the Potential Donor Audit 2017–2025.

| Calendar year | Total audited deaths number | Total potential donors number | Total number of potential donors where ODR registration known | ODR opt-in registration number/Total where ODR registration known (%) | ODR opt-out registration number/Total where ODR registration known (%) | Not registered number/Total where ODR registration known (%) | *Register not consulted or outcome not documented number/Total potential donors number (%) |
|---------------|-----------------------------|-------------------------------|---------------------------------------------------------------|-----------------------------------------------------------------------|------------------------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------------------------------------|
| 2017          | 28,507                      | 6,226                         | 1,786                                                         | 1,564/1,786 (87.6)                                                    | 7/1,786 (0.4)                                                          | 215/1,786 (12.0)                                             | 4,440/6,226 (71.3)                                                                         |
| 2018          | 27,926                      | 6,196                         | 1,772                                                         | 1,539/1,772 (86.9)                                                    | 24/1,772 (1.4)                                                         | 209/1,772 (11.8)                                             | 4,424/6,196 (71.4)                                                                         |
| 2019          | 27,631                      | 6,331                         | 1,965                                                         | 1,720/1,965 (87.5)                                                    | 42/1,965 (2.1)                                                         | 203/1,965 (10.3)                                             | 4,366/6,331 (69.0)                                                                         |
| 2020          | 30,960                      | 6,367                         | 2,743                                                         | 1,581/2,743 (57.6)                                                    | 100/2,743 (3.6)                                                        | 1,062/2,743 (38.7)                                           | 3,624/6,367 (56.9)                                                                         |
| 2021          | 35,239                      | 6,255                         | 4,707                                                         | 1,615/4,707 (34.3)                                                    | 123/4,707 (2.6)                                                        | 2,969/4,707 (63.1)                                           | 1,548/6,255 (24.7)                                                                         |
| 2022          | 31,195                      | 5,895                         | 5,035                                                         | 1,713/5,035 (34.0)                                                    | 148/5,035 (2.9)                                                        | 3,174/5,035 (63.0)                                           | 860/5,895 (14.6)                                                                           |
| 2023          | 29,975                      | 5,937                         | 5,153                                                         | 1,887/5,153 (36.6)                                                    | 130/5,153 (2.5)                                                        | 3,136/5,153 (60.9)                                           | 784/5,937 (13.2)                                                                           |
| 2024          | 29,888                      | 5,910                         | 5,123                                                         | 1,871/5,123 (36.5)                                                    | 139/5,123 (2.7)                                                        | 3,113/5,123 (60.8)                                           | 787/5,910 (13.3)                                                                           |
| 2025          | 27,865                      | 5,714                         | 5,024                                                         | 1,878/5,024 (37.4)                                                    | 169/5,024 (3.4)                                                        | 2,977/5,024 (59.3)                                           | 690/5,714 (12.1)                                                                           |

\*The ODR, is not consulted for all potential donors; where potential donors are assessed to be medically unsuitable for organ donation prior to the family approach, the ODR, register may not be checked.



recent years, DCD has exceeded DBD, with 678 DCD and 529 DBD donors recorded in 2025.

The Netherlands

Between 2010 and 2025, the number of deceased donors in the Netherlands increased following implementation of the opt-out system, with the highest number recorded in 2024 ( $n = 360$ ). However, donor numbers in recent years remain broadly comparable with earlier peaks, such as 2014 ( $n = 269$ ). A notable trend is the increasing number of donors following euthanasia, rising from 13 in 2022 to 34 in 2025. In total, 189 organ donors following euthanasia have been realised since 2012 (Figure 6).

Discussion

Reflections after 5 years of implementation of the opt-out consent system

England

A government-commissioned evaluation of deemed consent legislation in England examined the perspectives of families involved in donation discussions [7, 8]. Many families had limited understanding of deceased organ donation and perceived themselves as the principal decision-makers, rather than deemed

TABLE 3 Comparison of overall consent rates in England and the Netherlands before and after implementation of the Opt-Out Act.

| Hypothesis           | Group                          | Consent<br>(n) | Objection<br>(n) | Total  | Risk<br>[95% CI]    | Relative risk (RR);<br>[95%CI] | Chi   | P-value |
|----------------------|--------------------------------|----------------|------------------|--------|---------------------|--------------------------------|-------|---------|
| England              |                                |                |                  |        |                     |                                |       |         |
| Overall consent rate | Exposed (opt-out Act)          | 6,063          | 10,897           | 16,933 | 0.356 [0.349–0.363] | 0.961 [0.934–0.989]            | –2.65 | <0.05*  |
|                      | Unexposed (before opt-out Act) | 5,650          | 9,590            | 15,240 | 0.370 [0.363–0.378] |                                |       |         |
| Netherlands          |                                |                |                  |        |                     |                                |       |         |
| Overall consent rate | Exposed (opt-out Act)          | 1,022          | 2,001            | 3,023  | 0.338 [0.321–0.354] | 1.060 [0.995–1.128]            | 1.842 | >0.05   |
|                      | Unexposed (before opt-out Act) | 1,679          | 3,006            | 4,685  | 0.358 [0.344–0.372] |                                |       |         |

CI, confidence interval; \* = p-value &lt;0.05.

TABLE 4 Registration in the Donor Register of identified potential organ donors\* in the Netherlands in the intensive care unit 2017–2025.

| Calendar year | Total deaths in ICUs number | Total identified potential donors number | Yes, I want to be a donor number/ Total registered number (%) | No objection number/ Total registered number (%) | No, I do not want to be a donor number/ Total registered number (%) | Decision by next of kin number/ Total registered number (%) | Not registered number/ Total registered number (%) | *Register not consulted number/ Total potential donors number (%) |
|---------------|-----------------------------|------------------------------------------|---------------------------------------------------------------|--------------------------------------------------|---------------------------------------------------------------------|-------------------------------------------------------------|----------------------------------------------------|-------------------------------------------------------------------|
| 2017          | 7,755                       | 951                                      | 203/851 (23.6)                                                | NA                                               | 137/851 (16.1)                                                      | 67/851 (7.9)                                                | 444/851 (52.2)                                     | 100/951 (10.5)                                                    |
| 2018          | 7,990                       | 1,039                                    | 229/921 (24.9)                                                | NA                                               | 158/921 (17.2)                                                      | 64/921 (6.9)                                                | 470/921 (51)                                       | 118/1,039 (11.4)                                                  |
| 2019          | 7,373                       | 1,033                                    | 189/904 (20.9)                                                | NA                                               | 184/904 (20.4)                                                      | 62/904 (6.6)                                                | 469/904 (51.9)                                     | 129/1,033 (12.5)                                                  |
| 2020          | 7,618                       | 987                                      | 205/876 (23.4)                                                | NA                                               | 204/876 (22.8)                                                      | 52/876 (5.9)                                                | 415/876 (47.4)                                     | 111/987 (11.2)                                                    |
| 2021          | 7,750                       | 915                                      | 250/874 (28.6)                                                | 158/874 (18.1)                                   | 302/874 (34.6)                                                      | 80/874 (9.2)                                                | 84/874 (9.6)                                       | 41/915 (4.5)                                                      |
| 2022          | 6,981                       | 1,102                                    | 309/1,050 (29.4)                                              | 214/1,050 (20.4)                                 | 403/1,050 (38.4)                                                    | 79/1,050 (7.5)                                              | 45/1,050 (4.3)                                     | 52/1,102 (4.7)                                                    |
| 2023          | 6,810                       | 1,147                                    | 318/1,101 (28.9)                                              | 218/1,101 (19.8)                                 | 439/1,101 (39.9)                                                    | 77/1,101 (7)                                                | 49/1,101 (4.5)                                     | 46/1,147 (4)                                                      |
| 2024          | 6,793                       | 1,244                                    | 344/1,188 (29)                                                | 237/1,188 (19.9)                                 | 426/1,188 (35.9)                                                    | 110/1,188 (9.3)                                             | 71/1,188 (6)                                       | 56/1,244 (4.5)                                                    |
| 2025          | 6,732                       | 1,192                                    | 318/1,129 (28.2)                                              | 222/1,129 (19.7)                                 | 440/1,129 (39)                                                      | 78/1,129 (7)                                                | 71/1,129 (6.3)                                     | 63/1,192 (5.3)                                                    |

NA, not applicable.

\*The Donor Register was not consulted for all identified potential organ donors, such as in cases where donation had already been discussed with the family before consulting the register, the donor was from abroad, or the donor was a child.

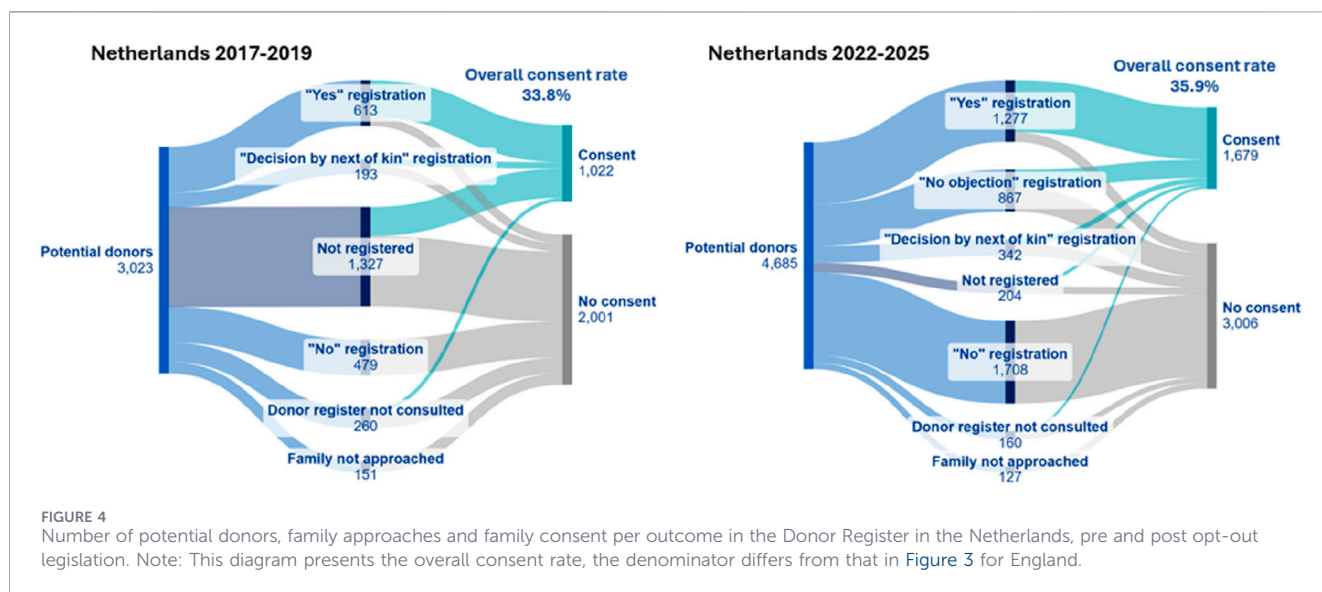
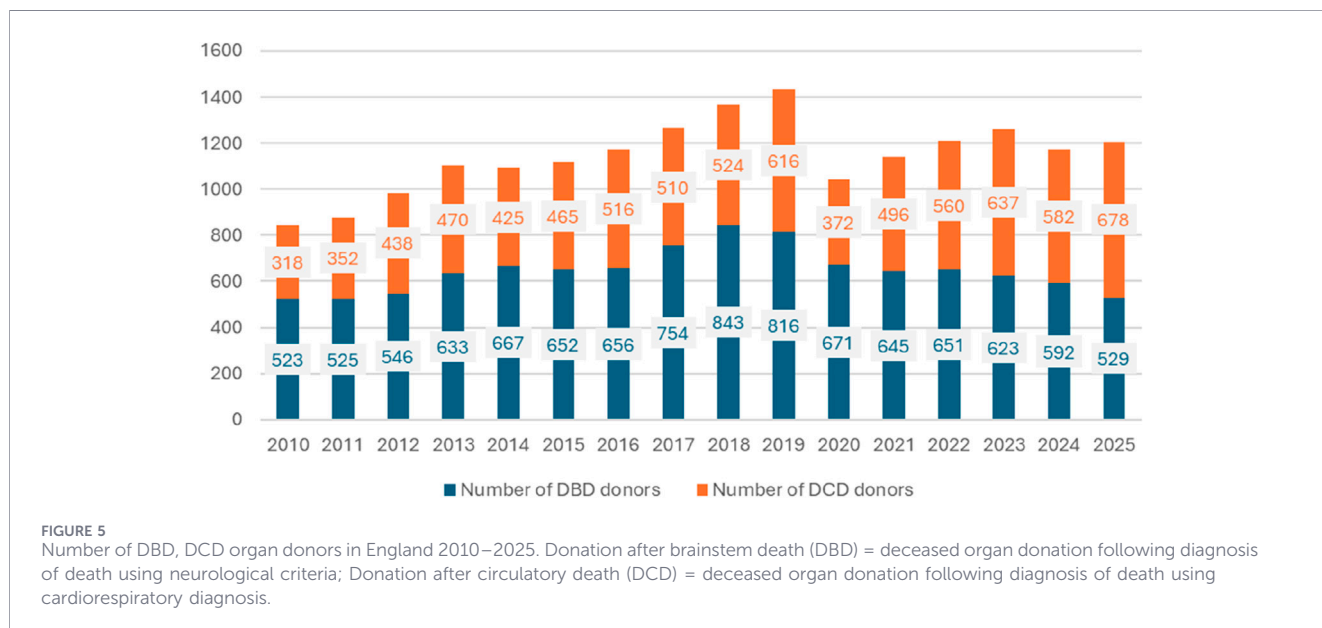
consent as an expression of the deceased's choice. Families relied heavily on specialist nurses in organ donation for guidance and emotional support and reported high satisfaction with their care. Healthcare professionals reported that the legislation had not reduced the complexity of family discussions or end-of-life care, and most remained unconvinced that legislation alone would improve consent rates. The evaluation concluded that deemed consent was unlikely to have caused the observed decline in consent rates, suggesting other factors influenced the reduction in consent and donor numbers.

Public attitudes towards the NHS have shifted markedly since the COVID-19 pandemic. YouGov® polling indicated 70% of respondents rated the NHS as “good” in 2020, whereas since

October 2022 more than 60% have consistently rated it as “bad” [9]. A preliminary analysis suggests a moderate positive correlation between positive public opinion of national NHS services and family consent rates (correlation coefficient +0.73; [Supplementary Figure 1](#)). Internal NHSBT surveys similarly show declining public awareness of the organ donation system and softening support for donation in principle.

In England, the coexistence of opt-in and opt-out pathways creates a complex dual consent system. Deemed consent may confuse the public, leading to misunderstanding or disengagement, where individuals neither opt-in nor opt-out. Registration patterns have remained relatively stable since implementation, suggesting limited sustained public engagement.





On the other hand, the inferential analysis demonstrated significant lower overall consent rates.

England operates a “soft” opt-out model in which families are expected to support the deceased’s decision. In practice, families may override both expressed opt-in decisions and deemed consent. In more than 50% of applicable deemed cases in recent years, families have not supported donation (Table 3). This highlights the continued centrality of the family at the bedside and raises important questions about the practical authority of individual decisions and “soft” opt-out legislation. Similar patterns across other UK nations suggest these challenges are systemic rather than purely legislative.

Together, these findings suggest that legislative reform alone is insufficient to create a supportive culture for deceased organ donation. Societal understanding, trust in healthcare institutions

and effective professional practice remain central to donation discussions and family consent.

## The Netherlands

Since implementation of the opt-out system in July 2020, active donor registration has doubled registrations in the Donor Register from 7 million to 14 million, covering the entire population aged 18 and older. Knowing the potential donor’s wishes provides greater clarity during the family approach. The annual family consent rate rose to approximately 56%, compared with 48% under the opt-in system. However, the large number of “No, I do not want to be a donor” registrations has considerably reduced the donor pool. When the entire consent process, from donor registration to family approach, is considered, the increase in overall consent

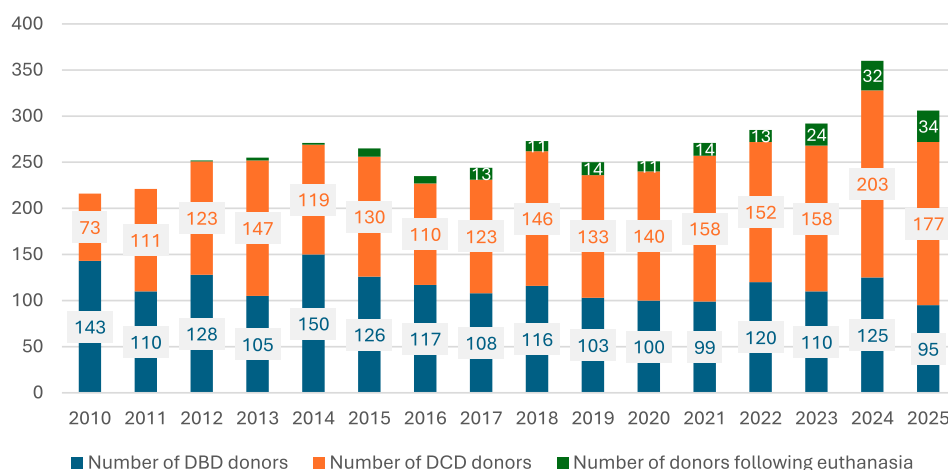


FIGURE 6

Number of DBD, DCD, and donors following euthanasia in the Netherlands 2010–2025. Donation after brain death (DBD) = deceased organ donation following diagnosis of death using neurological criteria; Donation after circulatory death (DCD) = deceased organ donation following diagnosis of death using cardiorespiratory diagnosis.

rate compared with the opt-in system is small, which was also the outcome of the inferential analysis.

The number of realised donors has nevertheless increased. This partly reflects increasing donation after euthanasia. In 2024, an exceptionally high number of potential donors, despite declining intensive care deaths, also contributed to a high number of realised donors. Greater opportunities for extended criteria donors, abdominal normothermic regional perfusion for DCD donors, machine preservation, and, since 2021, DCD heart donation have further expanded the potential donor pool. Given the limited increase in overall consent, the increase in realised donors cannot be attributed to the opt-out system alone.

A positive aspect of the Dutch system is that all 14 million adults are now registered, ensuring that family approaches are based on the potential donor's documented wishes. Under the opt-in system, families often faced donation decisions without knowing their loved one's preferences. The opt-out system has also prompted discussion within many families, with some now initiating donation conversations in intensive care.

## England

Faced with falling donor numbers in 2024, the Organ Donation Joint Working Group (ODJWG), jointly chaired by the Department of Health and Social Care and NHS Blood and Transplant produced *A Bolder, Braver Approach for Organ Donation in the UK*. [10]. The Group brought together UK clinical leaders, donor family representatives and international experts. It concluded that deemed consent legislation is positive and permissive, does not require amendment and has helped signal support for donation and enable system improvements. However, it found no reliable evidence that opt-out legislation alone increases consent rates and noted persistent public confusion about deemed consent. The report advises against using legislation in public-facing marketing, unless legally required, or in discussions with grieving families. Deemed consent was considered too complex and

potentially counterproductive in acute grief, with concern that referencing the law may provoke resistance or “reactance.” Instead, communication should focus on the individual's values, the positive impact of donation and supporting families. The Group recommends acting within the law but not leading with it, positioning legislation as a supportive framework rather than a tool of persuasion. Implementation of the Report is ongoing across the UK.

England's experience provides several lessons. We continue to consider the legislation valuable: it signalled societal and governmental support for donation while respecting individual autonomy through the mechanism of opt-out. It also enabled engagement with faith and community groups, broadening conversations beyond the clinical setting.

However, implementation could have been bolder. Campaigns emphasised choice and positioned opt-in and opt-out equally, rather than articulating a clear pro-donation societal norm. The law alone therefore did not generate a pro-donation culture, a factor that moderates policy effectiveness [11]. If opt-out legislation is intended as both a legal and cultural shift, it requires confident, sustained public messaging that supports pro-donation social norms while respecting individual autonomy. Low public acceptance may otherwise hinder successful implementation [12].

## The Netherlands

The opt-out system was introduced during the COVID-19 pandemic, limiting opportunities to train intensivists on approaching families about the new “No objection” category. In-person training was not possible, and even trained intensivists might wait months or years before encountering their first case. Van Oosterhout et al. found that donor conversations involve a complex interplay between donor registration and clinician-family interactions [13]. Clinicians' personal considerations, previous experiences with the family and professional context shaped four distinct approaches to discussing “Yes” or “No

objection” registrations, each reflecting increasing family influence by the family on the outcome of the donation decision.

The family’s role is also influenced by awareness and understanding of the opt-out system. Registration of 7 million previously unregistered residents occurred during the COVID-19 pandemic, a period of high mortality fear, that may not have been ideal for considering donation preferences. However, 31% registered “No, I don’t want to be an organ donor” suggesting that those strongly opposed could still record their preference.

Similar findings emerged in Nova Scotia, Canada, where deemed consent was also introduced during the pandemic. Sarti et al. found variable family awareness of the new law and their role in decision-making [14]. Some were optimistic about deemed consent, while others felt that continued family consultation limited its potential impact.

## Limitations

Our study has several limitations. First, implementation of opt-out legislation in both England and the Netherlands coincided with the COVID-19 pandemic, making it difficult to separate the effects of legislative change from wider impacts on healthcare systems, public attitudes and organ donation activity. Second, this observational analysis of national registry and audit data cannot establish causality. Third, organ donation activity is influenced by multiple factors beyond legislation. Fourth, differences in registration systems, data collection methods and consent processes limit direct comparisons between countries. Finally, donor registrations and consent rates may not fully capture wider societal attitudes towards donation.

## Conclusion

Five years on, our earlier conclusions remain broadly valid. Legislation alone has not transformed donation rates. Operational infrastructure, professional training, digital systems and stakeholder engagement remain essential. In both England and the Netherlands, opt-out legislation functioned as an enabler, signalling governmental commitment, unlocking investment, and creating opportunities for engagement with faith and community groups.

We underestimated the extent to which legislative change is symbolic rather than transformative in itself. While opt-out legislation signals a pro-donation stance, it does not automatically create a pro-donation culture. The Dutch experience illustrates this clearly: the new category “No objection” remains challenging, with fewer than half of families supporting donation.

We also underestimated the importance of narrative clarity and cultural positioning. In both countries, public campaigns were deliberately neutral, emphasising individual choice and avoiding pressure to donate. While principled, this limited the opportunity to present donation as an expected and valued social contribution. Legislative reform that aspires to cultural change requires sustained,

confident public messaging that supports donation as a shared societal good while respecting individual autonomy.

Taken together, the experiences of England and the Netherlands suggest that opt-out legislation should be understood not as a standalone solution, but as a structural platform from which supportive systems, public engagement and a pro-donation culture can be built.

## Data availability statement

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

## Author contributions

All authors participated in the study design, data analysis, and interpretation of the findings, as well as in the critical review and revision of the manuscript. NJ and DG drafted the manuscript; TW, MH, AH, CW, and SM contributed to data interpretation, provided expert input, and reviewed the final version. 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.2026.16664/full#supplementary-material>

## References

1. Parsons JA. Deemed consent for organ donation: a comparison of the English and Scottish approaches. *J L Biosciences* (2021) 8(1):lsab003. doi: 10.1093/jlb/lsab003
2. NHS Blood and Transplant. Annual activity report 2024/25. ODT clinical – NHS blood and transplant (2025). Available online at: <https://www.odt.nhs.uk/statistics-and-reports/annual-activity-report/> (Accessed March 3, 2026).
3. Jansen NE, Williment C, Haase-Kromwijk BJJM, Gardiner D. Changing to an opt out system for organ donation-reflections from England and Netherlands. *Transpl Int* (2022) 35(Jul 4):35. doi: 10.3389/ti.2022.10466
4. Office for National Statistics. *Office for National Statistics*. UK Government (2026). Available online at: <https://www.gov.uk/government/organisations/office-for-national-statistics> (Accessed March 3, 2026).
5. Donorregister. Cijfers Newport: Office for National Statistics (2026). Available online at: <https://www.donorregister.nl/over-het-donorregister/cijfers> (Accessed March 3, 2026).
6. Nederlandse Transplantatie Stichting. Cijfers over organen en weefsels (Power BI dashboards) (2026). Available online at: <https://www.transplantatiestichting.nl/cijfers-over-organen-en-weefsels> (Accessed March 3, 2026).
7. McLaughlin L, Williams L, Noyes J, Al-Haboubi M, Boadu P, Bostock J, et al. *Evaluation of the Organ Donation (Deemed Consent) Act, 2019 in England: Final Report*. London: National Institute for Health Research, Policy Innovation and Evaluation Research Unit (2024). Report to Department of Health and Social Care and. doi: 10.17037/PUBS.04673056
8. McLaughlin L, Mays N, Al-Haboubi M, Williams L, Bostock J, Boadu P, et al. Potential donor family behaviours, experiences and decisions following implementation of the organ donation (deemed consent) act 2019 in England: a qualitative study. *Intensive Crit Care Nurs* (2025) 86:103816. doi: 10.1016/j.iccn.2024.103816
9. YouGov. *How Good or Bad Are National NHS Services?* (2026). Available online at: <https://yougov.com/en-gb/trackers/how-good-or-bad-are-national-nhs-services> (Accessed March 3, 2026).
10. NHS Blood and Transplant. A bolder, braver approach for organ donation in the UK: final report of the organ donation joint working group (2025). Available online at: <https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/38066/odjwg-report.pdf> (Accessed March 3, 2026).
11. Johnson EJ, Goldstein D. Medicine. Do defaults save lives? *Science* (2003) 302(5649):1338–9. doi: 10.1126/science.1091721
12. Güntürkün P, Studte S, Winkler D, Clement M, Tan JHW, Merz EM, et al. Crowding-out effects of opt-out defaults: evidence from organ donation policies. *PNAS Nexus* (2025) 4(10):pgaf311. doi: 10.1093/pnasnexus/pgaf311
13. van Oosterhout SPC, van der Niet AG, Abdo WF, Boenink M, Cherpanath TGV, Epker JL, et al. How clinicians discuss patients' donor registrations of consent and presumed consent in donor conversations in an opt-out system: a qualitative embedded multiple-<sup>case</sup> study. *Crit Care* (2023) 27(1):299. doi: 10.1186/s13054-023-04581-9
14. Sarti AJ, Sutherland S, Weiss M, Landry A, Hemming H, Dirk J, et al. Nova Scotia's deemed consent for deceased organ donation: family member perspectives and experiences in the ICU setting. *Transpl Direct* (2024) 10(11):e1713. doi: 10.1097/TXD.0000000000001713





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# The SystemCHANGE™ intervention improves immunosuppressive medication-taking habit: a secondary analysis of the MAGIC studies

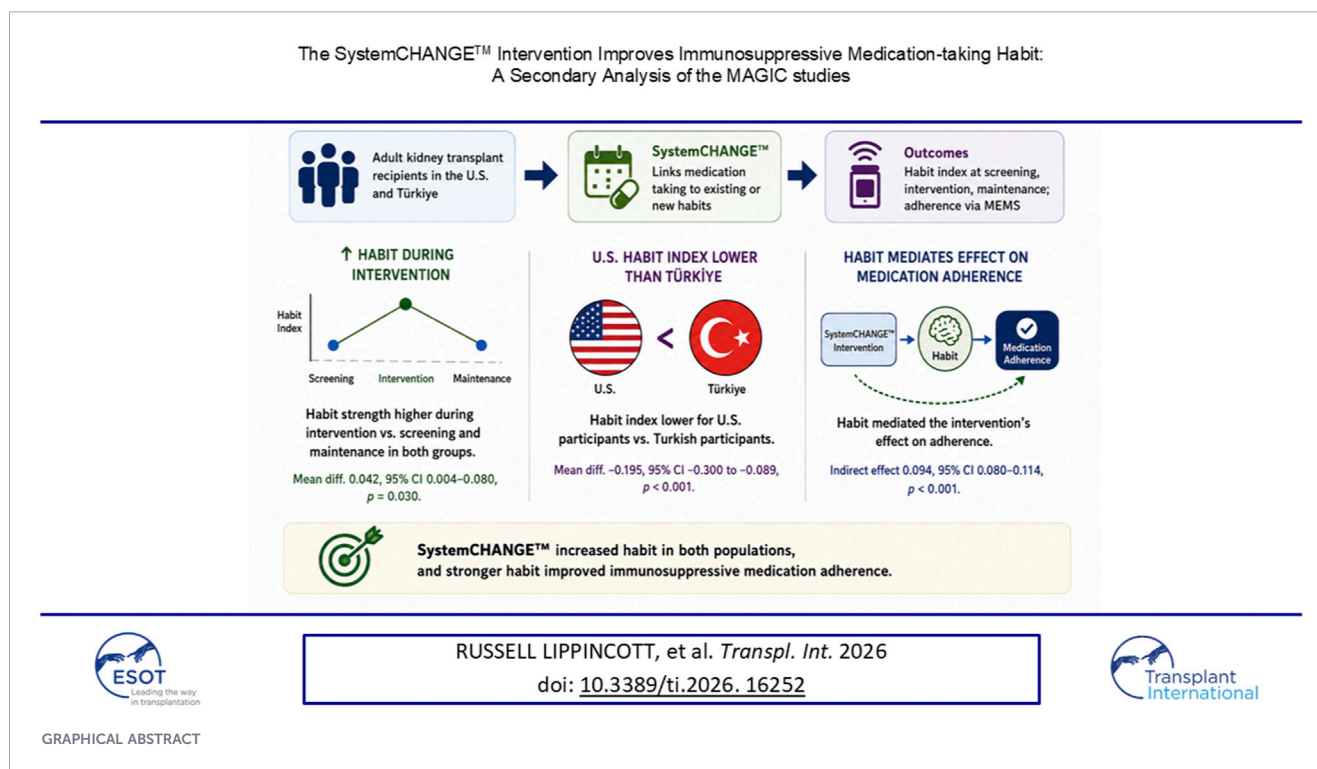
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Habit impacts healthy behaviors. The SystemCHANGE™ intervention supports associating medication taking with existing habits or creating new habits to improve medication adherence. This secondary analysis of United States and Türkiye MAGIC study data examines differences in habit between SystemCHANGE™ and attention control groups, differences in habit between countries, and habit as a relationship mediator between the SystemCHANGE™ intervention and immunosuppressive medication adherence in adult kidney transplant recipients. The Medication Event Monitoring System measured medication adherence. Objective habit index was computed during screening, intervention, and maintenance. During the SystemCHANGE™ intervention, habit strength was higher than during the screening and maintenance periods in both groups (mean difference 0.042, 95% confidence interval (CI): 0.004 to 0.080,  $p = 0.030$ ). Habit index was lower for US participants compared to Turkish participants (mean difference  $-0.195$ , 95% CI:  $-0.300$  to  $-0.089$ ,  $p < 0.001$ ). Habit was a mediator of the SystemCHANGE™ intervention on medication adherence (indirect effect was 0.094, 95% CI: 0.080 to 0.114,  $p < 0.001$ ). The SystemCHANGE™ intervention exerted an impact on habit in two different populations which then had a positive impact on medication adherence.

### KEYWORDS

adults, habit strength, kidney transplantation, medication adherence, secondary analysis



## Introduction

Health behavior change interventions have traditionally focused on motivation and intention with mixed results, particularly with difficult to change behaviors such as medication adherence. Such studies ignore routines and habits, which may account for their marginal effectiveness for individuals with acute and chronic illnesses [1–4] and equivocal findings for adult kidney transplant recipients [5].

Habit is critical in changing and maintaining healthy behaviors. Approximately 43% of daily behaviors are performed out of habit [6]. Habits are defined as automatic impulses to engage in a behavior in the presence of conditioned contextual cues. Habit formation is the process by which a behavior becomes automatic or habitual through regular repetition. Repetition of a behavior in a consistent context progressively leads to increased automaticity with which the behavior is performed when the situation is encountered [7, 8]. After some time, the automaticity of the behavior reaches a plateau and becomes a habit.

Duhigg theorized the “habit loop” to explain the process by which a habit is formed. The cue occurs first and “tells your brain to go into automatic mode and which habit to use”. [9], p.19 The routine happens next. It is the “physical, mental or emotional behavior that follows the cue”. [9], p.19 Finally, the reward occurs which “helps your brain figure out if this particular loop is worth remembering for the future”. [9], p.19 The loop becomes more automatic over time until a habit is created.

For a health behavior habit to form, it takes about 2 months, but the time is variable, with some habits taking nearly a year to form and become automatic [10]. Factors that positively influence habit

formation include habits selected by individuals themselves, more frequent habit practice, morning habits, habits that are more enjoyable, stability of the context in which the habit takes place, and planning specifically for implementation of the habit [10].

The association between habit and medication adherence is clear. In a systematic review of 11 studies, using self-report of habit and behavior automaticity, habit strength was strongly associated with medication adherence in 91% of the studies [11]. Recently, habit strength has been measured using more rigorous objective measures instead of self-report. In a secondary analysis of 108 studies and 15,818 participants, most of whom were being treated for hypertension and osteoporosis, medication adherence was associated with lower daily or weekly medication timing variability, so stronger habit strength [12]. Additionally, habit strength accounted for over 30% of the variability in medication adherence. Likewise, in 79 patients with type 2 diabetes receiving oral medications, higher habit strength using an objective measure, was significantly associated with medication adherence [13].

The Change Habits by Applying New Goals and Experience (SystemCHANGE™) intervention, has propelled adherence interventions in a new direction [14, 15]. This is a secondary analysis of the US and Türkiye MAGIC study data, which sought to examine 1) differences in habit strength between the SystemCHANGE™ intervention group and the attention control group, as well as demographic predictors of this difference, 2) differences in habit strength between the two countries, and 3) habit as a mediator of the relationship between the SystemCHANGE™ intervention and immunosuppressive medication adherence, using a new objective measure of habit called the “habit index”, in adult kidney transplant recipients.

## Materials and methods

### Design

This is a secondary analysis of the MAGIC ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study?term=MAGIC&rank=1) identifier NCT02416479) and MAGIC Türkiye ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study?term=MAGIC_Turkiye&rank=1) identifier NCT06106854) studies' data. The designs were single-blinded (participants), two-arm randomized controlled trials using repeated measures [14, 15]. Immunosuppressive medication intakes during the three-month screening phase were recorded using electronic monitoring. Immunosuppressive medication adherence during screening was quantified by computing the agreement of recorded intakes with the prescribed number and timing of intakes. After the screening phase, participants with an immunosuppressive medication adherence score of  $\geq 85\%$  exited the studies and those with a score of less than 85% were given the opportunity to enter the intervention phase of the studies where they were randomized to either the SystemCHANGE™ intervention (treatment) or the attention control intervention (control). Electronic monitoring continued in both groups. The 85% cut point was established with adult kidney transplant recipients using a cluster analysis approach where data from those who were adherent with morning and evening immunosuppressive medication doses had an electronic medication monitoring adherence score ranging from 85% to 100% [16]. Additionally, immunosuppressive medication nonadherence below 85% has been shown to impact clinical outcomes for adult kidney transplant recipients [17]. All participants received standard care. The attention control intervention delivered over 6 months included monthly receipt of an educational brochure addressing healthy living with a kidney transplant. After the six-month intervention phase a six-month maintenance phase followed during which there was no intervention. Electronic monitoring of both groups continued. The objective of the maintenance phase was to observe if the effects of the intervention remained once the intervention stopped.

The SystemCHANGE™ intervention is based on socio-ecological theory [18] and process improvement methods [19]. In an iterative process using a plan-do-check-act improvement process, patients create a 'personalized system solution' by embedding their medication-taking in existing daily routines, evaluating the solution's effect on consistent medication-taking guided by objective medication adherence data and an interventionist, then revising the solution as necessary and continuing the cycle.

### Setting and sample

For the MAGIC US study, participants were recruited from five transplant centers in the midwestern and southern US. For the MAGIC Türkiye study, participants were recruited from a transplant center in Izmir, Türkiye. The inclusion criteria were: individuals 18 years of age or older who had received a kidney-only transplant; who self-administered at least one prescribed immunosuppressive medication taken twice daily with a functioning kidney transplant; who were not hospitalized; had no diagnosis that would immediately shorten the lifespan; had access to a telephone; had the ability to speak, hear, and understand English or Turkish; were able to open an electronic monitoring cap on a medication bottle; had agreement from the transplant physician

and nephrologist to participate; and were not cognitively impaired. In both studies, all participants who met the study inclusion criteria were invited to be in the study and screened for 3 months for adherence. For the US study, 281 individuals were excluded due to high adherence while 130 participants with medication non-adherence were invited to be in the intervention; 41 of these declined and 89 were randomized. Five participants dropped out from the study during the intervention period. For the Türkiye study, 16 were excluded due to high adherence while 60 participants with medication non-adherence were invited to be in the intervention; 18 of these declined/were unable to continue in the study and 42 were randomized with no attrition during the intervention phase.

### Instruments

The following variables were collected at baseline: gender, age, relationship status, education level (as a 3-level categorical variable: primary school, secondary school, and college or higher), employment status, etiology of kidney disease, and type of transplant (from living or deceased donor).

Immunosuppressive medication adherence was measured using the Medication Event Monitoring System ([MEMS®], AARDEX Group, Belgium) which includes a bottle cap with a microprocessor that records the occurrence and time of each bottle opening [20].

Habit index was computed from the objective medication adherence data using a recently developed habit index [13], quantifying week-by-week consistency in the pattern of medication intakes. This habit index was previously shown to correlate with a widespread, self-reported habit metric [13]. Computation of the objective habit index is located in the [Supplementary Material](#). The habit index was computed for each participant during the entire 3-month screening, during each month of 6-month intervention and during each month of the 6-month maintenance period. The habit index ranges between 0 and 1, with 0 corresponding to taking medication at systematically different times (more than 2 h apart) from 1 week to the other, and 1 corresponding to a participant having a perfectly consistent medication intake pattern from week to week, e.g., taking the medication at 10:47 am every Monday, at 2:59 pm every Tuesday, not taking a dose on Wednesdays, etc.

### Procedure

For the MAGIC US study, the Institutional Review Board determined the present secondary analysis of the MAGIC US study data was exempt (Project number 2095804). For the MAGIC Türkiye study, the Institutional Review Board (Project number 2023/27-02) approved the study. Habit strength and immunosuppressive medication adherence data were extracted by a data analyst from the medication intake data.

### Data analysis

Demographic data were characterized using descriptive statistics. Chi-squared tests were used to compare count data between countries, and a Student's unpaired t-test was used to compare age and baseline habit between the two populations.

TABLE 1 Participant demographics (n = 126).

| Variable                      | Overall n, % or mean, $\pm$ SD | United States (n = 84) | Türkiye (n = 42)       | p-value for equality |
|-------------------------------|--------------------------------|------------------------|------------------------|----------------------|
| Gender                        |                                |                        |                        |                      |
| Male                          | 79 (62.7)                      | 49 (58.3)              | 30 (71.4)              | 0.152                |
| Female                        | 47 (37.3)                      | 35 (41.7)              | 12 (28.6)              |                      |
| Age in years                  | 49.7 $\pm$ 11.7                | 51.6 $\pm$ 10.6        | 46.1 $\pm$ 13.2        | 0.022                |
| Relationship status           |                                |                        |                        |                      |
| Living with someone           | 75 (59.5)                      | 48 (57.1)              | 27 (64.3)              | 0.441                |
| Single                        | 51 (40.5)                      | 36 (43.9)              | 15 (35.7)              |                      |
| Education                     |                                |                        |                        | <0.001               |
| Primary school                | 23 (18.3)                      | 5 (6.0)                | 18 (42.9)              |                      |
| Secondary school              | 59 (46.8)                      | 54 (64.3)              | 5 (11.9)               |                      |
| College or higher             | 44 (34.9)                      | 25 (29.7)              | 19 (45.2)              |                      |
| Employment status             |                                |                        |                        | <0.001               |
| Retired                       | 21 (16.7)                      | 9 (10.7)               | 12 (28.6)              |                      |
| Unemployed/Housewife          | 15 (11.9)                      | 7 (8.3)                | 8 (19.0)               |                      |
| Disabled/self employed/other  | 31 (24.6)                      | 31 (36.9)              | 0 (0.0)                |                      |
| Working full or part-time     | 59 (46.8)                      | 37 (44.0)              | 22 (52.4)              |                      |
| Etiology of kidney disease    |                                |                        |                        | 0.193                |
| Hypertension                  | 45 (35.7)                      | 30 (35.7)              | 15 (35.7)              |                      |
| Polycystic kidney disease     | 14 (11.1)                      | 10 (11.9)              | 4 (9.5)                |                      |
| Diabetes mellitus             |                                |                        |                        |                      |
| CKD/Other/Unknown/<br>Missing | 23 (18.3)<br>4 (34.9)          | 11 (13.0)<br>31 (39.4) | 12 (28.6)<br>11 (26.2) |                      |
| Type of transplant            |                                |                        |                        | <0.001               |
| Cadaveric/deceased donor      | 79 (62.7)                      | 62 (73.8)              | 17 (40.5)              |                      |
| Living donor                  | 47 (37.3)                      | 22 (26.2)              | 25 (59.5)              |                      |
| Baseline habit                | 0.517 $\pm$ 0.232              | 0.400 $\pm$ 0.146      | 0.764 $\pm$ 0.180      | <0.001               |

A mixed linear model with random intercepts was used to model habit strength, measured at several timepoints, as a function of the following covariates: habit during screening, treatment group, phase in the study (intervention or maintenance), country, gender, age, and type of transplant (from living or deceased donor). Interaction terms between group, phase, and country were also modelled. These variables were selected *a priori* for inclusion in the model. Addition of more demographic variables in the model was investigated using a bidirectional forward stepwise approach and likelihood ratio tests, as detailed in the [Supplementary Material](#). This procedure did not result in including additional demographic variables in the model.

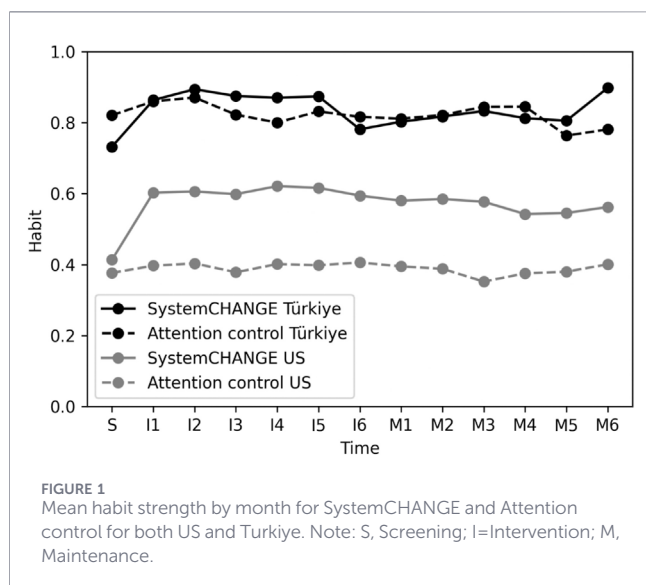
A mediation analysis was performed to investigate whether habit was a mediator of the effect of the intervention on medication adherence. Medication adherence, more precisely its implementation component [21], was quantified as the proportion of days with exactly two immunosuppressive medication intakes. The mediation analysis used several regressions to estimate the direct effect of habit on medication adherence and its indirect effect through the intervention. The previously selected demographics were included in these regression models. Computations were performed using Python version 3.7.3 [22]. Further details on statistical methodology are provided as [Supplementary Material](#).

## Results

Data from the MAGIC US study included 84 participants with mean age 51.6 years (standard deviation [SD] 10.6 years), with 58.3% (n = 49) male. The data from the MAGIC Türkiye study included 42 participants with mean age 46.1 years (SD 13.2 years), with 71.4% (n = 30) male. [Table 1](#) delineates the overall and country-specific sample demographics. The two populations are different in several aspects: age, education, employment status and type of transplant.

[Figure 1](#) shows the evolution of mean habit strength among the two groups (intervention versus attention control) among the two studies (US and Türkiye). The underlying data are provided in a [Supplementary Table](#). For participants in the intervention groups for both countries, habit strength was higher during the SystemCHANGE™ intervention than during screening and maintenance (regression coefficient for interaction between group and study phase = 0.042, 95% CI: 0.004 to 0.080, p = 0.030, with reference group being control and reference phase being maintenance). Participants from the US had a lower habit index overall when compared to Türkiye (regression coefficient for country being US = -0.195, 95% CI: -0.300 to -0.089, p < 0.001). Habit in US participants seems to remain higher even





during the maintenance phase (regression coefficient for interaction between country and group = 0.111, 95% CI: 0.002 to 0.221,  $p = 0.047$  with reference group being control and reference country being US). Finally, older participants had a slightly higher habit index (regression coefficient = 0.002, 95% CI: 0.000 to 0.005,  $p = 0.038$ ). No other demographic variable had a statistically significant impact.

The mediation analysis demonstrated that habit mediated the effect of intervention on medication adherence. The SystemCHANGE™ intervention had a significant effect on adherence (total effect = 0.215, 95% CI: 0.192 to 0.238,  $p < 0.001$ ). The direct effect of the intervention on adherence was 0.121 (95% CI: 0.102 to 0.140,  $p < 0.001$ ). The indirect effect of the intervention on adherence (through improving habit) was 0.094 (95% CI: 0.080 to 0.114,  $p < 0.001$ ), meaning that approximately 43% of the effect of the intervention is mediated by habit.

## Discussion

This is the first study to show that habit strength, measured using a new objective habit metric, was statistically significantly higher when participants were receiving the SystemCHANGE™ intervention, compared to those receiving an attention-control intervention. The SystemCHANGE™ intervention's impact on habit was immediately effective because the participants implemented the SystemCHANGE™ at the beginning of the intervention when the intervenor and the participant collaborated to explore habits around desired medication taking time. This included identifying important people for the medication taking process, examining the participant's life routines, habits and life cycles, and identifying and initiating a SystemCHANGE™ personalized system solution. For example, these solutions included placing medication next to regularly occurring habits such as near the coffee pot when making coffee, near the toothbrush when brushing teeth, near the television

remote control for watching favorite shows, or near pet medications when pets received their medications. Because these solutions embedded medications in existing habits and were implemented at the beginning of the intervention, habit strength and consequently, medication adherence, immediately improved and were maintained [14, 23, 24]. As such, this provides evidence for future testing of reduced 'doses' of the SystemCHANGE™ intervention or perhaps providing periodic boosters.

These findings are consistent with a recent systematic review of 11 studies including people taking medications for asthma, diabetes, hypertension, cystic fibrosis, psoriasis and contraception that found habit strength was strongly correlated with medication adherence, and stronger habit was associated with higher medication adherence rates [11]. These findings are also congruent with the recent study by Phillips et al. where the same objective habit metric was used to demonstrate an association between habit strength and medication adherence in adults with type 2 diabetes during a 1 month period [13].

This study is also the first to explore habit strength across two different countries, and therefore, cultural groups finding country-level differences in medication-taking habit during screening, during the SystemCHANGE™ intervention and the maintenance period between the US and Türkiye. These differences may originate from the different populations included in the two studies. The finding that habit strength was higher in the younger Turkish sample compared to the older US participants contrasts with the findings that older aged individuals, even with some cognitive decline, had higher medication taking automatized routines compared to healthy adults [25]. The routines and habits established in older individuals have been viewed as protective for medication adherence.

Other, more important differences than age between the two populations could explain the differences. The influence of the Turkish sample having significantly more living donor kidney transplants than the US sample on habit strength and thus immunosuppressive medication adherence could be explained by the findings that Türkiye has the highest rate of living-donor transplantation worldwide [26]. Moreover, nearly 80% of living-donor transplantations are performed using organs donated by first- or second-degree relatives. This unique characteristic may influence post-transplant outcomes, as family-member donors are often more actively involved in the recipient's care process. Living donors may monitor the recipients of their kidneys in matters such as taking medications [27]. This may have contributed to the high level of medication adherence among recipients. Such involvement may enhance the recipient's awareness of the donation process and foster a greater sense of responsibility toward protecting the transplanted organ, thereby promoting better adherence to medical recommendations and long-term graft care.

These results confirm the hypothesized effect of the SystemCHANGE™ intervention. Indeed, this intervention is designed to link medication-taking to a patient's established routines, to support improving medication-taking habit. The results of the present work demonstrated that habit was a mediator of the positive effect of the intervention on medication adherence.

## Strengths and limitations

This study's follow up of 6 months for the intervention period and 6 months for the maintenance period are much longer than previous habit strength studies [13]. The SystemCHANGE™ intervention taps into reliable personal systems for linkage of medication taking routines to habit so that medication taking happens at the same time every day without relying on intention or memory. Consequently, habit strength and thus medication adherence is sustained over long periods of time [14].

The original MAGIC studies aimed at testing whether the SystemCHANGE™ intervention improved medication adherence. Studies on behavioral interventions, by design, suffer a self-selection bias, as participants who may benefit the most from an intervention are also more likely to decline participation in the study [28].

The size of the US sample was determined through a power analysis aimed at detecting an effect of the SystemCHANGE™ intervention on medication adherence. This analysis is reported in the original article [14]. On the other hand, the Turkish sample was small, which might have prevented detecting an effect of the intervention during the maintenance period. This could limit the generalizability of the findings to the population. Additionally, important structural differences between the populations such as age, education level, employment status, and type of transplant donor may introduce confounding and limit the interpretation of country-level differences. The finding that the Turkish sample was younger, less educated, retired/less disabled, and received more living donor kidney transplants could have influenced the results and therefore, country-level differences should be interpreted with caution.

This analysis did not examine other variables that may influence immunosuppressive medication taking habit strength such as time of day during which the habit occurred and stability of the context in which the habit took place [10]. Additionally, depression and anxiety, two factors that may influence habit were also not studied [29].

The reference value of the habit index was computed over the entire 3-month screening period, to replicate the methodology that was used for the computation of the medication adherence score in the original MAGIC studies. However, habit (and medication adherence) may have changed during the 3-month screening period, because of the introduction of electronic monitoring of adherence causing a Hawthorne effect. The effect of the introduction of electronic monitoring on adherence is unclear [16, 30, 31] and, to the best of the authors' knowledge, the effect of the introduction of electronic monitoring on habit has not been studied.

Finally, because this work is a secondary analysis that was not prespecified in the design of the original MAGIC studies, it carries the associated limitations. Deciding upon this analysis *a posteriori*, once the trial results were already available, introduces a potential risk of analytical bias, meaning these findings should be considered exploratory.

## Clinical implications

These findings have important clinical implications. Briefly assessing habits of kidney transplant recipients around the prescribed times of immunosuppressive medication taking could

provide actionable strategies for linking medication taking to existing routines therefore establishing good medication taking habits. Additionally, conversations between the provider and the patient should happen early in the post-transplant period so positive medication taking habits can be established as soon as possible after transplant.

## Future research

Further trials are planned to examine whether these results are replicable in those with other chronic conditions such as chronic kidney disease and breast cancer using the SystemCHANGE™ intervention, the habit strength index, and objectively measured medication adherence using the MEMS®. Additionally, multi-level influences on habit strength should be explored, including the link between demographic variables and habit strength. Finally, the 6-month SystemCHANGE intervention involved monthly "checks" with participants collaboratively reviewing their medication adherence data and assessing whether the SystemCHANGE solution was continuing to be effective. The intervention was immediately effective within the first month with a slight drop in habit strength between months 5 and 6. Consequently, testing a 3 and 6 months SystemCHANGE "booster" seems like the next logical step in this line of research.

## Conclusion

This study's results support that objectively measured habit strength is higher at the completion of a six-month SystemCHANGE™ intervention when compared to a six-month attention-control intervention in two culturally diverse samples of adult kidney transplant recipients. This positive impact on habit strength is linked to a positive impact on medication adherence.

## Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The dataset is available upon contacting the corresponding author and with IRB approval. Requests to access these datasets should be directed to Cynthia Russell Lippincott russellc@umkc.edu.

## Ethics statement

This study involving humans was approved by University of Missouri-Kansas City and Dokuz Eylül University. This study was conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation for this study was not required by the IRBs.

## Author contributions

CR: Concept/design, Data analysis/interpretation, Drafting article, Critical revision of article, Approval of article, Funding

Secured; AP: Concept/design, Data analysis/interpretation, Drafting article, Critical revision of article, Approval of article; YS and RB: Concept/design, Data analysis/ interpretation, Critical revision of article, Approval of article, Funding Secured; WA: Data analysis/ interpretation, Drafting article, Critical revision of article, Approval of article; BC, EA, SY, PY, MW, and EH: Data analysis/ interpretation, Critical revision of article, Approval of article; MS: Critical revision of article, Approval of article.

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## Conflict of interest

Author AP was employed by the company AARDEX Group.

## References

- Russell CL, Conn V, Jantarakupt P. Older adult medication compliance: integrated review of randomized controlled trials. *Am J Health Behav* (2006) 30:636–50. doi: 10.5555/ajhb.2006.30.6.636
- Wilhelmsen NC, Eriksson T. Medication adherence interventions and outcomes: an overview of systematic reviews. *Eur J Hosp Pharm* (2019) 26(4):187–92. doi: 10.1136/ehjpharm-2018-001725
- Anderson LJ, Nuckols TK, Coles C, Le MM, Schnipper JL, Shane R, et al. A systematic overview of systematic reviews evaluating medication adherence interventions. *Am J Health Syst Pharm* (2020) 77(2):138–47. doi: 10.1093/ajhp/zxx284
- Yang C, Zhu S, Lee DTF, Chair SY. Interventions for improving medication adherence in community-dwelling older people with multimorbidity: a systematic review and meta-analysis. *Int J Nurs Stud* (2022) 126:104154. doi: 10.1016/j.ijnurstu.2021.104154
- De Bleser L, Matteson M, Dobbels F, Russell C, De Geest S. Interventions to improve medication-adherence after transplantation: a systematic review. *Transpl International: Official Journal Eur Soc Organ Transplant* (2009) 22(8):780–97. doi: 10.1111/j.1432-2277.2009.00881.x
- Wood W, Quinn JM, Kashy DA. Habits in everyday life: thought, emotion, and action. *J Pers Soc Psychol* (2002) 83(6):1281–97. doi: 10.1037/0022-3514.83.6.1281
- Verplanken B. Beyond frequency: habit as mental construct. *Br J Soc Psychol* (2006) 45(Pt 3):639–56. doi: 10.1348/014466605X49122
- Wood W, Neal DT. A new look at habits and the habit-goal interface. *Psychol Rev* (2007) 114(4):843–63. doi: 10.1037/0033-295X.114.4.843
- Duhigg C. *The Power of Habit*. New York: Random House (2012).
- Singh B, Murphy A, Maher C, Smith AE. Time to form a habit: a systematic review and meta-analysis of health behaviour habit formation and its determinants. *Healthcare (Basel)* (2024) 12(23):2488. doi: 10.3390/healthcare12232488
- Badawy SM, Shah R, Beg U, Heneghan MB. Habit strength, medication adherence, and habit-based Mobile health interventions across chronic medical conditions: systematic review. *J Med Internet Res* (2020) 22(4):e17883. doi: 10.2196/17883
- Pironet A, Phillips LA, Vrijens B. Correlation between objective habit metrics and objective medication adherence: Retrospective study of 15,818 participants from clinical studies. *Interact J Med Res* (2025) 14: e63987. doi: 10.2196/63987
- Phillips LA, Pironet A, Vrijens B. Evaluating objective metrics of habit strength for taking medications. *J Behav Med* (2023) 46(4):632–41. doi: 10.1007/s10865-023-00392-z
- Russell CL, Hathaway D, Remy LM, Aholt D, Clark D, Miller C, et al. Improving medication adherence and outcomes in adult kidney transplant patients using a personal systems approach: systemchange™ results of the MAGIC randomized clinical trial. *Am J Transplant* (2020) 20(1):125–36. doi: 10.1111/ajt.15528
- Sarigöl Ordin Y, Yildiz S, Kankaya EA, P1.07: a new intervention to increase medication adherence in kidney transplant recipients: investigation of effect of SystemCHANGE intervention. *Transplantation* (2022) 106(9S):S504. doi: 10.1097/01.tp.0000885232.46083.45
- Russell CL, Conn V, Ashbaugh C, Madsen R, Hayes K, Ross G. Medication adherence patterns in adult renal transplant recipients. *Res Nurs Health* (2006) 29(6):521–32. doi: 10.1002/nur.20149
- Russell CL, Ashbaugh C, Peace L, Cetingok M, Hamburger KQ, Owens S, et al. Time-in-a-Bottle (TIAB) study: patterns, predictors, and outcomes of medication adherence in adult kidney transplant recipients. *Clin Transpl* (2013) 27:E580–E590. doi: 10.1111/ctr.12203
- Bronfenbrenner U. Ecological models of human development. In: *International Encyclopedia of Education*. 3. 2nd ed. Pxford: Elsevier (1994).
- Russell CL. A clinical nurse Specialist-led intervention to enhance medication adherence using the plan-do-check-act cycle for continuous self-improvement. *Clin Nurse Spec* (2010) 24:69–75. doi: 10.1097/NUR.0b013e3181cf554d
- AARDEX Group (2026). *MEMS® Cap Smart Pill Bottle - AARDEX Group*. Available online at: <https://aardexgroup.com/solution/mems-hardware-ecosystem/>.
- Vrijens B, De Geest S, Hughes DA, Przemyslaw K, Demonceau J, Ruppar T, et al. A new taxonomy for describing and defining adherence to medications. *Br J Clin Pharmacol* (2012) 73:691–705. doi: 10.1111/j.1365-2125.2012.04167.x
- Van Rossum G, Drake FL. *Python 3 Reference Manual*. Scotts Valley, CA: CreateSpace (2009).
- Russell CL, Miller C, Remy LM, Wessol JL, Andrews AM, Aholt D, et al. Improvement of immunosuppressive medication adherence using a SystemCHANGE intervention: case study of an older adult kidney transplant recipient. *Nephrol Nurs J* (2018) 45(2):171–223.
- Russell CL, Moore S, Hathaway D, Cheng A-L, Chen G, Goggin K. MAGIC study: aims, design and methods using SystemCHANGE™ to improve immunosuppressive medication adherence in adult kidney transplant recipients. *BMC Nephrol* (2016) 17(1): 84. doi: 10.1186/s12882-016-0285-8
- van de Vijver I, Brinkhof LP, de Wit S. Age differences in routine formation: the role of automatization, motivation, and executive functions. *Front Psychol* (2023) 14: 1140366. doi: 10.3389/fpsyg.2023.1140366
- Andacoglu O, Aki FT. Global perspective on kidney transplantation: turkey. *Kidney360* (2021) 2(7):1160–2. doi: 10.34067/KID.0002542021
- Ordin YS, Karayurt Ö, Aksu Kul G, Kılıç M, Taylor LA. Exploration into donor-recipient relationship after living-donor liver transplantation using gift-exchange theory. *Florence Nightingale J Nurs* (2021) 29(2):150–7. doi: 10.5152/FNJJN.2021.20065

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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.2026.16252/full#supplementary-material>

28. Larzelere RE, Kuhn BR, Johnson B. The intervention selection bias: an underrecognized confound in intervention research. *Psychol Bull* (2004) 130(2): 289–303. doi: 10.1037/0033-2909.130.2.289
29. Uyar B. The analysis of immunosuppressant therapy adherence, depression, anxiety, and stress in kidney transplant recipients in the post-transplantation period. *Transpl Immunol* (2022) 75:101686. doi: 10.1016/j.trim.2022.101686
30. Acosta FJ, Hernández JL, Pereira J, Herrera J, Rodríguez CJ. Medication adherence in schizophrenia. *World J Psychiatry* (2012) 2(5):74–82. doi: 10.5498/wjp.v2.i5.74
31. Wagner GJ, Ghosh-Dastidar B. Electronic monitoring: adherence assessment of intervention? *HIV Clin Trials* (2002) 3(1):45–51. doi: 10.1310/XGXU-FUDK-A9QT-MPTF





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# The perfused liver utilisation study (PLUS): challenges and innovation in the evaluation of an emerging technology

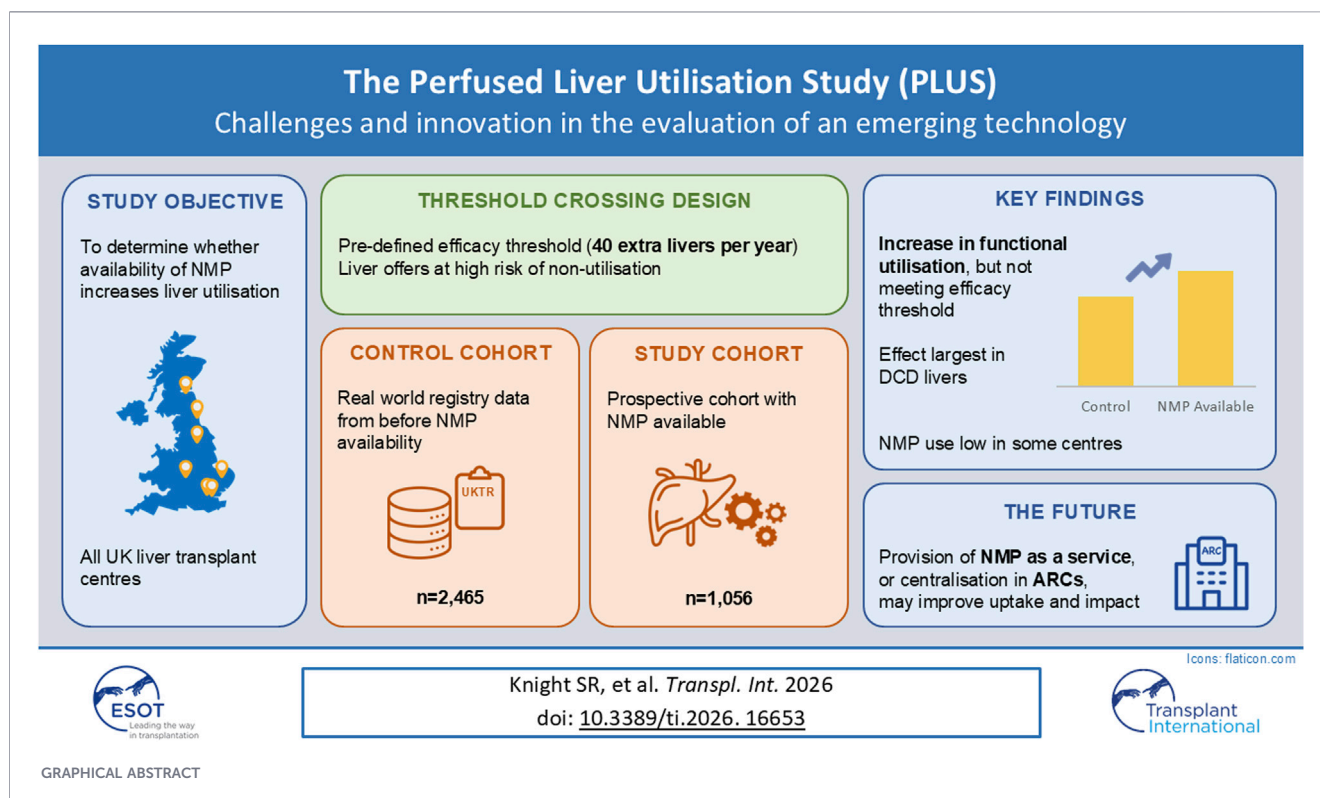
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Previous studies suggest that donor liver normothermic machine perfusion (NMP) reduces injury and enables functional assessment, resulting in an increase in utilisation. The PLUS study was designed to assess the impact of NMP availability on utilisation of livers at high risk of non-use. Using a threshold-crossing design, functional utilisation was assessed in a real-world retrospective registry-based control cohort. A predetermined efficacy threshold was set, and a prospective cohort was recruited in all UK liver transplant centres with NMP made available for all livers at high risk of non-utilisation. Availability of NMP resulted in a significant increase in functional utilisation (OR: 1.26, 95% CI: 1.04–1.53), but this did not exceed the pre-determined efficacy threshold. The threshold was exceeded in the subgroup of donation after circulatory death (DCD) livers, although this increase was reduced with adjustment for the use of normothermic regional perfusion. NMP was used in only 31% of retrieved livers, with variation between centres. The benefit of having NMP available was less than anticipated, likely due to low use in some centres associated with staff availability. Provision of NMP as a service, or centralisation of NMP use in assessment and recovery centres, may improve uptake.

### KEYWORDS

clinical study, liver transplantation, normothermic machine perfusion, organ utilization, threshold crossing



## Introduction

Liver transplant numbers in the UK have remained largely static over the past 10 years, whilst the number of patients waiting for liver transplantation has increased by 11% [1]. Around 70 patients die on the UK liver transplant waiting list each year. Only 57% of offered livers are transplanted, largely due to an increasing proportion of 'high-risk' organs and the lack of reliable preoperative viability assessment [2]. This is not just a UK phenomenon – this discrepancy between supply and demand is an international concern.

This organ shortage has led to increased interest in interventions with the potential to increase organ utilisation. Machine perfusion, either *in-situ* (normothermic regional perfusion; NRP) or *ex-situ* (normothermic or hypothermic machine perfusion) offers potential for reconditioning and functional assessment prior to transplant [3–5]. There is an increasing view that the ability to assess function *ex-situ* by means of normothermic machine perfusion (NMP) may improve confidence in transplanting more marginal organs, improving utilisation.

Randomised controlled trials comparing NMP to static cold storage (SCS) have shown reduced early allograft dysfunction with

lower incidence of reperfusion injury, especially for higher risk livers such as those from donors after circulatory death (DCD) [6, 7]. The pivotal multicentre European randomised controlled trial also demonstrated an increase in utilisation of retrieved livers with NMP (non-utilisation 11.7% vs. 24.1%), despite eligibility for the trial requiring all livers to be suitable for either preservation method [6]. This suggests that clinicians were using the technology to assess the suitability of livers prior to transplantation, improving confidence and utilisation without compromising post-transplant outcomes. Registry-based studies from the US have demonstrated similar improvements in liver utilisation with NMP, particularly from DCD donors, although historically the utilisation of DCD livers in the US has been lower than in European countries [8, 9]. The benefit of NMP appears to be greatest for patients with low model for end-stage liver disease (MELD) scores [10]. Implementation of NMP may also have the potential to reduce waiting times, particularly for patients with hepatocellular carcinoma [11]. Small-scale studies, including most notably the VITTAL study from Birmingham [12], suggest that a meaningful proportion of donor livers that are currently declined on conventional criteria could in fact be transplanted successfully.

Despite this potential beneficial effect of NMP use, there are no previous prospective studies designed to quantify the effect of NMP on organ utilisation. NMP is a resource-intensive alternative to SCS: high level evidence is important if this technology is to be funded by healthcare payers. There is a need to quantify the magnitude of this potential utilisation benefit in an appropriately-powered study in a real-world setting. The Perfused Liver Utilisation Study (PLUS) was designed to assess the impact of NMP availability (as opposed to NMP use) on the utilisation of livers at high risk of non-use in the UK transplant setting.

**Abbreviations:** ARC, Assessment and Recovery Centre; CI, Confidence Interval; DBD, Donation after Brain Death; DCD, Donation after Circulatory Death; DLI, Donor Liver Index; DUI, Donor Utilisation Index; HOPE, Hypothermic Oxygenated Perfusion; ITU, Intensive Care Unit; NMP, Normothermic Machine Perfusion; NRP, Normothermic Regional Perfusion; MEAF, Model for Early Allograft Function; MELD, Model for End-stage Liver Disease; OR, Odds Ratio; PLUS, Perfused Liver Utilisation Study; SAE, Serious Adverse Event; SCS, Static Cold Storage; SDC, Supplementary Digital Content; UKTR, UK Transplant Registry.

## Materials and methods

### Study design

The trial evaluated the effect of NMP availability on “functional utilisation”: the proportion of donor liver offers resulting in a functioning transplant at 12 months. To identify livers at greatest risk of non-use, a novel, regression-based score was developed using UK Transplant Registry (UKTR) data to estimate the probability of non-utilisation of individual livers offered for transplant (SDC1). This algorithm, the Donor Utilisation Index (DUI), identified the 60% of donors livers at greatest risk of decline for inclusion in the study [13].

PLUS was originally conceived as a randomised controlled trial, with eligible liver offers randomised to having NMP available or not. During sequential investigators’ meetings, surgeons in most centres became increasingly unwilling to randomise high-risk liver cohorts (particularly DCD) to SCS, preferring some form of machine preservation. The study was therefore revised to a “threshold-crossing” design, in which a real-world control cohort, selected consecutively according to the same objective inclusion/exclusion criteria to minimise bias, was used *a priori* to estimate the counterfactual (SCS cohort). A threshold was set for the primary outcome prior to recruiting a prospective single-arm study cohort (NMP available cohort) [14]. The lower bound of the 95% confidence interval for effect size had to exceed the pre-defined efficacy threshold to demonstrate superiority.

The study protocol was prospectively registered (16th November 2021; [ISRCTN11552402](#)). Ethical approval was granted by the South Central – Oxford C Research Ethics Committee (ref 21/SC/0297). Patients on the liver transplant list, liver recipients and family members were consulted during study design. A patient representative was part of the trial management group and trial steering committee, advising on conduct and dissemination.

### Inclusion/exclusion criteria

Eligible liver offers were DBD or DCD livers from UK donors aged 16 years or over, with a DUI >0.27, offered to any of the 7 UK liver transplant centres. This DUI threshold was selected as it identified the 60% most poorly utilised livers, with utilisation of 26% compared to 85% in livers with a DUI ≤0.27. Eligible offers were predominantly DCD livers (79%), reflecting lower utilisation compared to DBD livers. Offers from donors with HIV or hepatitis C were excluded, as were livers undergoing any other form of *ex-situ* machine perfusion or where the participating centre was unable to offer NMP at the time due to logistical reasons (unavailability of device, consumables or trained staff). Livers from donors undergoing NRP were eligible, with planned adjustment for NRP use in analysis. All eligible livers were enrolled to ensure comparability between cohorts.

Eligible recipients were adults (≥18 years) on the elective or super-urgent transplant waiting list. Those declining NMP use through local consent policy, receiving a split liver or reduced liver or multiorgan transplant were excluded.

### Real-world control cohort

To estimate the counterfactual, the primary outcome was assessed in a real-world control cohort where NMP was not routinely available, derived from the UKTR between 1st January 2018 and 31st December 2019 (SDC2). Identical inclusion/exclusion criteria were applied to this cohort as to the prospective study cohort, with stratified random sampling used to exclude a small proportion of patients to mimic the consent process used in the prospective cohort. A predefined efficacy threshold of at least 40 additional functional transplants per year was set, equivalent to an odds ratio of 1.22 for functional utilisation. This threshold was agreed by all seven participating centres to represent the minimum clinically-important increase in transplant rates that would justify the increased cost and logistical complexity of NMP use.

### Study cohort

A prospective cohort was recruited between 11th April 2022 and 3rd April 2023, with NMP made available for all eligible liver offers. Participating centres were informed of NMP availability at the time of offer, and the impact of availability on the primary outcome was assessed by comparing the two cohorts against the pre-defined efficacy threshold.

NMP was used at the discretion of the implanting surgeon. All participating centres had access to an OrganOx metra device and disposable kits at no additional cost, with limited per-case funding provided for staff time. NMP could be used either from the donor centre (device-to-donor) or following SCS and transport to the recipient centre (back-to-base) at the preference of the implanting team. The OrganOx metra device was used according to the current instructions for use and local protocols, with a minimum of 4 h and maximum of 24 h of perfusion time.

### Primary outcome

The primary outcome was defined as the proportion of livers with “functional utilisation”: transplantation of an offered liver with 1-year graft survival. This ensures that any increase in utilisation does not occur at the cost of inferior graft outcomes.

### Secondary outcomes

Pre-specified secondary outcomes included: proportion of non-utilisation, median biochemical liver function in the first 7 days post-transplant [bilirubin, GGT, ALT, INR, lactate (recorded during high-level care only)], and 12 months post-transplant (bilirubin, ALP, ALT, AST), median Model for Early Allograft Function score (MEAF), 12-month graft and patient survival, proportion of transplants with primary non-function, median length of ITU and hospital stay, proportion of recipients requiring renal replacement therapy after transplant and proportion of recipients experiencing adverse events in the first 3 months and 12 months after transplant. Outcomes for both cohorts were collected from the UK Transplant Registry (UKTR), with additional outcomes collected in study case report forms for the prospective cohort over a 12-month follow-up period. Unexpected serious adverse events during the 12-month follow-up period were collected for

the prospective cohort only. A health-economic analysis has been undertaken and will be presented in a separate manuscript.

## Sample size and statistical design

The sample size assumed a 50% increase in 1-year functional utilisation from 21.8% in the control cohort to 32.7% in the prospective NMP available cohort, representing 135 additional successful transplants per year. Allowing for cross-over for those in the prospective cohort not receiving NMP the study was powered to detect an increase in functional utilisation to 31.2%. To achieve 90% power using a 2.5% one-sided significance level to detect an increase of at least 40 additional transplants per year, 1,035 liver offers were required. This allowed for up to 25% loss due to logistical constraints, and 10% loss due to post-recruitment exclusions and loss to follow-up. Protocol v1.0 planned a smaller sample size of 799 liver offers, but this was revised in v2.0 to allow for higher loss to follow-up and NMP unavailability for logistical reasons. An embedded feasibility study assessed recruitment rates and NMP use at 6 months, but there was no formal interim outcome analysis.

Primary analysis was by modified intention-to-treat, where livers unable to be perfused for logistic reasons were excluded, along with any found to be ineligible after study entry (once recipient characteristics were known). Analyses were adjusted for donor type and use of NRP (DBD, DCD with NRP, DCD without NRP) as a fixed effect as these both have an important effect on organ utilisation.

Primary outcome results, from logistic regression models, are reported as odds ratios (OR) with 95% confidence intervals (CI), and *p*-values for the cohort effect under the null hypothesis of no difference. Pre-planned subgroup analyses for the primary outcome investigated the effect of donor type, donor liver index (DLI, a UK-derived algorithm that predicts transplant outcome based on donor parameters [15]) and DUI.

Sensitivity analyses and secondary outcome analyses are described in SDC3.

All analyses were performed in SAS v9.4M5.

## Results

### Study cohorts and demographics

The flow of liver offers through the study is shown in Figure 1. The control cohort comprised 2,465 liver offers where SCS was the routine method of preservation and NMP was unavailable. The prospective study cohort comprised 1,056 liver offers. Of this cohort, 983 met the eligibility criteria for the modified intention-to-treat analysis. 542 control offers and 278 study offers resulted in transplants.

Donor, liver and recipient characteristics were comparable between the two cohorts (Tables 1–3) and median follow-up time post-transplant was 365 days for both cohorts. For those livers resulting in a transplant, there was an increase in the proportion of DCD livers in the prospective cohort (61.2% vs. 51.5%), indicating an increased willingness to transplant DCD livers where machine perfusion is available. Use of NRP was also higher (29.0% vs. 12.4%), reflecting changes in practice as the prospective cohort was more recent than the control cohort.

### NMP use

In the prospective study cohort NMP was used for 31.1% of eligible livers that arrived at a transplant centre (12.4% of all livers offered). Of those where NMP was used, four livers (3.3%) used a device to donor approach, and 116 (96.7%) used a back to base approach. The approach was unknown for two livers (Table 2). There was considerable variation in NMP use between centres (SDC4) and the key reasons for non-use of NMP related to (i) livers being deemed untransplantable; (ii) surgeon deemed the liver clinically suitable for transplant without perfusion; and (iii) perfusion kit/staff unavailability (SDC10).

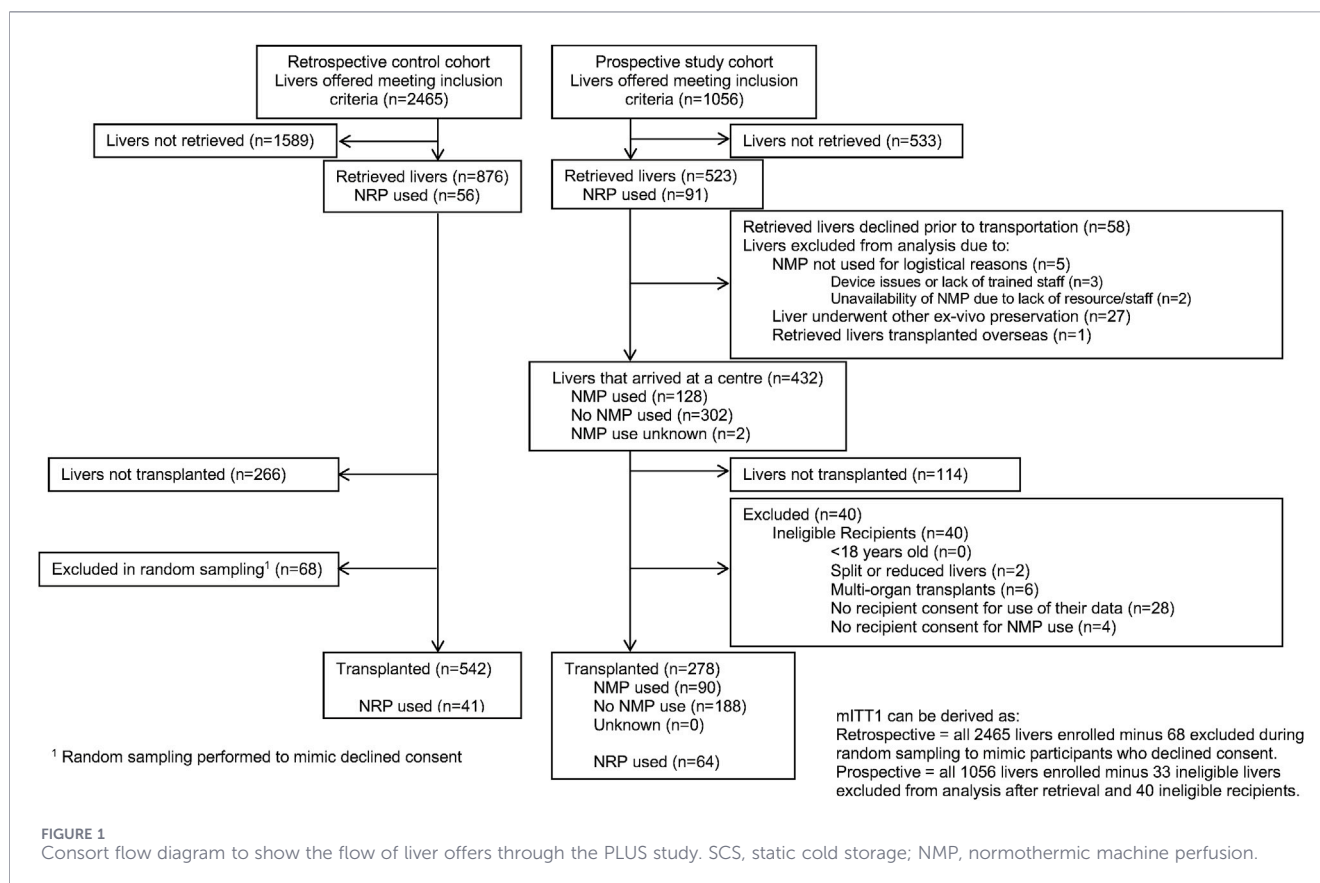
### Primary outcome

The primary outcome of functional utilisation was significantly higher in the prospective study cohort (NMP available) (25.2% vs. 19.6%, OR: 1.26, 95% CI: 1.04–1.53), representing 47 additional functional transplants per year (95% CI 8–90 transplants) (Figure 2). However, the lower bound of the 95% confidence interval did not exceed the pre-defined threshold of 40 additional liver transplants per year, therefore the intervention is not considered superior under the threshold-crossing design. The difference in functional utilisation was primarily due to increased organ utilisation in the cohort with NMP available (27.8% vs. 22.0%) with smaller improvements in patient (95.1% vs. 92.9%) and graft (90.8% and 89.1%) survival. Reasons for non-use of livers transported to the recipient centre for the prospective cohort are shown in SDC12; the most common reason for non-use in NMP treated livers was poor function on the device (78.1%).

Subgroup analyses demonstrated a statistically significant interaction when looking at the impact of NMP availability by donor type (*p* = 0.0245), with a greater difference in functional utilisation observed in the DCD group (OR: 1.70, 95% CI: 1.36–2.12), exceeding the efficacy threshold. However, this analysis did not include adjustment for NRP, which is not used for DBD donors. In a *post hoc* analysis which only included DCD donors and with risk adjustment for NRP the OR was 1.37 (95% CI 1.08–1.73; *p* = 0.0089), not exceeding the efficacy threshold. In livers from DCD donors, *post hoc* analysis demonstrated no significant interaction between the impact of NMP and use of NRP (*p* = 0.8861).

There were no statistically significant interactions between NMP availability and the DUI (*p* = 0.3087) or DLI (*p* = 0.0833), although for some subgroups the lower bound of the 95% confidence interval did exceed the efficacy threshold (Figure 2). A per-protocol analysis also did not exceed the efficacy threshold (OR: 1.18, 95% CI: 0.96–1.44). Two secondary, pre-specified propensity score matched analyses were also undertaken. The first mirrored the primary analysis and assessed the effect of NMP availability on functional utilisation of offered livers, and this also did not exceed the efficacy threshold (OR: 1.14, 95% CI: 0.93–1.39) (Figure 3). The second propensity score matched analysis instead assessed the impact of NMP usage (rather than availability) on functional utilisation. Post-hoc, this analysis was amended to avoid the bias whereby only retrieved livers could have received NMP and therefore analysed only retrieved livers in the prospective cohort, and compared between those where NMP was used, and propensity





score matched livers where NMP was not used. This analysis did exceed the efficacy threshold, indicating that NMP does increase functional utilisation of retrieved livers when it is used (*post hoc*, OR: 2.18, 95% CI: 1.35–3.51) (Figure 3). The sample size was lower for the propensity score matched analyses than for the primary analysis.

## Secondary outcomes

There were no statistically significant differences in biochemical liver function tests at 12 months post-transplant, graft and patient survival, primary non-function, length of hospital stay, length of ITU stay, and need for renal replacement therapy (SDC5). Sensitivity analyses of graft and patient survival outcomes adjusting for recipient risk factors did also not result in statistically significant differences between the two cohorts (SDC6).

Biochemical liver function on days 1–7 and MEAF score were only available for the prospective cohort with a mean MEAF score of 4.5 (SDC8).

A larger proportion of organs were retrieved in the prospective study cohort (NMP available) compared to the retrospective control cohort (SCS) (45.8% vs. 33.7%). The proportion of organs retrieved but not transplanted was also higher in the prospective study cohort (38.2% vs. 32.9%, respectively). Following adjustment for donor type and NRP, this yielded an adjusted odds ratio for organ non-utilisation of 1.33 (95% CI: 1.04–1.71). This was statistically significant at the 5% level ( $p = 0.0226$ ) (SDC9).

There was one positive perfusate culture reported. Using data collected through the UKTR the proportion of recipients

experiencing infections (41.0% vs. 22.9%, respectively), treated biopsy-proven rejection episodes (19.6% vs. 16.2%, respectively), and biliary complications requiring intervention (8.7% vs. 5.1%, respectively) in the first 3 months post-transplant was higher in the retrospective control cohort (SCS) than the prospective study cohort (NMP available). Slightly lower proportions were observed in the retrospective control cohort (SCS) for vascular complications, (9.0% vs. 12.7%) and reoperations (6.4% vs. 9.0%) at 3 months post-transplant. However, data completeness varied, so no formal statistical comparisons were performed (SDC5).

## Adverse events

SAEs were only reported in the prospective study cohort (NMP available). There were 156 SAEs reported in 94 recipients (36.9% of the cohort) (SDC7). 62 SAEs were reported for cases where NMP was used, but only 1 was reported to be linked to errors in device function and use, and only 4 possibly related to the intervention.

## Discussion

The perfused liver utilisation study (PLUS) was originally conceived as a randomised controlled trial. During study setup, clinicians became increasingly unwilling to transplant high-risk livers without perfusion, necessitating a change to a ‘threshold-crossing’ design in which a prospectively recruited cohort is compared with a retrospective real-world control cohort using



TABLE 1 Donor characteristics for enrolled and transplanted livers.

| Donor characteristic                 | Enrolled livers                 |                                       |                                              | Transplanted livers           |                                       |                                    |
|--------------------------------------|---------------------------------|---------------------------------------|----------------------------------------------|-------------------------------|---------------------------------------|------------------------------------|
|                                      | Retrospective (SCS) (n = 2,397) | Prospective (NMP available) (n = 983) | Total (n = 3,380)                            | Retrospective (SCS) (n = 542) | Prospective (NMP available) (n = 278) | Total (n = 820)                    |
| Age (years)                          | 60 (51–69)                      | 60 (51–68)                            | 60 (51–69)                                   | 58 (48–68)                    | 58 (46–65)                            | 58 (48–67)                         |
| Sex                                  |                                 |                                       |                                              |                               |                                       |                                    |
| Male                                 | 1,454/2,397 (60.7)              | 614/983 (62.5)                        | 2,068/3,380                                  | 311/542 (57.4)                | 162/278 (58.3)                        | 473/820 (57.7)                     |
| Female                               | 943/2,397 (39.3)                | 369/983 (37.5)                        | (61.2)<br>1,312/3,380<br>(38.8)              | 231/542 (42.6)                | 116/278 (41.7)                        | 347/820 (42.3)                     |
| BMI (kg/m <sup>2</sup> )             | 27.8 (24.2–32.0)                | 28.1 (24.3–31.9)                      | 28.0 (24.2–31.9)                             | 27.1 (23.6–31.3)              | 27.7 (23.4–30.9)                      | 27.4<br>(23.6–31.2)                |
| Ethnic origin                        |                                 |                                       |                                              |                               |                                       |                                    |
| Asian                                | 56/2,375 (2.4)                  | 27/959 (2.8)                          | 83/3,334 (2.5)                               | 19/536 (3.5)                  | 6/274 (2.2)                           | 25/810 (3.1)                       |
| Black                                | 25/2,375 (1.1)                  | 13/959 (1.4)                          | 38/3,334 (1.1)                               | 9/536 (1.7)                   | 7/274 (2.6)                           | 16/810 (2.0)                       |
| White                                | 2,249/2,375 (94.7)              | 909/959 (94.8)                        | 3,158/3,334                                  | 495/536 (92.4)                | 259/274 (94.5)                        | 754/810 (93.1)                     |
| Other                                | 45/2,375 (1.9)                  | 10/959 (1.0)                          | (94.7)<br>55/3,334 (1.6)                     | 13/536 (2.4)                  | 2/274 (0.7)                           | 15/810 (1.9)                       |
| Donor type                           |                                 |                                       |                                              |                               |                                       |                                    |
| DBD                                  | 519/2,397 (21.7)                | 201/983 (20.4)                        | 720/3,380 (21.3)                             | 263/542 (48.5)                | 108/278 (38.8)                        | 371/820 (45.2)                     |
| DCD                                  | 1,878/2,397 (78.3)              | 782/983 (79.6)                        | 2,660/3,380<br>(78.7)                        | 279/542 (51.5)                | 170/278 (61.2)                        | 449/820 (54.8)                     |
| Smoker                               | 1,552/2,383 (65.1)              | 593/979 (60.6)                        | 2,145/3,362<br>(63.8)                        | 334/541 (61.7)                | 173/276 (62.7)                        | 507/817 (62.1)                     |
| History of diabetes                  | 339/2,377 (14.3)                | 151/973 (15.5)                        | 490/3,350 (14.6)                             | 79/538 (14.7)                 | 34/277 (12.3)                         | 113/815 (13.9)                     |
| History of tumour                    | 178/2,365 (7.5)                 | 65/971 (6.7)                          | 243/3,336 (7.3)                              | 44/537 (8.2)                  | 18/274 (6.6)                          | 62/811 (7.6)                       |
| Cardiac/respiratory arrest           | 1,191/2,334 (51.0)              | 477/958 (49.8)                        | 1,668/3,292<br>(50.7)                        | 271/532 (50.9)                | 136/274 (49.6)                        | 407/806 (50.5)                     |
| Cause of death                       |                                 |                                       |                                              |                               |                                       |                                    |
| CVA                                  | 1,080/2,298 (47.0)              | 454/947 (47.9)                        | 1,534/3,245                                  | 282/524 (53.8)                | 140/272 (51.5)                        | 422/796 (53.0)                     |
| Anoxia                               | 909/2,298 (39.6)                | 371/947 (39.2)                        | (47.3)                                       | 170/524 (32.4)                | 103/272 (37.9)                        | 273/796 (34.3)                     |
| Trauma                               | 48/2,298 (2.1)                  | 18/947 (1.9)                          | 1,280/3,245                                  | 11/524 (2.1)                  | 5/272 (1.8)                           | 16/796 (2.0)                       |
| Other                                | 261/2,298 (11.4)                | 104/947 (11.0)                        | (39.4)<br>66/3,245 (2.0)<br>365/3,245 (11.2) | 61/524 (11.6)                 | 24/272 (8.8)                          | 85/796 (10.7)                      |
| Length of ITU stay (days)            | 3.4 (1.8–5.8)                   | 3.8 (2.2–5.9)                         | 3.5 (1.9–5.8)                                | 2.4 (1.4–4.0)                 | 3.1 (1.9–4.6)                         | 2.6 (1.5–4.2)                      |
| Donor utilisation index <sup>1</sup> |                                 |                                       |                                              |                               |                                       |                                    |
| DBD                                  | 0.41 (0.33–0.55)                | 0.40 (0.31–0.54)                      | 0.41 (0.32–0.55)                             | 0.38 (0.32–0.48)              | 0.35 (0.29–0.44)                      | 0.37                               |
| DCD                                  | 0.76 (0.59–0.89)                | 0.77 (0.61–0.89)                      | 0.76 (0.60–0.89)                             | 0.61 (0.46–0.75)              | 0.62 (0.47–0.76)                      | (0.31–0.47)<br>0.61<br>(0.46–0.75) |

(Continued)

TABLE 1 Continued

| Donor characteristic           | Enrolled livers                 |                                       |                   | Transplanted livers           |                                       |                                    |
|--------------------------------|---------------------------------|---------------------------------------|-------------------|-------------------------------|---------------------------------------|------------------------------------|
|                                | Retrospective (SCS) (n = 2,397) | Prospective (NMP available) (n = 983) | Total (n = 3,380) | Retrospective (SCS) (n = 542) | Prospective (NMP available) (n = 278) | Total (n = 820)                    |
| Donor liver index <sup>2</sup> |                                 |                                       |                   |                               |                                       |                                    |
| DBD                            | 1.28 (1.10–1.42)                | 1.21 (1.06–1.36)                      | 1.25 (1.10–1.40)  | 1.25 (1.09–1.40)              | 1.20 (1.04–1.38)                      | 1.23                               |
| DCD                            | 2.28 (1.99–2.57)                | 2.25 (1.99–2.53)                      | 2.27 (1.99–2.56)  | 2.10 (1.81–2.45)              | 2.11 (1.91–2.37)                      | (1.09–1.39)<br>2.10<br>(1.84–2.41) |

*Summary of missing data:*

*Enrolled livers:* Age, sex, BMI, donor type, DUI, 0; Ethnic origin, 46 (22 SCS, 24 NMP available); Smoking status, 18 (14 SCS, 4 NMP available); History of diabetes, 30 (20 SCS, 10 NMP available); History of tumour, 44 (32 SCS, 12 NMP available); Cardiac/respiratory arrest, 88 (63 SCS, 25 NMP available); Cause of death, 135 (99 SCS, 36 NMP available); Length of ITU, stay, 22 (13 SCS, 9 NMP available); DLI, 25 donors (3 DBD SCS, 18 DCD SCS, 2 DBD NMP available, 2 DCD NMP available).

*Transplanted livers:* Age, sex, BMI, donor type, DUI, 0; Ethnic origin, 10 (6 SCS, 4 NMP- available); Smoking status, 3 (1 SCS, 2 NMP available); History of diabetes, 5 (4 SCS, 1 NMP available); History of tumour, 9 (5 SCS, 4 NMP available); Cardiac/respiratory arrest, 14 (10 SCS, 4 NMP available); Cause of death, 24 (18 SCS, 6 NMP available). Length of ITU, stay, 4 (2 SCS, 2 NMP available). DLI, 4 (0 DBD SCS, 2 DCD SCS, 2 DBD NMP available, 0 DCD NMP available).

Data are n/N (%) for categorical variables, and median (IQR) for continuous variables. SCS, static cold storage; NMP, normothermic machine perfusion; BMI, body mass index; DBD, donor after brainstem death; DCD, donor after circulatory death; CVA, cerebrovascular accident.

<sup>1</sup>See SDC for definition of the donor utilisation index (Mehew et al. [13]).

<sup>2</sup>UK donor liver index, Collett et al. [15].

the same pre-defined inclusion/exclusion criteria. All seven UK centres agreed to take part and recruitment was rapid.

PLUS demonstrated a statistically significant increase in functional utilisation of poorly utilised liver offers with availability of NMP (from 19.6% to 25.2%), but this did not exceed our pre-defined efficacy threshold. The threshold crossing design sets a higher burden of proof than a typical randomised controlled trial, requiring the lower bound of the confidence interval to exceed a predefined effect size to account for potential bias from the non-randomised design [14]. Although the effect size equates to an additional 47 successful liver transplants per year in the UK (a 4% increase), the lower confidence bound (8 transplants) did not meet the threshold of 40. Whilst the study design is sensitive to the threshold selected at the outset, these conclusions would not have changed unless the threshold has been selected to be below 8 additional transplants per year, which represents a less than 1% increase in UK transplant numbers.

The most likely explanation for the smaller than expected effect size was lower-than-anticipated uptake of NMP (12% of livers offered, 31% of eligible retrieved livers) and varied greatly between centres (ranging from 2.4% to 60.3% of eligible retrieved livers). Post-hoc propensity matched analysis suggests that when NMP is used, its effect on utilisation is greater, indicating that its full potential was likely not realised.

Several factors likely contributed to lower uptake than anticipated. Whilst the device and consumables were funded, the study budget did not cover the cost of 24/7 staffing infrastructure. In centres with existing infrastructure, NMP use was far higher than in more resource constrained centres. Similar patterns have been reported in the US, with lower use seen in low-volume centres lacking a dedicated perfusionist [16] as well as disparities related to insurance provider [17]. This disparity in access to new perfusion technologies is concerning and may have a knock-on impact on waiting times for patients in low-volume centres. Although logistical

constraints were anticipated in the study design, it appears very likely that “clinician decision” was recorded as the reason not to undertake NMP in many cases where “logistical constraints” was the actual reason.

In the original European randomised controlled trial of NMP, the impact on utilisation was larger than that seen in the current study [6]. That study recruited all retrieved livers regardless of quality, with a higher baseline chance of utilisation. NMP was managed by a dedicated research fellow who travelled with the device to the donor hospital, removing the logistical burden from the receiving centre. In the PLUS study, NMP use was almost entirely back-to-base. Whilst there are no prospective randomised trials comparing the device-to-donor and back-to-base approaches, non-randomised studies suggest broadly similar outcomes [18, 19]. Uptake of NMP may be improved by provision as a service (with device and perfusionist included), or by centralisation of perfusion in dedicated Assessment and Recovery Centres (ARCs). Organs that should be considered for ARC assessment include (i) those with the highest risk of decline (perhaps defined by the DUI derived for this study) to allow further viability assessment and improve chances of utilisation, (ii) those where more time is required for logistical reasons, and (iii) organs declined by all centres, for assessment and reoffering if they meet pre-defined viability criteria. Embedding ARCs within dedicated organ recovery centres is a viable option that is already gaining traction in the US [20]. Centralised provision of perfusion technologies also has the benefit of consistent protocols and data collection, and the potential to reduce the evolving disparities in access.

Other factors affecting NMP uptake include a preference to proceed straight to transplant following SCS (with or without NRP) for lower-risk livers, or for other machine perfusion technologies including hypothermic oxygenated machine perfusion (HOPE), which is logistically much simpler. Indeed, the most common reason documented for NMP non-use during

TABLE 2 Retrieval and perfusion characteristics for enrolled and transplanted livers.

| Liver characteristic                                                         | Enrolled                        |                                       |                    | Transplanted                  |                                       |                 |
|------------------------------------------------------------------------------|---------------------------------|---------------------------------------|--------------------|-------------------------------|---------------------------------------|-----------------|
|                                                                              | Retrospective (SCS) (n = 2,397) | Prospective (NMP available) (n = 983) | Total (n = 3,380)  | Retrospective (SCS) (n = 542) | Prospective (NMP available) (n = 278) | Total (n = 820) |
| Livers retrieved                                                             | 808/2,397 (33.7)                | 450/983 (45.8)                        | 1,258/3,380 (37.2) |                               |                                       |                 |
| Degree of steatosis <sup>1</sup>                                             |                                 |                                       |                    |                               |                                       |                 |
| Mild                                                                         | 283/497 (56.9)                  | 152/299 (50.8)                        | 435/796 (54.6)     | 210/298 (70.5)                | 105/157 (66.9)                        | 315/455         |
| Moderate                                                                     | 180/497 (36.2)                  | 114/299 (38.1)                        | 294/796 (36.9)     | 83/298 (27.9)                 | 50/157 (31.8)                         | (69.2)          |
| Severe                                                                       | 34/497 (6.8)                    | 33/299 (11.0)                         | 67/796 (8.4)       | 5/298 (1.7)                   | 2/157 (1.3)                           | 133/455 (29.2)  |
|                                                                              |                                 |                                       |                    |                               |                                       | 7/455 (1.5)     |
| NRP used <sup>2</sup>                                                        | 54/436 (12.4)                   | 84/290 (29.0)                         | 138/726 (19.0)     | 41/279 (14.7)                 | 64/170 (37.6)                         | 105/449 (23.4)  |
| Cold ischaemic time (hours)                                                  |                                 |                                       |                    | 7.9 (6.4–9.5)                 | 7.3 (6.0–9.3)                         | 7.7 (6.3–9.5)   |
| NMP used <sup>3</sup>                                                        |                                 | 122/392 (31.1)                        | 122/392 (31.1)     |                               | 90/278 (32.4)                         | 90/278 (32.4)   |
| NMP used (% enrolled)                                                        |                                 | 122/983 (12.4)                        | 122/983 (12.4)     |                               |                                       |                 |
| NMP approach                                                                 |                                 |                                       |                    |                               |                                       |                 |
| Back to base                                                                 |                                 | 116/120 (96.7)                        | 116/120 (96.7)     |                               | 84/88 (95.5)                          | 84/88 (95.5)    |
| Device to donor                                                              |                                 | 4/120 (3.3)                           | 4/120 (3.3)        |                               | 4/88 (4.5)                            | 4/88 (4.5)      |
| Time between donor perfusion and NMP start (hours)                           |                                 | 6.5 (5.4–7.5)                         | 6.5 (5.4–7.5)      |                               | 6.4 (5.0–7.1)                         | 6.4 (5.0–7.1)   |
| Time on NMP (hours)                                                          |                                 |                                       |                    |                               |                                       |                 |
| Median (IQR)                                                                 |                                 | 7.4 (5.7–10.2)                        | 7.4 (5.7–10.2)     |                               | 8.1 (6.1–10.5)                        | 8.1 (6.1–10.5)  |
| Range                                                                        |                                 | 1.4–21.8                              | 1.4–21.8           |                               | 3.1–21.8                              | 3.1–21.8        |
| Categories                                                                   |                                 |                                       |                    |                               |                                       |                 |
| ≤4 h                                                                         |                                 | 6/119 (5.0)                           | 6/119 (5.0)        |                               | 3/87 (3.4)                            | 3/87 (3.4)      |
| 4 - ≤8 h                                                                     |                                 | 57/119 (47.9)                         | 57/119 (47.9)      |                               | 40/87 (46.0)                          | 40/87 (46.0)    |
| 8 - ≤12 h                                                                    |                                 | 39/119 (32.8)                         | 39/119 (32.8)      |                               | 30/87 (34.5)                          | 30/87 (34.5)    |
| >12 h                                                                        |                                 | 17/119 (14.3)                         | 17/119 (14.3)      |                               | 14/87 (16.1)                          | 14/87 (16.1)    |
| Time between removal from NMP and recipient reperfusion (hours) <sup>4</sup> |                                 |                                       |                    |                               | 0.9 (0.7–1.1)                         | 0.9 (0.7–1.1)   |

Summary of missing data (where expected):

Enrolled livers: Retrieval status, NRP usage, NMP usage, 0; Steatosis, 7 (5 SCS, 2 NMP available); Degree of steatosis, 2 (1 SCS, 1 NMP available); NMP approach, Time between donor perfusion and NMP, 2; NMP duration, 3; Time between NMP and recipient reperfusion, 42.

Transplanted livers: NRP usage, NMP usage, 0; Steatosis, 4 (2 SCS, and 2 NMP available); Degree of steatosis, 2 livers (1 SCS, 1 NMP available); Cold ischaemia time, 9 (3 SCS, 6 NMP available); NMP approach, Time between donor perfusion and NMP, 2; NMP duration, 3; Time between NMP and recipient reperfusion, 10.

Data are n/N (%) for categorical variables, and median (IQR) for continuous variables. SCS, static cold storage; NMP, normothermic machine perfusion, NRP, normothermic regional perfusion.

<sup>1</sup>Degree of steatosis is only reported for livers reported as steatotic.

<sup>2</sup>Denominator is retrieved DCD livers.

<sup>3</sup>Denominator is livers received by a centre.

<sup>4</sup>Arterial or portal reperfusion, whichever occurred first.

the PLUS study was clinician preference to proceed directly to transplant, although this may also partly reflect logistical/resource challenges with use of NMP. Livers undergoing HOPE were excluded from the current study, although the number was relatively small. DCD livers undergoing NRP were included, with adjustment in analysis, and NRP use

increased in the prospective cohort. Notably, the effect of NMP availability exceeded our pre-defined efficacy threshold in the DCD subgroup, although this effect on utilisation was reduced when corrected for NRP use in *post hoc* analysis. This suggests that the significant improvement in functional utilisation of DCD livers seen between the two cohorts

TABLE 3 Recipient characteristics (pre-transplant).

| Recipient characteristic                                                        | Retrospective control (SCS)<br>(n = 542) | Prospective study (NMP available)<br>(n = 278) | Total<br>(n = 820) |
|---------------------------------------------------------------------------------|------------------------------------------|------------------------------------------------|--------------------|
| Age (years)                                                                     | 57 (50–63)                               | 58 (49–63)                                     | 58 (50–63)         |
| Sex                                                                             |                                          |                                                |                    |
| Male                                                                            | 372/542 (68.6)                           | 196/272 (72.1)                                 | 568/814 (69.8)     |
| Female                                                                          | 170/542 (31.4)                           | 76/272 (27.9)                                  | 246/814 (30.2)     |
| BMI (kg/m <sup>2</sup> )                                                        | 28.4 (25.0–32.4)                         | 28.8 (24.4–32.0)                               | 28.4 (24.8–32.3)   |
| Ethnic origin                                                                   |                                          |                                                |                    |
| Asian                                                                           | 27/533 (5.1)                             | 20/250 (8.0)                                   | 47/783 (6.0)       |
| Black                                                                           | 6/533 (1.1)                              | 7/250 (2.8)                                    | 13/783 (1.7)       |
| White                                                                           | 480/533 (90.1)                           | 219/250 (87.6)                                 | 699/783 (89.3)     |
| Other                                                                           | 20/533 (3.8)                             | 4/250 (1.6)                                    | 24/783 (3.1)       |
| Urgency status                                                                  |                                          |                                                |                    |
| Elective                                                                        | 523/542 (96.5)                           | 264/272 (97.1)                                 | 787/814 (96.7)     |
| Super-urgent                                                                    | 19/542 (3.5)                             | 8/272 (2.9)                                    | 27/814 (3.3)       |
| HCV status positive                                                             | 65/530 (12.3)                            | 17/269 (6.3)                                   | 82/799 (10.3)      |
| Encephalopathy present                                                          | 202/539 (37.5)                           | 90/264 (34.1)                                  | 292/803 (36.4)     |
| Renal support                                                                   | 25/541 (4.6)                             | 21/269 (7.8)                                   | 46/810 (5.7)       |
| Primary liver disease                                                           |                                          |                                                |                    |
| Acute hepatic failure                                                           | 14/540 (2.6)                             | 6/272 (2.2)                                    | 20/812 (2.5)       |
| Autoimmune and cryptogenic disease                                              | 29/540 (5.4)                             | 14/272 (5.1)                                   | 43/812 (5.3)       |
| Cancer                                                                          | 146/540 (27.0)                           | 44/272 (16.2)                                  | 190/812 (23.4)     |
| Fatty liver disease – non-alcohol related                                       | 62/540 (11.5)                            | 42/272 (15.4)                                  | 104/812 (12.8)     |
| Fatty liver disease – alcohol related                                           | 133/540 (24.6)                           | 75/272 (27.6)                                  | 208/812 (25.6)     |
| Hepatitis B                                                                     | 8/540 (1.5)                              | 5/272 (1.8)                                    | 13/812 (1.6)       |
| Hepatitis C                                                                     | 12/540 (2.2)                             | 6/272 (2.2)                                    | 18/812 (2.2)       |
| Metabolic liver disease                                                         | 8/540 (1.5)                              | 5/272 (1.8)                                    | 13/812 (1.6)       |
| Primary sclerosing cholangitis                                                  | 36/540 (6.7)                             | 35/272 (12.9)                                  | 71/812 (8.7)       |
| Primary biliary cholangitis                                                     | 39/540 (7.2)                             | 13/272 (4.8)                                   | 52/812 (6.4)       |
| Regraft                                                                         | 29/540 (5.4)                             | 12/272 (4.4)                                   | 41/812 (5.0)       |
| Other                                                                           | 24/540 (4.4)                             | 15/272 (5.5)                                   | 39/812 (4.8)       |
| UK end stage liver disease (UKELD score) prior to transplant <sup>1</sup>       |                                          |                                                |                    |
| Elective                                                                        | 53 (50–57)                               | 54 (50–58)                                     | 53 (50–57)         |
| Super-urgent <sup>3</sup>                                                       | 61 (60–64)                               | 62 (60–67)                                     | 61 (60–64)         |
| Model for end stage liver disease (MELD score) prior to transplant <sup>2</sup> |                                          |                                                |                    |
| Elective                                                                        | 13 (10–17)                               | 15 (11–19)                                     | 14 (10–18)         |
| Super-urgent <sup>3</sup>                                                       | 36 (31–40)                               | 24 (22–38)                                     | 35 (24–39)         |
| INR                                                                             | 1.3 (1.2–1.6)                            | 1.4 (1.2–1.7)                                  | 1.3 (1.2–1.6)      |
| Creatinine (μmol/L)                                                             | 75 (62–93)                               | 74 (60–91)                                     | 75 (61–92)         |
| Bilirubin (μmol/L)                                                              | 36 (19–79)                               | 41 (20–86)                                     | 37 (19–81)         |

(Continued)



TABLE 3 Continued

| Recipient characteristic | Retrospective control (SCS)<br>(n = 542) | Prospective study (NMP available)<br>(n = 278) | Total<br>(n = 820) |
|--------------------------|------------------------------------------|------------------------------------------------|--------------------|
| Sodium (mmol/L)          | 137 (134–140)                            | 137 (134–140)                                  | 137 (134–140)      |

**Summary of missing data:**

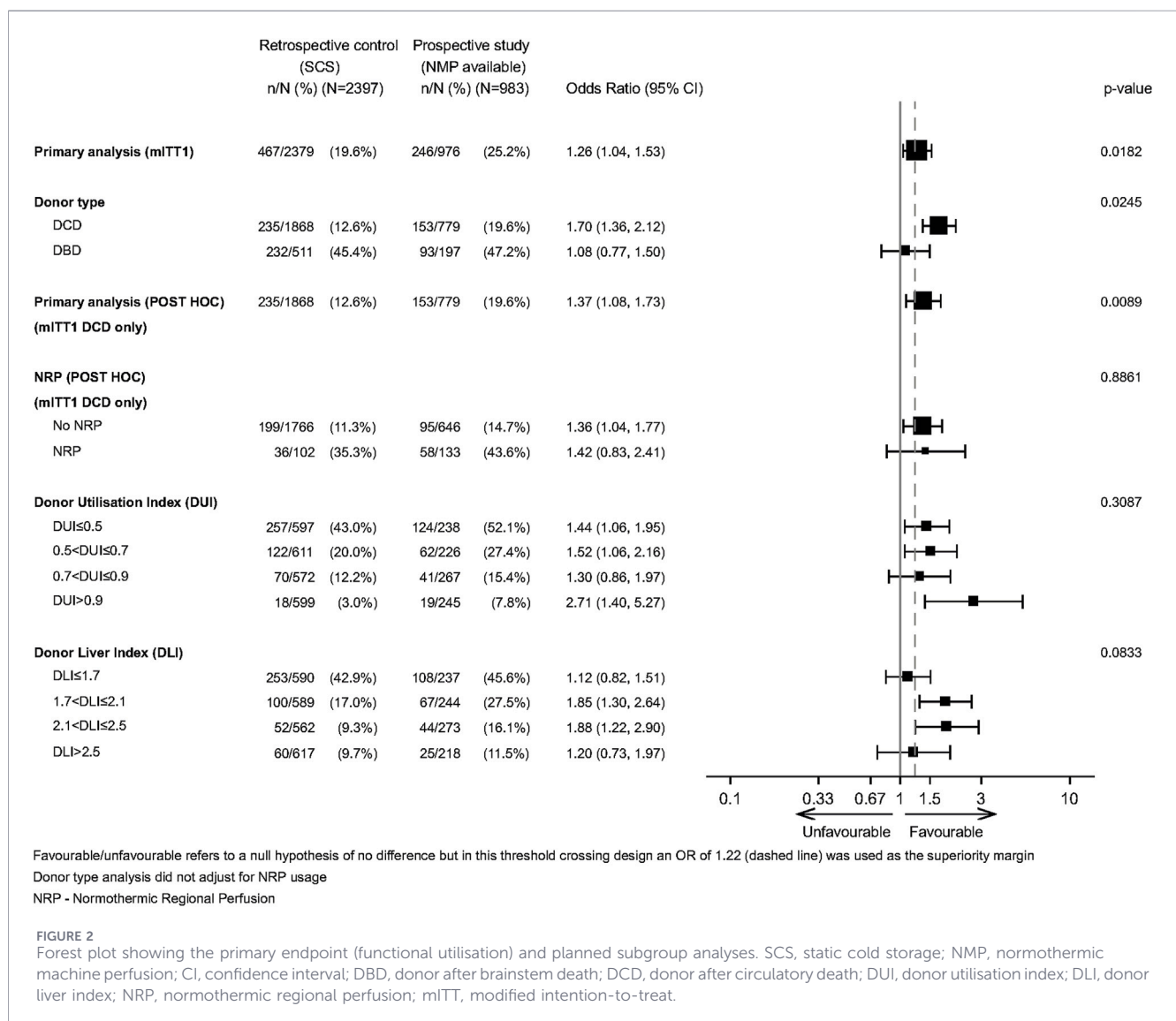
Age, 0; Sex, BMI, and urgency status, 6 (0 SCS, 6 NMP available); Ethnic origin, 37 (9 SCS, 28 NMP available). HCV status, 21 (12 SCS, 9 NMP available); Presence of encephalopathy, 17 (3 SCS, 14 NMP available); Renal support, 10 (1 SCS, 9 NMP available); Primary liver disease 8 (2 SCS, 6 NMP available); UKELD, 34 elective (30 SCS, 4 NMP available), 2 super-urgent (1 SCS, 1 NMP available); MELD, 34 elective (30 SCS, 4 NMP available), 2 super-urgent (1 SCS, 1 NMP available); INR, 40 (29 SCS, 11 NMP available); Creatinine, bilirubin, and sodium, 9 (1 SCS, 8 NMP available).

<sup>1</sup>UKELD,  $5.395 \ln(\text{INR}) + 1.485 \ln(\text{creatinine } \mu\text{mol/L}) + 3.130 \ln(\text{bilirubin } \mu\text{mol/L}) - 81.565 \ln(\text{sodium mmol/L}) + 435$ .

<sup>2</sup>MELD,  $9.57 \ln(\text{creatinine mg/dL}) + 3.78 \ln(\text{bilirubin mg/dL}) + 11.2 \ln(\text{INR}) + 6.43$ .

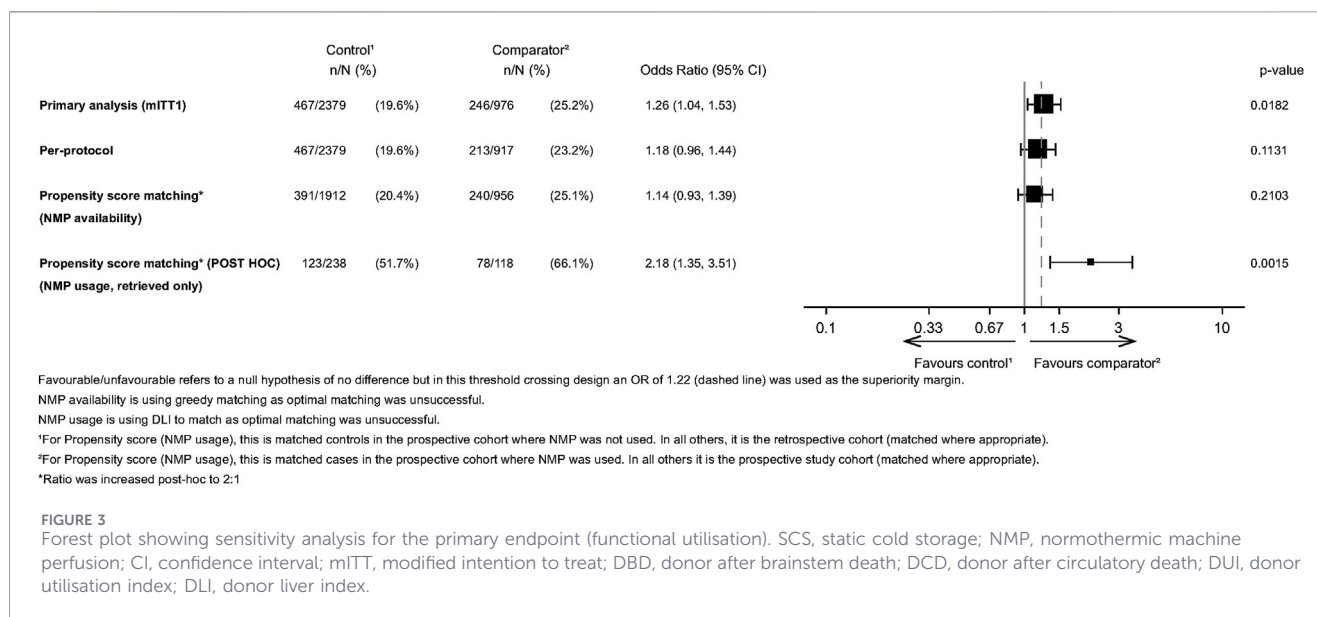
<sup>3</sup>Small number of super urgent recipients (25 total, 18 in SCS, and 7 in NMP available).

Data are n/N (%) for categorical variables, and median (IQR) for continuous variables. SCS, static cold storage; NMP, normothermic machine perfusion; BMI, body mass index; HCV, Hepatitis C virus; UKELD, UK End stage Liver Disease; MELD, Model for End stage Liver Disease; INR, international normalised ratio.



resulted from a combination of the two technologies in DCD donation. Indeed, there is already evidence from the UK that NRP can improve liver utilisation [3], and so in many cases (especially given resource constraints) additional NMP

following NRP may have limited impact. However, even with the use of NRP, NMP allows additional opportunity to recondition and assess function *ex-situ* prior to transplant, and the two technologies are increasingly seen as



complementary rather than exclusive particularly for livers failing NRP criteria [21, 22]. The increase in the proportion of livers retrieved, and the proportion of DCD livers transplanted, in the prospective cohort in our study suggest that with the combined use of these technologies transplant clinicians are more likely to accept organs for assessment that would previously not have been retrieved.

The liver non-utilisation rate (of retrieved livers) increased in the prospective cohort. This was accompanied by a significant increase in the liver retrieval rate, suggesting an increased willingness to retrieve and assess higher-risk organs in this cohort, possibly due to increased availability of both NMP and NRP allowing preservation and assessment.

No significant differences were seen in secondary outcomes. One-year graft and patient survival were consistent with national data, despite the cohort consisting of relatively high DLI livers. Despite an increase in the proportion of DCD livers transplanted in the NMP cohort, a smaller proportion reported biliary complications. The VITTAL study has previously suggested a high rate of non-anastomotic biliary strictures when transplanting very high-risk declined livers following use of NMP, albeit with long pre-perfusion cold ischaemic times [12]. Improved definition of cholangiocyte viability markers during NMP (including bile production, bile pH and bicarbonate levels) may help to mitigate this risk [23]. The current results suggest that in this cohort of livers at high risk of non-utilisation, functional assessment during NMP allows safe transplantation without an increase in the risk of post-transplant complications.

PLUS addressed an important question—what would the effect be of making of NMP available routinely? Strengths of the study are the real-world nature of the liver and patient cohorts, involving all UK transplant centres. The use of registry data for follow-up improved the efficiency of the study, allowing direct comparison to the retrospective control cohort.

The experience of running this study illustrates the challenges of conducting any trial of a novel (but available)

intervention in which the judgement of investigators may be in flux (i.e., loss of equipoise leading to unwillingness to recruit consistently). We believe that the use of a rigorous but non-randomised design of the type used in PLUS may prove to be a useful precedent in future transplant studies. The use of a threshold-crossing design is novel in transplantation: it removes the need to randomise patients to receive conventional therapy (including no intervention), but requires the assumption that the retrospectively-recruited cohort remains typical of current practice. Indeed, one of the main weaknesses of the threshold-crossing design is that changes in practice over time may favour better outcomes in the study cohort. In PLUS, pre-study analysis demonstrated that the primary outcome of functional utilisation had remained remarkably static over time in the 4 years prior to the study, and the donor and recipient characteristics were also very similar between the cohorts. In fact, as seen in SDC12, the functional utilisation of DCD livers had been falling slightly over time, making the observation of increased utilisation in the prospective study cohort even more important. The main differences seen were in the characteristics of livers retrieved and transplanted, which are likely due to a combination of the study intervention and increased use of NRP between the cohorts, which we corrected for in the analysis.

In summary, PLUS supports the hypothesis that NMP availability increases functional utilisation of high-risk livers, but that the benefit of this new technology may be restricted unless it is properly resourced. Future implementation requires consideration of provision of NMP as a service, or centralised delivery of NMP in assessment and repair centres.

The main conclusions of this study, therefore, are that: (i) NMP availability increases organ utilisation, although less than expected; (ii) alternative trial designs are feasible and may be required where equipoise is lacking; (iii) evaluation of new technologies must include the infrastructure needed for effective implementation.

## 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 South Central – Oxford C Research Ethics Committee (ref 21/SC/0297). 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

PF was CI, SK was co-CI and operational lead for the PLUS study, sharing responsibility for overall design and conduct. CW, BD, RR, DN, CC, and HT were co-investigators, and members of the trial management group. BF, AS, CW, J-MP, RM, WJ, CC, and GO were local PIs, responsible for recruitment and study conduct at the individual sites. HT, RB, CB, KB, EA, LS, AN, JM, AI, and KK are members of the NHSBT Clinical Trials Unit, responsible for database design, quality assurance and statistical analysis. AH, BM, and SB assisted in site data collection and data queries. CM represents OrganOx Ltd., the SME partner for the study. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that this study received support-in-kind from OrganOx limited, who provided discounted disposable sets for use in the study. OrganOx was not involved in the study design, collection, analysis or interpretation of data. PF and CC are co-founders and shareholders in OrganOx (a University of Oxford spinout company). At the time of this study, they were in receipt of consultancy payments as non-executive Chief Medical and Technical Officers (respectively) of the company. CW receives consultancy income from OrganOx as Consulting Medical Director, but was not employed at the time of study recruitment. SK, CC, and DN have received consultancy income from OrganOx for assisting with the design and conduct of previous trials. J-MP has received a speaker fee for presenting at an OrganOx symposium. CM is CEO of OrganOx Limited, SME partner for this study.

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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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2026.16653/full#supplementary-material>

## References

1. NHS Blood and Transplant. Annual report on liver transplantation - report for 2023/2024 (2024). Available online at: <https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/34641/nhsbt-liver-transplantation-report-2324.pdf> (Accessed April 16, 2025).
2. NHS Blood and Transplant. Organ and tissue donation and transplantation activity report 2023/2024 (2024). Available online at: <https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/33778/activity-report-2023-2024.pdf> (Accessed April 16, 2025).
3. 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
4. Patrono D, Del Prete L, Eden J, Dutkowski P, Guarrera JV, Quintini C, et al. Machine perfusion of liver grafts: hypothermic versus normothermic versus normothermic regional perfusion. *Int J Surg* (2025) 111(9):5768–82. doi: 10.1097/JIS.0000000000002648
5. Jaber F, Abuelazm M, Soliman Y, Madi M, Abusuilik H, Mazen Amin A, et al. Machine perfusion strategies in liver transplantation: a systematic review, pairwise, and network meta-analysis of randomized controlled trials. *Liver Transplant* (2025) 31(5): 596–615. doi: 10.1097/LVT.0000000000000567
6. Nasralla D, Coussios CC, Mergental H, Akhtar MZ, Butler AJ, Ceresa CDL, 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. Chapman WC, Barbas AS, D'Alessandro AM, Vianna R, Kubal CA, Abt P, et al. Normothermic machine perfusion of donor livers for transplantation in the United States: a randomized controlled trial. *Ann Surg* (2023) 278(5):e912–21. doi: 10.1097/SLA.0000000000005934
8. Yamamoto T, Koizumi N, Markmann JF. The impact of over three years commercial use of *Ex Vivo* normothermic machine perfusion for liver transplantation in the USA: a UNOS/OPTN database analysis. *Artif Organs* (2025) 49:1030–45. doi: 10.1111/aor.14975
9. Wisel SA, Steggerda JA, Kim IK. Use of machine perfusion in the United States increases organ utilization and improves DCD graft survival in liver transplantation. *Transpl Direct* (2024) 10(12):e1726. doi: 10.1097/TXD.0000000000001726
10. Wehrle CJ, Kusakabe J, Gross A, Esfeh JM, Udeh B, Fujiki M, et al. Programmatic normothermic machine perfusion and association with liver transplant waitlist outcomes for patients with lower model for end-stage liver disease score. *J Am Coll Surg* (2025) 241(1):486–96. doi: 10.1097/XCS.0000000000001398
11. Rivera B, Cojuc-Konigsberg G, Canizares S, Chumdermpadetsuk RR, Dib M, Lee D, et al. Normothermic machine perfusion and liver transplant waitlist times: a single-center matched cohort study. *J Surg Res* (2025) 30(25):00209–4. doi: 10.1016/j.jss.2025.03.064
12. 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
13. Mehew J, Thomas H, Friend P, Knight S. Acceptance of deceased donor livers in the United Kingdom – development of a liver “donor utilisation index”. *Transpl Int*. (2026) 39:16484. doi: 10.3389/ti.2026.16484
14. Eichler H, Bloechl-Daum B, Bauer P, Bretz F, Brown J, Hampson L, et al. “Threshold-crossing”: a useful way to establish the counterfactual in clinical trials? *Clin Pharmacol Ther* (2016) 100(6):699–712. doi: 10.1002/cpt.515
15. Collett D, Friend PJ, Watson CJE. Factors associated with Short- and long-term liver graft survival in the United Kingdom. *Transplantation* (2017) 101(4):786–92. doi: 10.1097/TP.0000000000001576
16. Angelico R, Sensi B, Quaranta C, Orsi M, Parente A, Schlegel A, et al. The impact of center volume on the utilization and outcomes of machine perfusion technology in liver transplantation: an international survey. *Artif Organs* (2023) 47(11):1773–85. doi: 10.1111/aor.14635
17. Lopimpisuth C, Goldberg DS. Center-level variability in use of normothermic machine perfusion and the association with recipient insurance. *Clin Transpl* (2025) 39(4):e70140. doi: 10.1111/ctr.70140
18. Ceresa CDL, Nasralla D, Watson CJE, Butler AJ, Coussios CC, Crick K, et al. Transient cold storage prior to normothermic liver perfusion may facilitate adoption of a novel technology. *Liver Transpl* (2019) 25(10):1503–13. doi: 10.1002/lt.25584
19. Hallesy J, Sambommatsu Y, Imai D, Savsani K, Tarashi J, Wolfe S, et al. Comparison of back-to-base and upfront normothermic machine perfusion in liver transplantation: a retrospective cohort study. *Am J Transplant* (2025) 25(8):S237. doi: 10.1016/j.ajt.2025.07.521
20. Marklin GF, Brockmeier D, Spector K. The 20-year paradigm shift toward organ recovery centers: 2500 donors at mid-america transplant and broader adoption across the United States. *Am J Transplant* (2023) 23(7):891–903. doi: 10.1016/j.ajt.2023.01.010
21. Gaurav R, Paul S, Swift L, Martin J, Philipoff A, Webster R, et al. Sequential *in situ* normothermic regional perfusion and *Ex Situ* normothermic machine perfusion in controlled donation after circulatory death liver transplantation: a retrospective single-center experience. *Transplantation* (2026) 110:e1424–e1436. doi: 10.1097/TP.0000000000005659
22. Croome KP, Subramanian V, Mathur AK, Aql B, Mao SA, Clendenon JN, et al. Outcomes of DCD liver transplant using sequential normothermic regional perfusion and normothermic machine perfusion or NRP alone *versus* static cold storage. *Transplantation* (2025) 109(7):1184–90. doi: 10.1097/TP.0000000000005301
23. Mergental H, Laing RW, Kirkham AJ, Clarke G, Boteon YL, Barton D, et al. Discarded livers tested by normothermic machine perfusion in the VITTAL trial: secondary end points and 5-year outcomes. *Liver Transpl* (2024) 30(1):30–45. doi: 10.1097/LVT.0000000000000270



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# Improved access to kidney transplantation with favorable graft survival in highly sensitized candidates under the French acceptable mismatch program

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The scarcity of the human leukocyte antigen (HLA)-compatible donor pool, which leads to a long waiting time for transplantation, often requires specific national or supranational strategies to improve kidney graft access for highly sensitized (HS) patients. Acceptable national mismatch priority is a way to increase the number of potential matched donors without increasing the immunologic graft failure risk. In 2005, the French Acceptable Mismatch program (AMP) was successfully introduced as a strategy to improve kidney allocation for HS patients. The AMP was enhanced in 2015, authorizing up to four HLA-A, -B, -DR, and -DQβ mismatches, provided that each mismatch was an acceptable antigen. Our study compared graft survival between AMP allocation and other kidney allocation modalities among HS recipients between 2016 and 2022 (N = 2,985). After adjustment for donor and recipient characteristics using Cox regression, we found that the risk of graft failure was higher for HS non-AMP transplantations (hazard ratio 1.19 [95% CI 1.04–1.36]; 1.31 [95% CI 1.08–1.59] with death censored). The French AMP improves access to kidney transplantation for HS patients. Post-transplant survival is comparable to other kidney allocation modalities, possibly because of the absence of preformed donor-specific antibodies in recipients' historical and recent serum, which is associated with reduced risk of rejection.

### KEYWORDS

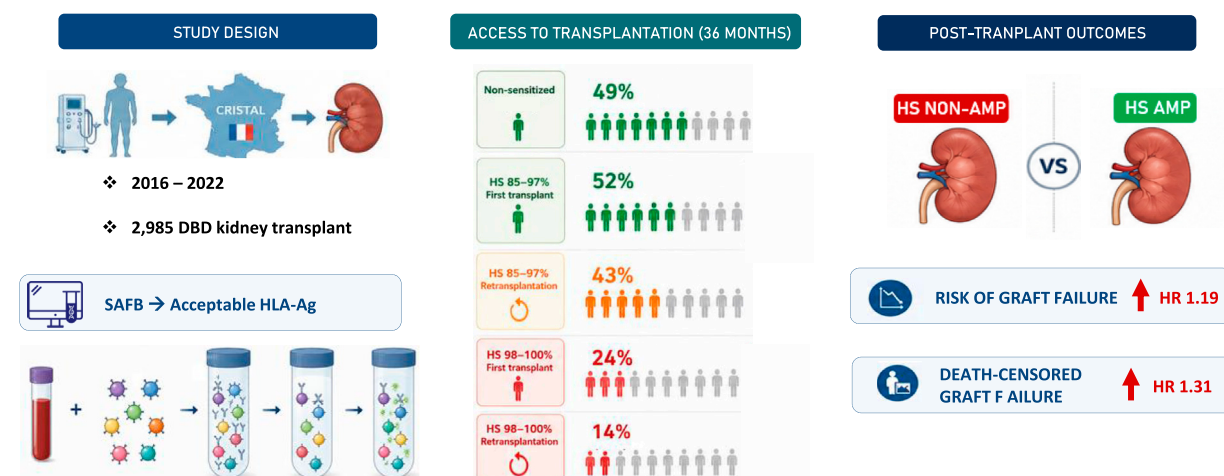
acceptable mismatch program, allocation, highly sensitized, human leukocyte antigen, kidney transplantation

## Introduction

Highly sensitized (HS) patients, who have high levels of preformed anti-HLA (human leukocyte antigen) antibodies, face significant challenges in accessing kidney transplantation because of the limited pool of HLA-compatible donors. The definition of a candidate considered HS varies across countries and national consensus statements. Currently, two



## Improved access to kidney transplantation with favorable graft survival in highly sensitized candidates under the French acceptable mismatch program



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GRAPHICAL ABSTRACT

main criteria are used to assess the severity of sensitization: Virtual Panel-Reactive Antibodies<sup>1</sup> and calculated Reaction Frequency (cRF)<sup>2</sup>. Both estimate the proportion of deceased donors incompatible with a patient owing to pre-formed HLA antibodies [1]. This immunologic barrier leads to prolonged waiting times and increased morbidity and mortality while on a waiting list.

To address this issue, several complementary strategies exist worldwide: 1) national priorities for candidates with high cRF and long dialysis duration, including Acceptable Mismatch Programs (AMPs); 2) expansion of kidney-exchange programs and cross-border sharing, both expanding donor options without increasing rejection risk or compromising long-term graft survival; 3) immunologic forgiveness policies (unacceptable antigen delisting for antibodies that declined in level or disappeared over time or that do not activate complement); and 4) desensitization strategies.

1 Virtual Panel-Reactive Antibodies is based on the frequency of unacceptable antigens in the theoretical donor population.

2 cRF estimates the proportion of HLA-incompatible deceased donors among all transplanted kidney deceased donors (or actual deceased donors within the same blood group, over a specific period).

**Abbreviations:** AMP, Acceptable Mismatch Program; cDCDD, controlled donation after circulatory determination of death; cRF, calculated Reaction Frequency; DBDD, donation after brain determination of death; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; HLA Ab, anti-HLA antibodies; HLA Ag, HLA antigens; HS, highly sensitized; MFI, mean fluorescence intensity; PMD, potential matched donor; SAFB, single antigen flow beads.

In France, national priorities in the allocation rules are based on two principles: difficulty in access to transplantation and the excellence of HLA matching. A kidney recipient is considered HS if their peak cRF is  $\geq 85\%$ ; these patients automatically receive national HS priority, provided that their current cRF remains  $>70\%$ . The system operates via three sequential, prioritized allocation pathways, with systematic age matching and ABO blood-group compatibility exemption in certain circumstances (see below). The pathways are 1) full-match HLA-A, -B, and -DR priority; 2) the AMP; and 3) a priority allowing no more than one mismatch for HLA-A, -B, and -DR between the donor and the recipient. In 2024, 9% of new registrations and 17% of active prevalent candidates were considered hyperimmunized [2].

In some cases, a long list of unacceptable HLA antigens (Ag) or high cRF is insufficient to explain immunologic difficulties in accessing a transplant. This is particularly true because the national score for allocating kidney grafts favors a good HLA match, especially for class-II Ag in recipients  $<45$  years old. This situation may be due to rare haplotypes, including those with no linkage disequilibrium or partial or complete homozygosity. Therefore, another indicator is used: the Potential Matched Donor (PMD). The PMD estimates the number of compatible and well-HLA matched donors among all utilized kidney donations after brain determination of death (DBDD) donors within the same blood group over the past 5 years [3, 4].

A low PMD score indicates that the patient has very few suitable donors. Consequently, these patients receive additional points in the French National Kidney Allocation Score (NKAS) whenever a

compatible and well-matched donor becomes available (see [Table 1](#) for a description of kidney transplantation allocation pathways). This final point explains why a certain number of HS patients undergo transplantation via the NKAS system.

France introduced its AMP in 2005 ([Table 2](#)). An initial evaluation in 2010 demonstrated improved access to kidney transplantation for HS patients, with no difference in graft survival from other patients. Within 2 years, access to transplantation for highly immunized incident patients increased, but access decreased for non-immunized or slightly immunized patients during the same period [5]. The AMP was significantly enhanced in 2015 with the adoption of advanced solid-phase assays, such as Lumindex, which have revolutionized HLA antibody (Ab) screening and identification, particularly for class-II HLA Ag. Single Antigen Flow Bead (SAFB) assays enable precise, semi-quantitative detection of HLA Ab and acceptable HLA Ag, in accordance with national guidelines issued by the French Society of Histocompatibility and Immunogenetics (SFHI) [6].

The « virtual cross match » is routinely implemented in all transplant centers in France to decrease the duration of cold ischemia and the risk of delayed refusal for immunologic reasons [6]. The conditions for performing a pretransplant virtual crossmatch in an emergency are standardized by the national scientific committee of the *Agence de la Biomédecine* (official referral procedure 7 June 2016, 2016G-04 deliberation). HLA-C, -DP, and -DQa typing is now available for each deceased donor, which improves the pretransplant virtual crossmatch accuracy [6].

The aim of this study was to 1) evaluate the transplant activity of HS patients and 2) compare post-transplant outcomes of HS kidney transplant recipients according to whether they benefited from the French AMP or other allocation modalities for HS transplantation.

## Materials and methods

### Study design, endpoints, and populations

For all analyses, HS recipients were defined as those with peak cRF  $\geq 85\%$  and current cRF  $>70\%$ . Non-sensitized recipients were defined as those with current cRF 0% or without registered HLA antibodies. Moderately sensitized (MS) recipients were defined as those with peak cRF  $<85\%$  and current cRF 1%–84% or peak cRF  $\geq 85\%$  and current cRF  $\leq 70\%$ . MS recipients were divided into two groups: group A, recipients with peak cRF  $<85\%$  and current cRF  $<85\%$ , and group B, recipients with peak cRF  $\geq 85\%$  and current cRF  $\leq 70\%$ .

Data on the kidney waiting list and transplant activity were available for HS and MS recipients from 2016 to 2024. Additionally, longitudinal analyses of new registrations from 2005 to 2024 by transplant number, sensitization, and age were available.

For the analysis of access to transplantation, we included candidates registered on the waiting list from 2016 to 2023. Candidates were stratified by transplant number (first vs. retransplantation) and sensitization (non-sensitized, peak cRF 85%–97%, and peak cRF 98%–100%). Data for MS groups A and B are available in the [Supplementary Materials](#).

For the post-transplant survival analysis, we included all adult single-organ kidney transplant recipients from DBDDs in

France from 2016 to 2022, with a mean follow-up of 45.5 months. The analysis focused on non-sensitized and HS recipients. Cox regression was restricted to HS recipients to ensure that the analysis focused on risk factors relevant to this population. HS recipients were stratified by allocation modality (AMP vs. non-AMP). In a supplementary analysis, HS non-AMP recipients were further divided into two groups: those with and without the presence of subsequently delisted preformed donor-specific antibodies (DSAs). Different survival outcomes were studied: death-censored graft loss and overall graft failure, including patient death.

For [Supplementary Materials](#), the frequency of acute or chronic rejection frequency was calculated for HS AMP, HS non-AMP without DSA, and HS non-AMP with DSA recipients. Please note that this data entry is not exhaustive and the data quality is not audited.

Patients were followed until 1 January 2025.

### Data collection and definition of variables

The French national transplant registry (CRISTAL, *Agence de la Biomédecine*, Saint-Denis, France) prospectively collects demographic, clinical, and laboratory data for all organ transplant recipients and donors in France, as well as transplant outcomes. Data are recorded at the time of registration (inclusion in the transplant waiting list), procurement, and transplantation and then annually thereafter. Data collection is mandatory, and research technicians double-check the completeness and accuracy of data. In accordance with French law, research studies based on this national registry are part of transplant assessment and do not require additional institutional review board approval. The database has been reported to the French National Commission on Computing and Liberty.

Donor estimated glomerular filtration rate (eGFR) values were estimated with the CKD-EPI formulas [7].

### Statistical methods

Data are expressed as mean (SD, standard deviations) or median ([IQ], interquartile ranges), depending on the data distribution or number (%), percentage). For the multivariate analysis, missing data were imputed to the least risky and most frequent category when possible: body mass index ( $<1\%$ , 2 recipients) imputed to the median, missing HLA-DQ mismatches ( $<1\%$ , 2 transplantations) imputed to 0, and donor hypertension ( $<1\%$ , 17) imputed to no. A complete-case analysis was performed as a sensitivity analysis.

Access to kidney transplantation was estimated using the cumulative incidence function, with death or delisting for worsening condition treated as competing risks, and group comparisons performed with Gray's test. Data were censored 3 years after registration or at the time of delisting for reasons other than worsening condition or kidney transplantation, whichever occurred first.

Post-transplant survival curves were estimated with the Kaplan-Meier method and compared with the log-rank test. Factors associated with post-transplant failure were assessed with multivariable Cox proportional-hazards models. Candidate covariates for multivariable analysis were selected by an adjustment strategy that combined statistical and clinical

TABLE 1 Kidney transplantation allocation pathways for highly sensitized (HS) candidates and the approximate share of kidney transplants for each.

| Allocation pathway                    | Approximate share | Priority level | Description                                                                                                                                                     |
|---------------------------------------|-------------------|----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Full HLA match (A, B, DR)***          | ~7%               | Priority #1    | Allocation to recipients with full HLA A, B, DR match with the donor                                                                                            |
| Acceptable mismatch program (AMP)* ** | ~47%              | Priority #2    | Access to grafts with each HLA mismatch considered “acceptable” based on individual antibody profiles (SAFB profile) and ≤4 HLA-A, -B, -DR, and -DQβ mismatches |
| ≤1 HLA mismatch (A, B, DR)***         | ~9%               | Priority #3    | For recipients with ≤1 mismatch among HLA-A, -B, and -DR                                                                                                        |
| Score-based allocation                | ~25%              | Standard       | Allocation according to the national scoring system that integrates waiting time, age matching, HLA matching, and sensitization factors such as PMD.            |
| Other and combined graft              | ~6%               |                |                                                                                                                                                                 |
| Living donor                          | ~6%               |                |                                                                                                                                                                 |

\*Age matching: the donor is not >15 years younger if the recipient is ≥50 years old).  
\*\*Blood group exemptions: restricted blood group exemption in group A for AB HS, candidates and in group O for B HS, candidates as soon as the cRF, reaches 85%; ABO, compatible exemption for all HS, candidates when the cRF, reaches 95%.  
Remark: Non-AMP, recipients belong to Priority #1, Priority #2, and Standard Score-based Allocation.  
HLA: human leukocyte antigen; PMD: potential matched donor; SAFB: single antigen flow beads.

TABLE 2 French acceptable mismatch program.

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Eligible patient criteria                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <ul style="list-style-type: none"><li>• Waiting time &gt;18 months</li><li>• Peak cRF &gt;85%, based on results from SAFB assays; current cRF &gt;70%</li><li>• List of acceptable HLA mismatches based on both current and historical sera, with normalized MFI of less than 500, updated every 3 months in the CRISTAL database</li></ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Kidney offer conditions                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <ul style="list-style-type: none"><li>• If the recipient is &gt;50 years, the donor must not be &gt;15 years younger than the recipient</li><li>• Restricted derogation to group A for AB patients and to group O for B patients is granted once cRF reaches 85%. Derogation to group O for A and AB recipients is allowed only when cRF ≥95%</li><li>• Institutional minimum match criteria must be fulfilled, especially regarding the number of HLA class-II mismatches: ≤4 HLA-A, -B, -DR, or -DQβ mismatches, ≤1 HLA-DQβ mismatch, and ≤1 HLA-DR mismatch</li><li>• Each mismatch at HLA-A, B, DR, or DQβ must be acceptable</li><li>• Negative pretransplant virtual crossmatch required before kidney graft transfer</li><li>• Negative T and B cell crossmatch required before kidney transplantation</li></ul> |

cRF: calculated reaction frequency; HLA: human leukocyte antigen; MFI: mean fluorescence intensity.

considerations. Variables associated with the outcome at  $p < 0.20$  in univariable analyses, along with those deemed clinically relevant based on transplant expertise and the literature, were considered for inclusion in the multivariable model. A stepwise selection procedure with a large  $p$ -value threshold ( $p < 0.20$ ) was used to derive the final model while ensuring retention of clinically important covariates. The hazard ratio (HR) for the outcome with continuous variables was explored graphically with restricted cubic splines; graphs were defined according to spline plots and clinical relevance. The primary objective was to estimate the total effect of the AMP protocol on post-transplant outcomes. Consequently, we excluded variables considered potential mediators of this effect, including HLA matching, DSAs, and cold ischemia time from the adjustment set to avoid overadjustment. Adjustment for these variables could have accounted for mechanisms by which the AMP exerts its effect on

outcomes. The selection of adjustment variables was guided by a prespecified causal framework, summarized in a directed acyclic graph (Supplementary Figure S1).

Statistical analyses were performed with SAS enterprise guide 9.3 (SAS Institute, Cary, NC, USA). All  $p$ -values were two-tailed and  $p < 0.05$  was considered statistically significant.

## Results

### Kidney waiting list and transplant activity of HS recipients

From 2016 to 2025, the number of HS patients active on the kidney waiting list decreased from 2,024 to 1,866 (from 28% to 17%,

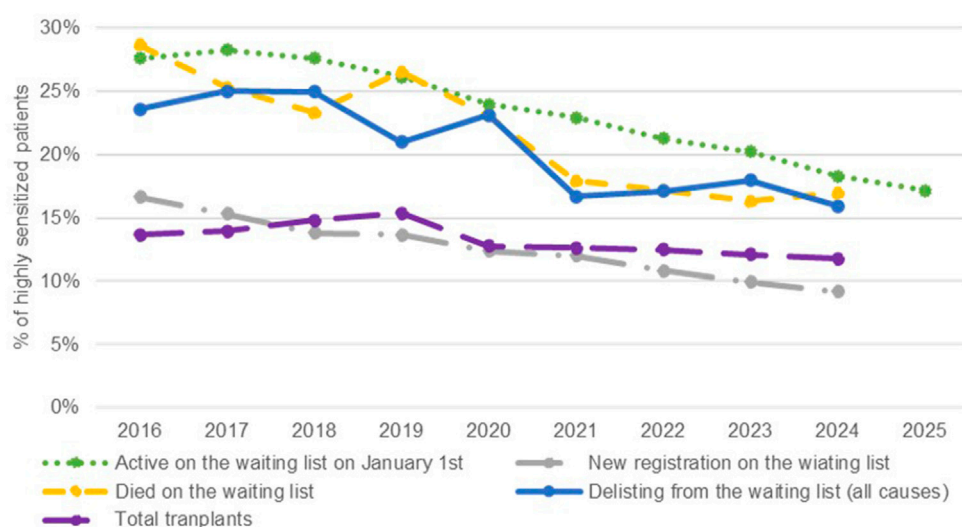


FIGURE 1  
Waiting list and transplant activity of highly sensitized (HS) patients.

Figure 1; Supplementary Table S1; MS recipients in Supplementary Table S2; reasons for delisting in Supplementary Table S3). Annual new registrations also decreased steadily, representing a decline from 17% to 9%. These decreases were observed without any modification to the method used to calculate cRF. The total number of transplants ranged from 331 to 560 annually, with a marked decline after 2019. Transplants were mainly from DBDD; living donor and controlled donation after circulatory determination of death represented a small but stable minority throughout the period. When restricting the analysis to new registration candidates to avoid the effect of long-waiting patients with very high cRF, we found a reduction in the number of eligible HS candidates as first-transplant (−35%) and retransplant patients (−43%) (Figure 2A). This decrease in proportion was more pronounced for male than female candidates (−55% vs. −27%).

Among prevalent patients actively listed on January 1 of each year, the clinical characteristics differed significantly between HS and non-sensitized candidates. The distribution of sensitization levels varied markedly across recipient subgroups (Figure 2B; Supplementary Figure S2). Non-sensitized recipients predominated among first transplant candidates and preemptive recipients, whereas HS patients (HS 98%–100%) were overrepresented among retransplantation and long-term dialysis (>2 years) groups. Moderate sensitization (MS) and intermediate highly sensitization (cRF 85%–97%) accounted for substantial proportions in female and male recipients as well as those with 1–2 years of dialysis. Overall, retransplantation and prolonged dialysis were associated with an increased burden of sensitization as compared with first transplants or shorter dialysis exposure. Accordingly, retransplantation accounted for 69% of listings among HS candidates versus only 12% among non-sensitized candidates. Women represented 57% of HS candidates versus 34% of non-sensitized candidates. Furthermore, 36% of HS patients had been on dialysis for >2 years versus 21% of non-sensitized candidates.

Longitudinal analyses of new registrations are in Supplementary Figures S3–S5.

## Access to kidney transplantation

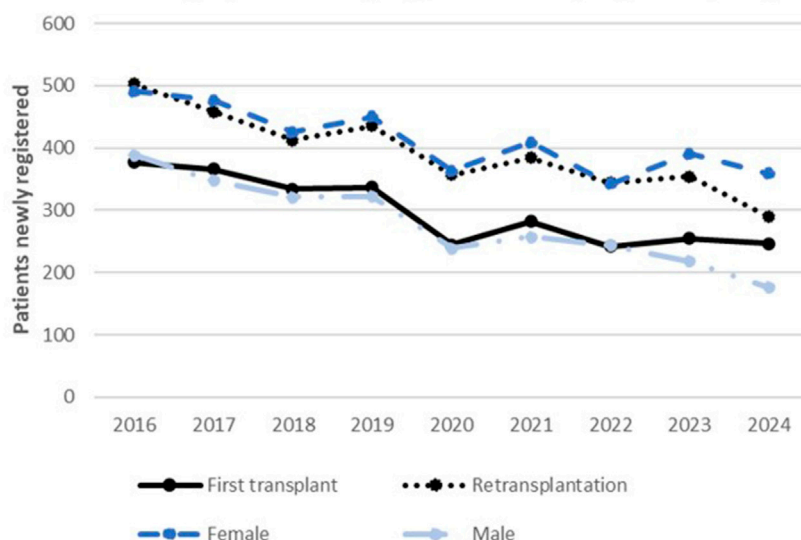
The cumulative incidence of kidney transplantation varied markedly by cRF level and transplant number ( $p < 0.001$ ) (Figure 3). Non-sensitized recipients showed the highest access to transplantation: 49% [49–50] at 36 months. Among HS recipients, those with peak cRF 85%–97% undergoing a first transplant had comparable access: 52% [50–55] transplanted at 36 months. In contrast, retransplant candidates with peak cRF 85%–97% had lower access at 43% [40–45]. Outcomes were poorest for patients with peak cRF 98%–100%: 24% [21–28] for first transplants and only 14% [12–16] for retransplants at 36 months. Overall, access decreased with higher cRF and was consistently lower with retransplantation versus first transplantation. We note a clear inflection in the access curve at about 18 months after waitlist registration, corresponding to the time when patients become eligible for the national AMP. Notably, despite substantial differences in access to transplantation, the cumulative incidence of delisting for death or worsening condition remained low across sensitization categories at 36 months, from 8% [7–10] to 10% [8–13] frequencies. The cumulative incidence of delisting for death or worsening condition is detailed in Supplementary Figure S6. The cumulative incidence of kidney transplantation for MS group A and B is detailed in Supplementary Figure S7.

## Post-transplant survival analysis

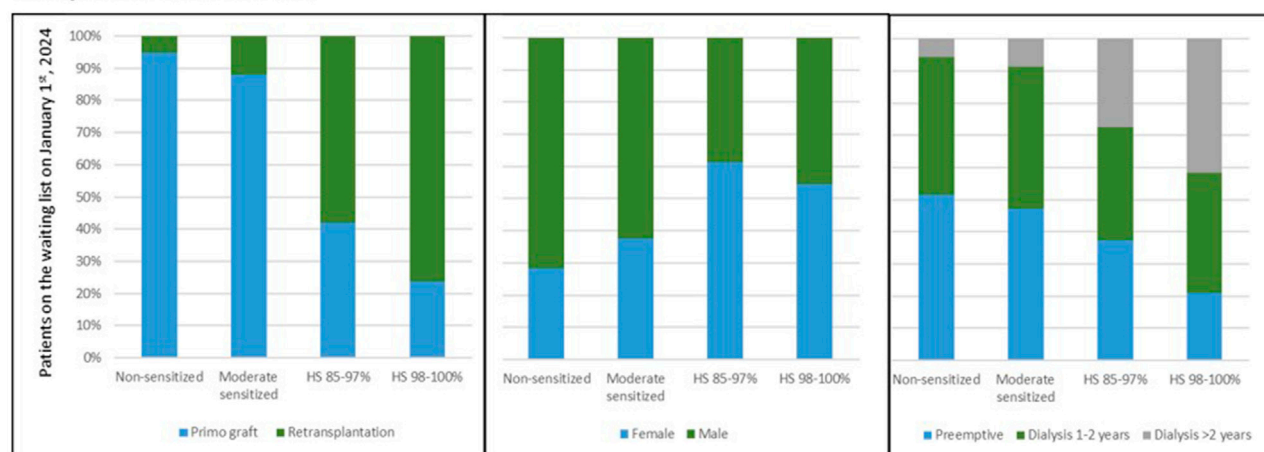
From 2016 to 2022, 2,985 single-organ kidney transplantations were performed in adult HS recipients from DBDDs in France (Supplementary Figure S8).



### A – Evolution of highly sensitized (HS) patients newly registered; analysis by transplant number and sex



### B – Distribution of patients on the waiting list on January 1, 2024 by sensitization status: analysis by transplant number and sex



HS: highly sensitized

FIGURE 2

Characteristics of patients by sensitization. (A) Evolution of highly sensitized (HS) patients newly registered; analysis by transplant number and sex. (B) Distribution of patients on the waiting list on January 1, 2024 by sensitization status: analysis by transplant number and sex.

Known risk factors for graft failure were not equally distributed between non-sensitized and HS recipients or between AMP and non-AMP recipients (Supplementary Table S4).

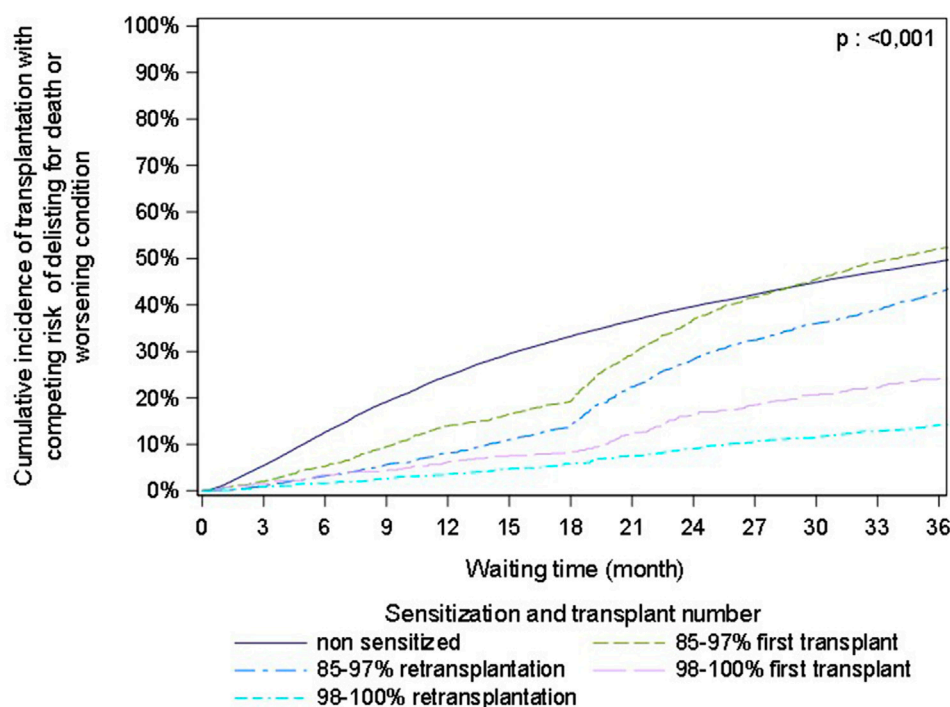
As compared with non-sensitized recipients, HS recipients were more frequently younger, female, undergoing retransplantation, and on dialysis; they more frequently had blood group B and a longer dialysis duration. They less often had a body mass index  $>30$  kg/m<sup>2</sup>, comorbidities, and a diabetic cause for end-stage kidney disease. They more often received right-side kidneys, received kidneys from donors who were 45–69 years old, had died from vascular causes, and had blood group O, and more often had longer cold ischemia times than non-sensitized recipients.

As compared with HS non-AMP recipients, HS AMP recipients were older and had more comorbidities. They were less often pre-

emptive but had shorter dialysis duration. HS AMP recipients more often received kidneys from older donors and had longer cold ischemia times. Non-AMP recipients more often had previous history of kidney transplants, more frequently a peak cRF of 99%–100% (26% vs. 14%), and transplantations with more HLA mismatches.

Overall survival death censored rates were comparable for HS AMP and non-sensitized recipients during follow-up, with no significant difference on log-rank testing ( $p = 0.1185$ ) (Figure 4). In contrast, survival was significantly lower among HS non-AMP than HS AMP recipients ( $p = 0.0125$ ). Kidney graft survival with death censored for MS groups A and B is detailed in Supplementary Figure S9. Patient survival by sensitization is detailed in Supplementary Figure S10.





| Recipients                    | N     | Cumulative incidence of transplantation with competing risk of delisting for death or worsening condition in % [95% CI] |             |              |              |              |
|-------------------------------|-------|-------------------------------------------------------------------------------------------------------------------------|-------------|--------------|--------------|--------------|
|                               |       | at 3 months                                                                                                             | at 6 months | at 12 months | at 24 months | at 36 months |
| Non-sensitized                | 19011 | 5 [5-6]                                                                                                                 | 13 [12-13]  | 25 [24-25]   | 40 [39-40]   | 49 [49-50]   |
| 85-97% and first transplant   | 1560  | 2 [1-3]                                                                                                                 | 5 [4-6]     | 14 [12-16]   | 37 [34-39]   | 52 [50-55]   |
| 85-97% and retransplantation  | 1542  | 1 [1-2]                                                                                                                 | 3 [2-4]     | 8 [7-9]      | 28 [26-31]   | 43 [40-45]   |
| 98-100% and first transplant  | 623   | 1 [1-3]                                                                                                                 | 3 [2-5]     | 6 [5-8]      | 16 [14-20]   | 24 [21-28]   |
| 98-100% and retransplantation | 1351  | 1 [0-1]                                                                                                                 | 2 [1-3]     | 4 [3-5]      | 9 [8-11]     | 14 [12-16]   |

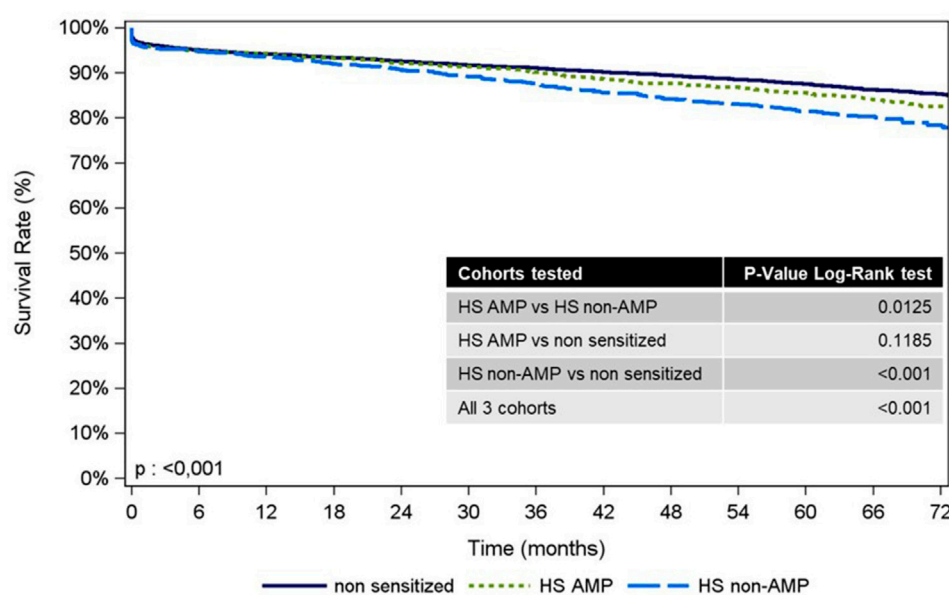
FIGURE 3  
Cumulative incidence of access to transplantation by sensitization status and transplant number.

After adjustment for donor and recipient characteristics with a Cox model, risk of graft failure was associated with HS non-AMP allocations (HR 1.19 [95% CI 1.04–1.36]; 1.31 (95% CI 1.08–1.59) with death censored; [Supplementary Figure 11](#) and [Figure 5](#)). Three sensitivity analysis were performed. First, a complete-case analysis excluded transplants with missing data for 1,398 HS non-AMP and 1,553 HS AMP recipients. The risk of graft failure was associated with HS non-AMP allocations (HR 1.19 [95% CI 1.04–1.36]; 1.30 [95% CI 1.07–1.58] with death censored). Second, we added a center effect. Again, the risk of graft failure was associated with HS non-AMP allocations (HR 1.17 (95% CI 1.02–1.34); 1.28 [95% CI 1.05–1.55] with death censored). Finally, in an analysis restricted to right-side donor donation, the risk of graft failure was associated with HS non-AMP allocations (HR 1.20 [95% CI 1.01–1.42]; 1.31 [95% CI 1.04–1.65] with death censored). Other independent predictors of death-censored graft loss included young recipient age, high body mass index, multiple comorbidities, dialysis  $\geq 3$  years, transplant number  $\geq 2$ , older donor age, donor hypertension, and low donor eGFR.

The kidney graft survival with death censored for HS AMP, HS non-AMP without DSA, and HS non-AMP with DSA recipients is detailed in [Supplementary Figure S12](#). Acute or chronic rejection frequency for HS AMP, HS non-AMP without DSA, and HS non-AMP with DSA recipients is detailed in [Supplementary Table S4](#).

## Discussion

This study demonstrates that improved death-censored graft survival in HS patients is achieved with the French AMP, offering post-transplant survival comparable to that for non-HS recipients transplanted under the standard allocation scheme. Despite an increased risk of rejection, HS recipients transplanted under the AMP achieved significantly better 5-year graft survival than HS patients transplanted outside the AMP, without additional desensitization strategies. At comparable cRF levels, HS AMP candidates awaiting a first transplant had better access to transplantation than those awaiting retransplantation, which reflects higher class-II HLA immunization and fewer permissible class-II HLA Ag in the latter group. The profile of patients who benefited most from access programs included candidates with common HLA phenotypes with cRF  $> 97\%$ , first-transplant recipients, patients with lower class-II HLA sensitization, particularly against HLA-DQ $\beta$  Ag, and those with a greater number of permissible mismatches in class-II HLA, including DR and DQ $\beta$ . In contrast, HS non-AMP patients more frequently had a history of prior kidney transplantation. This observation reflects listing for retransplantation commonly being associated with increased class-II HLA immunization and DSAs. In this context, selected patients might be under an



|                                  | N    | Survival at 1 month      | Survival at 1 year       | Survival at 5 years      |
|----------------------------------|------|--------------------------|--------------------------|--------------------------|
| <b>Non-sensitized recipients</b> | 8395 | 96,6%<br>[96,1% - 96,9%] | 94,2%<br>[93,6% - 94,7%] | 87,5%<br>[86,7% - 88,3%] |
| Patients at risk                 |      | 8008                     | 7313                     | 3016                     |
| <b>HS AMP recipients</b>         | 1575 | 96,2%<br>[95,2% - 97,1%] | 94,3%<br>[93,0% - 95,3%] | 85,6%<br>[83,4% - 87,5%] |
| Patients at risk                 |      | 1495                     | 1373                     | 533                      |
| <b>HS non-AMP recipients</b>     | 1410 | 95,9%<br>[94,7% - 96,8%] | 93,6%<br>[92,1% - 94,7%] | 81,5%<br>[78,9% - 83,8%] |
| Patients at risk                 |      | 1333                     | 1218                     | 463                      |

HS: highly sensitized; [ ]: 95% Confidence Interval

FIGURE 4

Kidney graft survival with death censored in highly sensitized (HS) Acceptable Mismatch Program (AMP) recipients and HS non-AMP recipients vs. non-sensitized recipients (observation end date: January 1, 2025, Kaplan-Meier method).

immunologic forgiveness policy to facilitate access to a subsequent graft.

Over the past 10 years, the proportion of HS patients on the active waiting list decreased markedly, from 29% to 17%. This improvement is mainly the result of two combined factors.

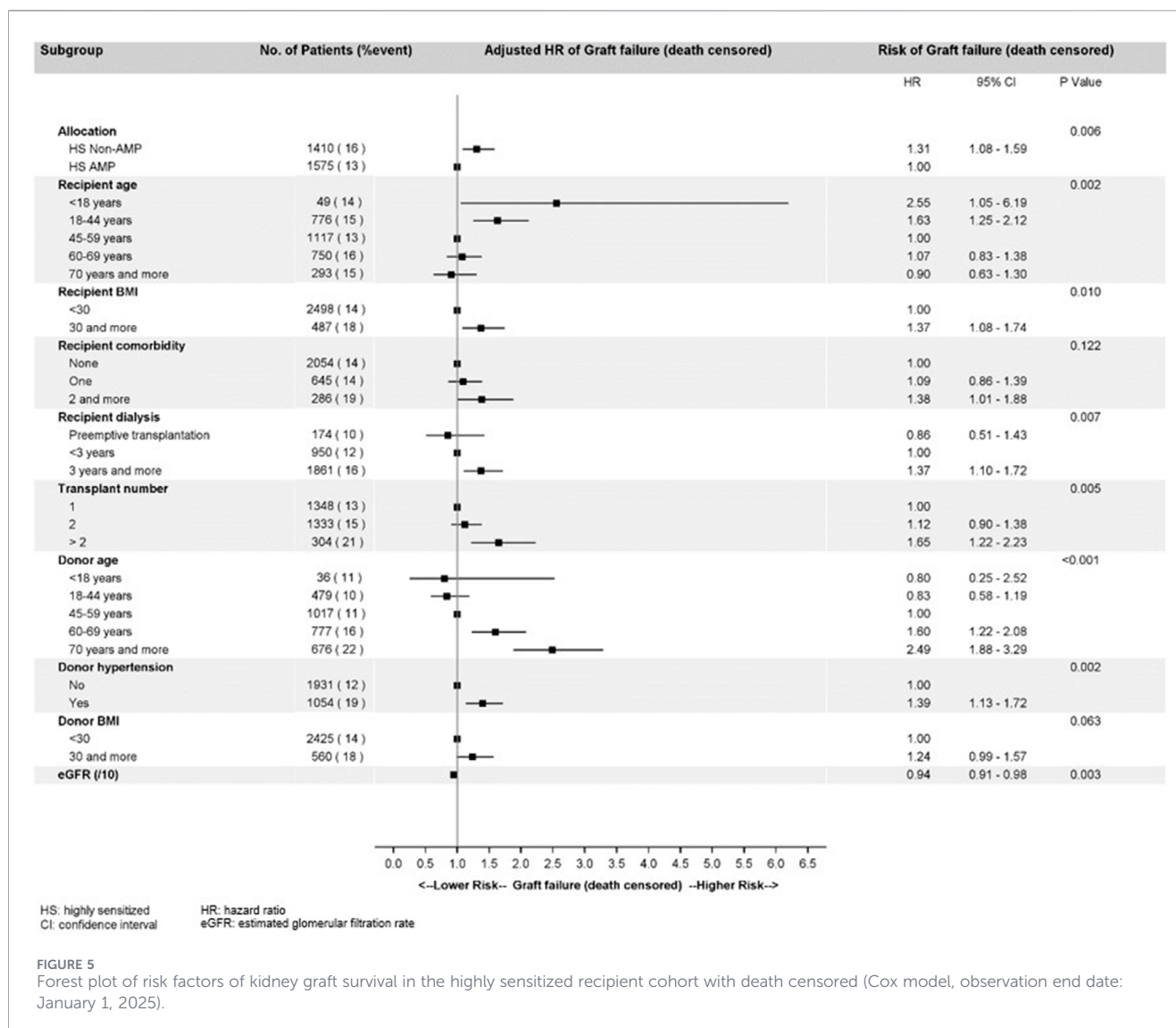
The first factor is better HLA matching strategies, especially the emphasis on high-quality class-II matching, which is included in our French National Kidney Allocation Score and is strongly weighted for younger recipients. This strategy, implemented since the nationalization of the kidney allocation score [3], helps reduce the creation of hyperimmunized patients after a previous graft failure by introducing immunologic sparing for the youngest recipients. HLA compatibility, particularly class-II HLA, carries a high coefficient in the score, especially for the youngest patients; the weight then decreases with recipient age. By promoting HLA class-II matching among young adult recipients, the number of hyperimmunized re-registrants has been limited and post-transplant sensitization reduced.

The second factor in the reduced proportion of HS patients on the active waiting list is improved allelic resolution of anti-HLA antibody testing and donor HLA typing and, a better interpretation based on SFHI and European Federation for Immunogenetics recommendations [8]. Recent recommendations have helped refine HLA antibody characterization by encouraging high-resolution, allele-level

analysis while maintaining a critical approach to the risk of false-positive SAFB results, particularly via the evaluation of autoreactivity and assay artefacts [9–14]. These guidelines also emphasize avoiding the blanket exclusion of all DQβ Ag carrying a given DQα epitope and instead recommend focusing on the corresponding frequent (haplotype-based) DR-associated specificities in the presence of clinically relevant anti-DQα reactivity. Additional measures, such as improved technical standardization across laboratories, reduction of inter-assay variability, and the use of epitope-based interpretation tools, have been implemented, thereby enhancing the accuracy of unacceptable Ag assignment [12].

Collectively, these advances have substantially reduced the number of listed unacceptable HLA Ag and, consequently, the proportion of candidates classified as HS. These developments could be effectively implemented with the national adoption of the pretransplant virtual crossmatch, which serves as the final and essential safety check before accepting a graft. This implementation was facilitated by the integration, within the CRISTAL donor-management platform, of allele-level donor HLA typing together with national guidelines defining interpretation criteria and technical warning thresholds [6].

The French experience can be viewed as a succession of major immunologic milestones: 1) nationwide implementation of SAFB-based unacceptable Ag in 2009; 2) introduction of a cRF directly



derived from SAFB-defined unacceptable Ag in late 2009; 3) incorporation of anti-HLA-DQ antibodies into kidney allocation and cRF calculation in 2009; 4) introduction of the new French Kidney Allocation System in 2015 with improved HLA matching, particularly for younger recipients; 5) nationwide implementation of virtual crossmatch and intermediate/high-resolution HLA typing from 2016 onward; and 6) progressive refinement of antibody interpretation by the exclusion of denatured HLA antibodies and isolated allele-specific sensitizations. Together, these successive developments likely explain why France experienced an early increase in the proportion of HS candidates, followed by a sustained decline over the last few years. Future changes in national consensus regarding the integration of anti-HLA-Cw and -DP antibodies into cRF calculation and the allocation algorithm for HS patients may again modify the epidemiology of HLA sensitization, particularly among retransplant candidates.

Although the cRF remains the cornerstone indicator for defining immunologic sensitization and access to national priority, it does not fully capture the structural difficulty of identifying a compatible

donor for a given candidate. In this respect, complementary indicators such as the PMD, which estimates the number of compatible and well-matched donors among actually utilized deceased donors in recent years, provide additional insight into real-world transplantability. Patients with similar cRF values may exhibit markedly different PMD profiles, reflecting differences in HLA phenotype frequency and compatibility constraints. This distinction is particularly relevant for candidates with rare HLA phenotypes or limited acceptable mismatches, who may remain disadvantaged despite comparable cRF levels.

HS patients with a cRF >85% but <98% have good access to kidney transplantation. Their transplant rate increases markedly after 18 months on the waiting list and eventually reaches that of non-immunized first transplant candidates. Among candidates with cRF 98%–100%, most are awaiting retransplantation. Of the quarter of candidates who are first-transplant patients, most are women sensitized (90%) through pregnancy and/or transfusion, with broad class-I HLA immunization. In this group, first-time registrants have higher transplant rates, reaching 24% by 3 years. Overall, patients with common HLA phenotypes, particularly first-transplant

candidates, including women, benefit most, because they have fewer unacceptable class-II Ag and more acceptable mismatches, especially regarding HLA-DQ $\beta$  Ag. Finally, the AMP program also addresses sex inequities, because women are more frequently sensitized than men.

After adjustment, risk of death-censored graft failure was 31% higher for HS non-AMP than HS AMP recipients. Other significant risk factors included young recipient age, high body mass index, multiple comorbidities, prolonged dialysis duration (>3 years), retransplantation, and older donors with hypertension. Young recipient age has consistently emerged as a risk factor in numerous studies, because immune responsiveness tends to be more vigorous in younger individuals and this group also has a higher likelihood of non-adherence. Cold ischemia time also plays an important role, as it does in all kidney transplants, by inducing damage-associated molecular patterns via ischemia-reperfusion injury, leading to the overexpression of HLA Ag on endothelial cells. Moreover, recent mechanistic reviews highlight that ischemia-reperfusion injury triggers endothelial activation and complex innate-adaptive immune interactions that contribute to both acute and antibody-mediated rejection in kidney transplantation [15].

The French allocation framework has historically embedded the principle that priority for HS candidates should be granted only when the proposed transplant is expected to carry an acceptable immunologic risk and outcomes comparable to those observed under standard allocation rules. Therefore, national priority has never been conceived as an unconditional advantage but rather as a mechanism to restore equity while preserving optimal graft utility. In this context, the AMP fits precisely within this regulatory philosophy because it is able to expand donor options: it allows for improved access for difficult-to-match candidates without increasing rejection risk or compromising long-term graft survival. Several notable differences distinguish the French AMP from the Eurotransplant AMP. Both rely on a cRF threshold of 85% based on real donor data, although the Eurotransplant AMP is accessible after 2 years of waiting time on dialysis and centralizes eligibility assessment within a single reference laboratory in Leiden, where the immunologic profile is determined not just from SAFB testing but also includes the HLA Matchmaker [1, 16, 17]. This algorithm identifies acceptable Ag predicted to carry a very low risk of *de novo* DSAs formation, based on shared or non-permissive eplets, whereas the French AMP prioritizes identifying donors without any historical or current DSA on the day of transplantation. Despite these methodological differences, outcomes remain excellent in both programs, as shown in the most recent Eurotransplant reports [11, 18, 19]. A final distinction is that the French AMP does not incorporate anti-HLA-Cw or -DP antibodies in its exclusion criteria because of the lack of national consensus on their interpretation, particularly given the high proportion of false-positive anti-HLA-Cw results in first kidney transplant candidates [10, 20]. These antibodies are instead managed at the time of organ offer via pretransplant virtual crossmatch assessment and, when necessary, confirmatory cellular testing.

Nevertheless, a subset of candidates remains poorly served by current allocation strategies, including those with extremely high cRF values combined with rare HLA phenotypes, absence of

common linkage-disequilibrium haplotypes, or homozygosity at multiple HLA loci. For these patients, even acceptable mismatch-based approaches may fail to generate compatible offers. These limitations underscore the need to shift the focus upstream, by preventing the emergence of such extreme immunologic profiles via improved HLA matching, particularly at the class-II level, beginning at the time of the first transplant for the youngest recipients. In the future, the number of such patients could be reduced if a minimum level of HLA class-II compatibility, or the inclusion of less immunogenic class-II matching, is systematically implemented in standard allocation systems. Another elegant approach would be to preferentially allocate both kidneys from a single donor with homozygous HLA class-II typing to HS candidates with cRF 99%–100% who have been on dialysis for several years. From the French CRISTAL Registry, we found 193 DBDDs aged 18–65 years who matched this hypothesis from 2021 to 2023. An analysis of the fate of these “golden” grafts showed that a significant proportion are often transplanted locally to less sensitized recipients with few or no years on dialysis, even though, because of the homozygous HLA DR-DQ $\beta$  haplotype, the graft may represent the only compatible organ for HS patients waiting for >5 years.

Recent recommendations have also proposed strategies for these long-waiting patients without an immediately compatible donor, including immunologic forgiveness policies (Ag delisting after antibody decline or disappearance over time or antibodies that do not activate complement) [21, 22] or carefully monitored desensitization protocols [23, 24], based on simulations from deceased donor databases. Indeed, HS non-AMP recipients are at increased immunologic risk and require more intensive immunosuppressive regimens, with associated infectious or oncologic complications, yet outcomes remain acceptable, providing a viable option when no alternative is available.

A key limitation of this study is its observational design, which precludes definitive causal inference and leaves the possibility of residual confounding despite adjustment for measured covariates. In addition, mechanistic interpretations should be approached with caution because the present analysis could not reliably assess rejection episodes, DSAs, *de novo* DSAs, or immunologic causes of graft loss comprehensively. The CRISTAL registry, managed by the *Agence de la biomédecine*, offers nationwide coverage of organ allocation and transplantation activity in France but is primarily designed to support allocation processes and long-term outcome monitoring rather than detailed clinical research. As a result, although the registry includes fields for acute and chronic rejection, antibody-mediated rejection, and cellular rejection, with or without DSAs, completion of these fields is not mandatory and the data quality is not subject to the same level of control as allocation-related data. Likewise, the specific cause of graft loss is incompletely recorded. Consequently, the reporting of immunologic complications is not exhaustive, which limits the robustness of any mechanistic analysis between preformed sensitization and graft outcomes. To further explore this issue, we performed an additional analysis by the presence of historical preformed DSAs that had been subsequently removed from the list of unacceptable antigens during the waiting period. This analysis showed a significant difference in death-censored graft survival between HS AMP recipients, HS non-AMP recipients without such historical DSA, and HS non-AMP recipients with historical DSAs, with 5-year



death-censored graft survival rates of 85.6% [83.4–87.5], 81.7% [79.0–84.0], and 75.4% [61.2–85.0], respectively ( $p = 0.024$ ). In addition, among graft losses for which rejection was reported as acute or chronic, the corresponding proportions were 30%, 38%, and 43%, although these data should be interpreted with caution given incomplete reporting. Taken together, these findings are consistent with a possible role of immunologic memory and preformed DSAs, but they do not allow firm mechanistic conclusions.

In conclusion, the French AMP represents a major advancement in kidney transplantation for HS patients, substantially improving their access to suitable donors while maintaining post-transplant outcomes comparable to those of non-sensitized recipients. More broadly, our findings illustrate that acceptable mismatch-based allocation represents an effective compromise between equity and utility. By selectively expanding donor compatibility while maintaining stringent immunologic safety criteria, the AMP restores access to transplantation for HS candidates without compromising graft outcomes. This balance is particularly critical in systems in which organ scarcity mandates that increased equity for disadvantaged patients must not come at the expense of predictable graft failure. By facilitating transplantation for women, who are disproportionately represented among HS candidates due to prior pregnancies, the program also contributes to reducing sex-related inequities in organ allocation [25]. More broadly, these results underscore that the most effective long-term strategy remains careful immunologic management at the time of transplantation, particularly through high-quality HLA class-II matching in young recipients, which minimizes alloimmune risk and maximizes graft longevity. Overall, the AMP exemplifies how tailored allocation policies, informed by precise immunologic profiling, can optimize both equity and clinical outcomes in transplantation.

## Data availability statement

All data used in this study were extracted from the CRISTAL registry, coordinated and supported by the French Agence de la biomédecine. External access for research to national data is regulated by a scientific committee of the French Agence de la biomédecine, which analyses each request. Thus, data cannot be made publicly available due to legal restrictions. Data are available upon reasonable request. If readers need information about the data from the CRISTAL registry, they can contact Nicolas Chatauret (nicolas.chatauret@biomedecine.fr).

## Ethics statement

This study was based on data from the French national CRISTAL observational transplant registry. In accordance with French law, research studies based on this national registry are part of transplant assessment and do not require additional institutional review board approval. The CRISTAL database has

been declared to the French National Commission on Computing and Liberty (CNIL) and complies with the General Data Protection Regulation (GDPR) and the Council of Europe Convention 108+. Therefore, no additional ethics committee approval or specific written informed consent was required for this study.

## Author contributions

CA and ES: study concept and design, statistical analysis, interpretation of results, writing of the manuscript. Others: critical revision of the manuscript and final approval of the version to be published.

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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

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2026.16762/full#supplementary-material>



## References

- Mamode N, Bestard O, Claas F, Furian L, Griffin S, Legendre C, et al. European guideline for the management of kidney transplant patients with HLA antibodies: by the european society for organ transplantation working group. *Transpl Int* (2022) 35:10511. doi: 10.3389/ti.2022.10511
- Agence de la biomédecine. *Rapport Annuel Médical Et Scientifique De L'Agence De La Biomédecine* (2024). Available online at: <https://rams.agence-biomedecine.fr/> (Accessed September 30, 2025).
- Audry B, Savoye E, Pastural M, Bayer F, Legeai C, Macher MA, et al. The new French kidney allocation system for donations after brain death: rationale, implementation, and evaluation. *Am J Transplant* (2022) 22(12):2855–68. doi: 10.1111/ajt.17180
- Lefaucheur C, Antoine C, Suberbielle C, Glotz D. Mastering the risk of HLA antibodies in kidney transplantation: an algorithm based on pretransplant single-antigen flow bead techniques. *Am J Transplant* (2011) 11(8):1592–8. doi: 10.1111/j.1600-6143.2011.03560.x
- Antoine C. Les stratégies de greffe chez les patients immunisés ou hyperimmunisés. *Néphrologie and Thérapeutique* (2008) 4:S174–S178. doi: 10.1016/S1769-7255(08)74230-5
- Cargou M, Bardy B, Moalic V, Liby MT, Lacraz SF, Hau F, et al. Guidelines from the french-speaking society of histocompatibility and immunogenetics for virtual crossmatching for deceased donor kidney transplantation and the use of wet crossmatch in the decision-making process. *HLA* (2025) 105(4):e70171. doi: 10.1111/tan.70171
- Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* (2009) 150(9):604–12. doi: 10.7326/0003-4819-150-9-200905050-00006
- European Federation for Immunogenetics. *Standards for Histocompatibility and Immunogenetics testing* (2024). Available online at: <https://efi-web.org/committees/standards-committee> (Accessed January 15, 2026).
- Tambur AR, Bestard O, Campbell P, Chong AS, Barrio MC, Ford ML, et al. Sensitization in transplantation: assessment of risk 2022 working group meeting report. *Am J Transplant* (2023) 23(1):133–49. doi: 10.1016/j.ajt.2022.11.009
- Visentin J, Bachelet T, Aubert O, Del Bello A, Martinez C, Jambon F, et al. Reassessment of the clinical impact of preformed donor-specific anti-HLA-Cw antibodies in kidney transplantation. *Am J Transplant* (2020) 20(5):1365–74. doi: 10.1111/ajt.15766
- Furian L, Bestard O, Budde K, Cozzi E, Diekmann F, Mamode N, et al. European consensus on the management of sensitized kidney transplant recipients: a Delphi study. *Transpl Int* (2024) 37:12475. doi: 10.3389/ti.2024.12475
- Reed EF, Rao P, Zhang Z, Gebel H, Bray RA, Guleria I, et al. Comprehensive assessment and standardization of solid phase multiplex-bead arrays for the detection of antibodies to HLA. *Am J Transplant* (2013) 13(7):1859–70. doi: 10.1111/ajt.12287
- Visentin J, Couzi L, Taupin J. Clinical relevance of donor-specific antibodies directed at HLA-C: a long road to acceptance. *HLA* (2021) 97(1):3–14. doi: 10.1111/tan.14106
- Visentin J, Guidicelli G, Bachelet T, Jacquelinet C, Audry B, Nong T, et al. Denatured class I human leukocyte antigen antibodies in sensitized kidney recipients: prevalence, relevance, and impact on organ allocation. *Transplantation* (2014) 98(7):738–44. doi: 10.1097/TP.0000000000000229
- Troise D, Infante B, Mercuri S, Lindholm B, Kublickiene K, Stallone G. Exploring the immunological landscape of ischemia/reperfusion injury and graft rejection in kidney transplantation: shared mechanisms and insights. *Cells* (2025) 14(18):1443. doi: 10.3390/cells14181443
- Heidt S, Haasnoot GW, Van Der Linden-van Oevelen MJH, Claas FHJ. Highly sensitized patients are well served by receiving a compatible organ offer based on acceptable mismatches. *Front Immunol* (2021) 12:687254. doi: 10.3389/fimmu.2021.687254
- Kramer CSM, Heidt S, Tieken CM, de Boer J, Doxiadis I. Eurotransplant manual (2024). Available online at: <https://webshare.zenya.work/zfstn9tqqqt1kgs1/Document.aspx?websheredocumentid=5654432d-58cc-4812-aa04-4381f044532f> (Accessed December 29, 2025).
- Heidt S, Haasnoot GW, Witvliet MD, van der Linden-van Oevelen MJH, Kamburova EG, Wisse BW, et al. Allocation to highly sensitized patients based on acceptable mismatches results in low rejection rates comparable to nonsensitized patients. *Am J Transplant* (2019) 19(10):2926–33. doi: 10.1111/ajt.15486
- Heidt S, Haasnoot GW, Van Rood JJ, Witvliet MD, Claas FHJ. Kidney allocation based on proven acceptable antigens results in superior graft survival in highly sensitized patients. *Kidney Int* (2018) 93(2):491–500. doi: 10.1016/j.kint.2017.07.018
- Devriese M, Usureau C, Lion J, Sayegh C, Carmagnat M, Caillat-Zucman S, et al. Assessing the allogenic realness of the Cw1/12/15 pattern occurring in the LABScreen single antigen assay. *HLA* (2023) 102(2):157–67. doi: 10.1111/tan.15025
- Cozzi E. Delisting of HLA antigens as a possible strategy to enable transplantation in highly sensitized patients with a cPRA  $\geq$  99.9. *Kidney Int* (2025) 108(5):748–51. doi: 10.1016/j.kint.2025.08.003
- Bestard O, Couzi L, Crespo M, Kassarir N, Thauinat O. Stratifying the humoral risk of candidates to a solid organ transplantation: a proposal of the ENGAGE working group. *Transpl Int* (2021) 34(6):1005–18. doi: 10.1111/tri.13874
- Couzi L, Malvezzi P, Amrouche L, Anglicheau D, Blanco G, Caillard S, et al. Imlifidase for kidney transplantation of highly sensitized patients with a positive crossmatch: the French consensus guidelines. *Transpl Int* (2023) 36:11244. doi: 10.3389/ti.2023.11244
- De Weerd AE, Roelen DL, Van De Wetering J, Betjes MGH, Heidt S, Reinders MEJ. Imlifidase desensitization in HLA-incompatible kidney transplantation: finding the sweet spot. *Transplantation* (2024) 108(2):335–45. doi: 10.1097/TP.0000000000004689
- Cozzi E, Carella C, Puoti F, Masiero L, Forsythe J, Manas D, et al. The sex of donor and recipients in solid organ transplantation: an in depth analysis across the council of Europe member states. *Transpl Int* (2026) 39:15711. doi: 10.3389/ti.2026.15711



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# Bortezomib in combination with standard-of-care for late active antibody-mediated rejection with de novo donor-specific antibodies after kidney transplantation: a multicenter randomized trial

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Bortezomib has been proposed as a treatment for antibody-mediated rejection (AMR) after kidney transplantation; however, its efficacy remains uncertain. We conducted a multicenter, randomized, open-label trial in France between February 2015 and July 2019 in adult kidney transplant recipients with late active AMR associated with *de novo* donor-specific antibodies (dnDSA). Sixty of the planned 100 patients were randomized 1:1 to receive standard-of-care consisting of corticosteroids, plasmapheresis, and intravenous immunoglobulin, either alone or with bortezomib administered as two cycles of four infusions. The primary endpoint was a composite of a >50% reduction in immunodominant DSA mean fluorescence intensity and stabilization or improvement of microvascular inflammation and transplant glomerulopathy on graft biopsy at 12 months. This endpoint was achieved in 40.0% of patients receiving bortezomib and 33.3% receiving standard-of-care alone in the intention-to-treat analysis (RR = 1.20, 95% CI 0.61–2.34; *p* = 0.59), and in 50.0% and 42.1%, respectively, in the per-protocol analysis (RR = 1.19, 95% CI 0.60–2.36; *p* = 0.62). Neither component differed between groups. Serious adverse events occurred in 23 patients (76.7%) receiving bortezomib and 14 patients (46.7%) receiving standard-of-care alone (*p* = 0.017).

Adding bortezomib did not demonstrate a significant efficacy benefit and was associated with a higher incidence of serious adverse events (ClinicalTrials.gov number: NCT02201576).

## KEYWORDS

antibody-mediated rejection, bortezomib, donor-specific antibodies, immunosuppression, kidney transplantation

## Introduction

Within ten years of kidney transplantation, up to 25% of recipients develop *de novo* donor-specific antibodies (dnDSA), most of which are class II anti-human leukocyte antigen (HLA) antibodies [1]. The occurrence of dnDSA is associated with a 40% reduction in 10-year graft survival [2] and can trigger active or chronic active antibody-mediated rejection (AMR). These late AMR episodes frequently occur subclinically several years post-transplantation, unlike AMR associated with pre-existing donor-specific antibodies [3]. They are now considered one of the leading causes of long-term graft failure [4, 5].

To date, no approved treatments exist for AMR. The most widely accepted standard-of-care consists of corticosteroids, plasmapheresis, and intravenous immunoglobulin (IVIg), although this approach is supported by low-quality evidence. Several additional therapies are frequently used by transplant centers as adjuncts to standard-of-care including anti-CD20 monoclonal antibodies (mAb), complement inhibitors, anti-IL-6 antibodies, and plasma cell-targeting agents, for which evidence also remains low and anecdotal.

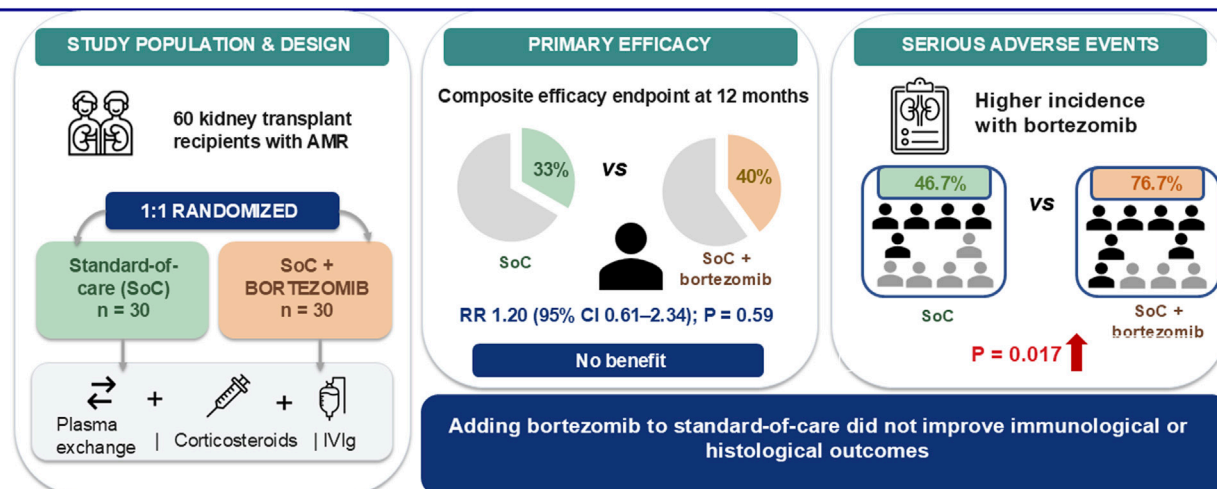
Among plasma cell-targeting therapies, bortezomib, a proteasome inhibitor indicated for multiple myeloma, has been

proposed to treat AMR because of its ability to induce apoptosis of plasma cells, which produce anti-HLA antibodies. Uncontrolled studies have shown contrasting results with bortezomib in the context of pre-transplant desensitization [6–9].

Small-scale, single-center retrospective studies conducted in patients with AMR have reported reductions in immunodominant DSA (idDSA) following bortezomib treatment [10, 11], whereas others have found no such effect [12, 13]. Thus, no definitive conclusions can be drawn regarding its effect on AMR outcomes. More recently, the randomized BORTEJECT study demonstrated no benefit of bortezomib monotherapy over placebo in slowing the decline of glomerular filtration rate (GFR) in patients screened for DSA and displaying biopsy-proven AMR [14]. However, in that study, bortezomib was used as monotherapy.

To better assess the role of bortezomib, our objective was to perform a randomized controlled trial in a homogeneous cohort of kidney transplant recipients with active AMR and dnDSA. Specifically, we evaluated the efficacy of adding bortezomib to standard-of-care, including plasmapheresis, corticosteroids, and IVIg. We hypothesized that proteasome inhibitor therapy could be beneficial when combined with interventions targeting circulating alloantibodies (plasmapheresis and IVIg), while

### Bortezomib in Combination with Standard-of-Care for Late Active Antibody-Mediated Rejection With De Novo Donor-Specific Antibodies After Kidney Transplantation: A Multicenter Randomized Trial



GRAPHICAL ABSTRACT

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TABLE 1 Study treatment protocol.

| Treatment component               | Bortezomib group                                                                                                                                                                         | Standard-of-care group                                        |
|-----------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------|
| Plasma exchange                   | Five sessions within 10 days after inclusion                                                                                                                                             |                                                               |
| Maintenance immunosuppression     | Prednisone 10 mg/day; mycophenolate mofetil 500 mg twice daily or mycophenolate sodium 360 mg twice daily; tacrolimus (target trough level 6–10 ng/mL)                                   | Same protocol, adjusted if necessary                          |
| Bortezomib                        | Two cycles, each consisting of four intravenous injections of 1.3 mg/m <sup>2</sup> administered every 3 days, repeated after 3 weeks                                                    | -                                                             |
| Dexamethasone                     | 20 mg orally on each day of bortezomib administration                                                                                                                                    | 20 mg orally, four doses every 3 days, repeated after 3 weeks |
| Intravenous immunoglobulin (IVIg) | Four infusions of 2 g/kg at 3-week intervals                                                                                                                                             |                                                               |
| Infection prophylaxis             | Cotrimoxazole and valaciclovir for 6 months after inclusion                                                                                                                              |                                                               |
| Treatment discontinuation         | Temporary: neutropenia, thrombocytopenia, abnormal liver function tests, or neuropathy. Permanent: patient withdrawal, severe irreversible toxicity, or graft failure requiring dialysis |                                                               |
| Follow-up                         | 12 months                                                                                                                                                                                |                                                               |

corticosteroids may enhance the pro-apoptotic effects of proteasome inhibitor therapy on plasma cells.

## Materials and methods

### Study design

The last version of the protocol is available in [Supplementary Material](#). A randomized controlled, non-blinded multicenter superiority trial was conducted at 15 centers in France between February 12, 2015, and July 25, 2019. Among the centers, 11 sites enrolled patients including Paris (three sites), Lyon, Nantes, Toulouse, Montpellier, Suresnes, Créteil, Strasbourg, and Amiens. Eligible patients were randomized in a 1:1 ratio to receive bortezomib or to the standard-of-care group using a centralized web-based randomization system (CleanWeb<sup>TM</sup>; Telemedicine Technologies SAS, France). Randomization occurred after AMR was confirmed by an inpatient hospital transplant biopsy, eligibility criteria were checked, and informed consent was obtained. Randomization was stratified according to the center and type of rejection, clinical or subclinical. AMR was classified as clinical if the biopsy was performed for graft dysfunction or new-onset proteinuria, and subclinical if performed as a screening biopsy or in response to the detection of dnDSA.

### Inclusion and exclusion criteria

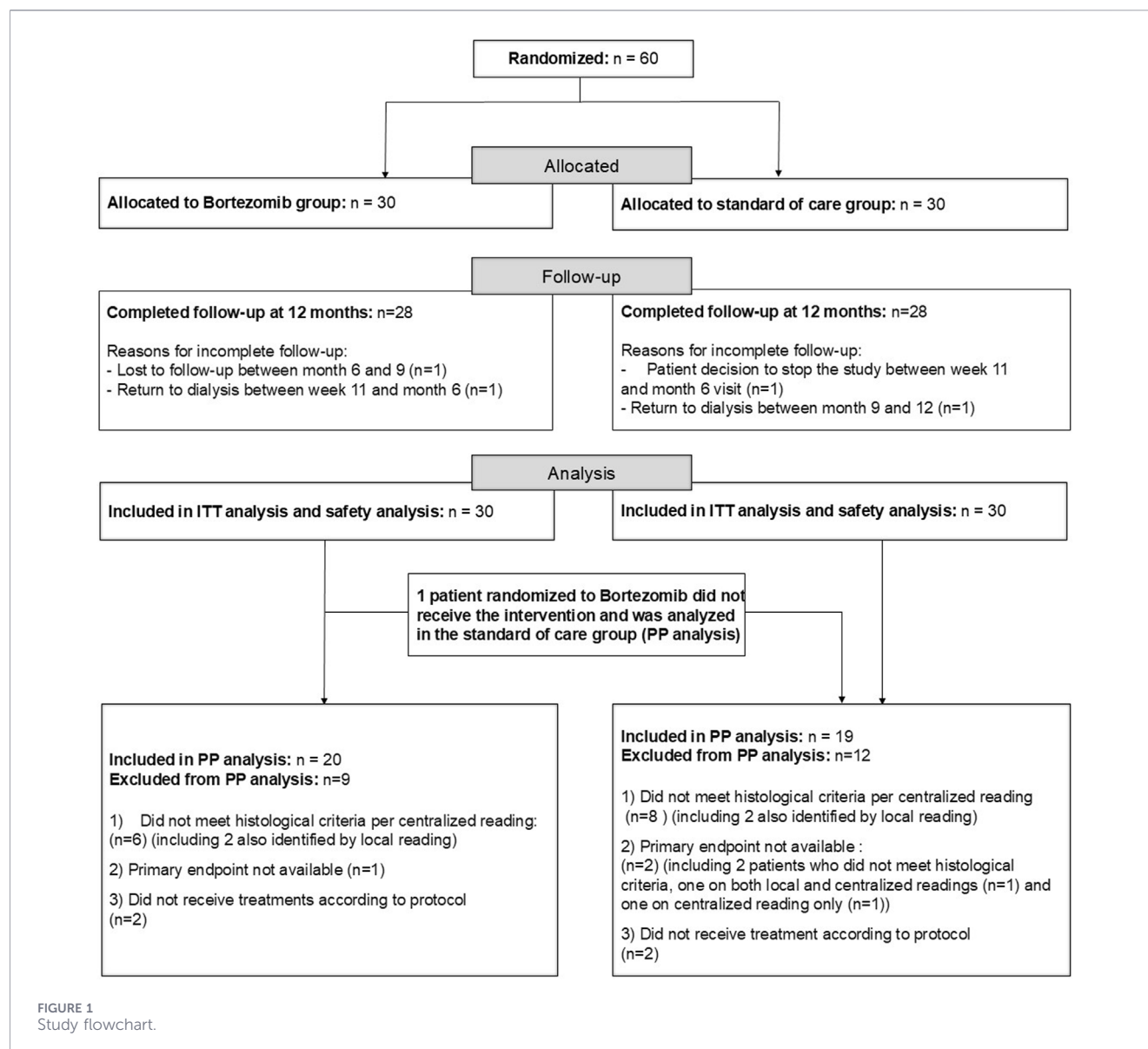
Patients were included if they met the following criteria: i) aged at least 18 years, ii) had received a first or second ABO-compatible kidney transplant either from a deceased donor after brain death or circulatory death, or from a living donor, performed at least 90 days earlier, iii) presence of one or more dnDSAs, iv) evidence of microvascular inflammation (sum of glomerular inflammation (g score) and peritubular

capillary inflammation (ptc score), g + ptc ≥ 2), and chronic glomerulopathy (cg score < 3) on graft biopsy, and v) provided prior written informed consent. Female patients of childbearing potential were required to have a negative pregnancy test on the day of enrollment, and all participants (male and female) were required to use at least one effective contraceptive method before the initiation of treatment, throughout treatment and for the duration of the study. Patients with concomitant borderline lesions or T-cell-mediated rejection, corresponding to mixed rejection phenotypes, were eligible for inclusion.

Exclusion criteria were as follows: i) presence of a DSA before or at the time of transplantation, ii) patients receiving a third or fourth kidney transplant, iii) receiving combined transplantation with a non-renal organ, iv) previous history of AMR during the current transplantation, v) severe graft dysfunction, defined as an estimated GFR (eGFR) < 20 mL/min/1.73 m<sup>2</sup>, vi) severe histological lesions of chronic AMR, defined by the cg score of 3/3, vii) severe neuropathy or hematologic disturbances (platelet count < 100 × 10<sup>9</sup>/L or absolute neutrophil count < 1,000 × 10<sup>6</sup>/L), viii) active uncontrolled infection, chronic active hepatitis B or C, or HIV infection, ix) severe hepatic impairment or abnormal liver function tests, and x) acute diffuse infiltrative pneumonitis or pericardial involvement. Patients unable to understand or comply with the study protocol, as well as those enrolled in another therapeutic trial or within its exclusion period, were also excluded.

### Study intervention

All patients initially received five plasma exchange sessions within 10 days of inclusion. During this period, maintenance immunosuppression was adjusted, if necessary, to include prednisone (10 mg/day), mycophenolate mofetil (500 mg twice



daily) or mycophenolate sodium (360 mg twice daily), and tacrolimus with target trough levels of 6–10 ng/mL (Table 1).

Patients in the bortezomib group received two cycles of intravenous bortezomib, each consisting of four injections (1.3 mg/m<sup>2</sup> every 3 days), administered at three-week intervals. In addition, these patients received oral dexamethasone (20 mg) on each day of bortezomib administration and four IVIg infusions (2 g/kg body weight) at three-week intervals. Patients in the standard-of-care group received dexamethasone according to the same schedule as the bortezomib group (20 mg orally, four doses every 3 days, repeated after 3 weeks) along with four IVIg infusions (2 g/kg body weight) at three-week intervals. The planned follow-up duration was 12 months (Table 1).

All patients received prophylaxis with cotrimoxazole and valaciclovir for 6 months after inclusion to prevent *Pneumocystis jirovecii*, herpes simplex virus, and varicella-zoster virus infections. The bortezomib treatment was temporarily discontinued in the event of toxicity including neutropenia, thrombocytopenia,

abnormal liver function tests and/or neuropathy. Permanent discontinuation was required in cases of voluntary withdrawal by the patient, severe irreversible toxicity and/or terminal graft dysfunction requiring return to dialysis.

## Outcome measures

The primary endpoint was a composite of two conditions assessed at 12 months: i) a >50% decrease from baseline in the mean fluorescence intensity (MFI) of the idDSA and, ii) stabilization or improvement of histological AMR lesions on the 12-month biopsy, defined as a difference in MVI score, defined as g + ptc, ≤1, and in cg score <1 compared with baseline. This composite endpoint was chosen to capture both immunological and histological responses to treatment.

Secondary endpoints included: i) each component of the primary endpoint analyzed separately, ii) in patients with multiple DSA, relative change from baseline in the MFI of each



TABLE 2 Baseline characteristics.

| Variable                                             | Bortezomib group (n = 30) | Standard-of-care group (n = 30) |
|------------------------------------------------------|---------------------------|---------------------------------|
| <b>Sex</b>                                           |                           |                                 |
| Male                                                 | 14 (46.7)                 | 26 (86.7)                       |
| Female                                               | 16 (53.3)                 | 4 (13.3)                        |
| Age at inclusion (years)                             | 44.4 ± 13.8               | 51.3 ± 13.0                     |
| <b>Type of nephropathy</b>                           |                           |                                 |
| Diabetic                                             | 0 (0.0)                   | 6 (20.0)                        |
| Hypertensive                                         | 1 (3.3)                   | 2 (6.6)                         |
| Polycystic kidney disease                            | 4 (13.3)                  | 3 (10.0)                        |
| Pyelonephritis or chronic interstitial nephritis     | 4 (13.3)                  | 3 (10.0)                        |
| Glomerulonephritis                                   | 11 (36.7)                 | 11 (36.7)                       |
| Other                                                | 5 (16.7)                  | 3 (10.0)                        |
| Unknown                                              | 5 (16.7)                  | 2 (6.7)                         |
| <b>Rank of transplantation</b>                       |                           |                                 |
| First transplant                                     | 26 (86.7)                 | 30 (100.0)                      |
| Second transplant                                    | 4 (13.3)                  | 0 (0.0)                         |
| Duration at inclusion since transplantation (months) | 76 [25: 104]              | 76 [30: 111]                    |
| <b>Donor type</b>                                    |                           |                                 |
| Living                                               | 8 (26.7)                  | 6 (20.0)                        |
| Deceased (brain death)                               | 21 (70.0)                 | 21 (70.0)                       |
| Deceased (circulatory death)                         | 1 (3.3)                   | 3 (10.0)                        |
| History of TCMR before inclusion                     | 1 (3.3)                   | 4 (13.3)                        |
| <b>Renal function at inclusion</b>                   |                           |                                 |
| Serum creatinine (μmol/L)                            | 149.2 ± 61.0              | 149.7 ± 50.4                    |
| eGFR (mL/min/1.73 m <sup>2</sup> )                   | 44.0 ± 14.6               | 47.5 ± 19.3                     |
| Urine protein/creatinine ratio (g/g)*                | 0.48 [0.11: 0.73]         | 0.23 [0.11: 1.20]               |
| <b>Maintenance immunosuppression at inclusion</b>    |                           |                                 |
| Corticosteroid (prednisone)                          | 24 (80.0)                 | 24 (80.0)                       |
| Ciclosporin                                          | 6 (20.0)                  | 6 (20.0)                        |
| Tacrolimus                                           | 18 (60.0)                 | 18 (60.0)                       |
| No ciclosporin or tacrolimus                         | 6 (20.0)                  | 6 (20.0)                        |
| Azathioprine                                         | 0 (0.0)                   | 1 (3.3)                         |
| Mycophenolate†                                       | 30 (100.0)                | 27 (90.0)                       |
| No mycophenolate or azathioprine                     | 0 (0.0)                   | 2 (6.7)                         |
| Sirolimus                                            | 1 (3.3)                   | 1 (3.3)                         |
| Everolimus                                           | 2 (6.7)                   | 2 (6.7)                         |
| No sirolimus or everolimus                           | 27 (90.0)                 | 27 (90.0)                       |

eGFR; estimated glomerular filtration rate, TCMR; T-cell-mediated rejection, SD; standard deviation. Data are presented as n (%) for categorical variables, mean ± SD, for normally distributed variables, or median [Q1: Q3] for non-normally distributed variables.

\*Missing data for urine protein-to-creatinine ratio: bortezomib group n = 10 and standard-of-care group n = 4.

†Data were unavailable for one patient in the standard-of-care group.

TABLE 3 Characteristics of donor specific antibodies (DSA) at inclusion.

| Variable                                                              | Bortezomib group (n = 30) | Standard-of-care group (n = 29*) |
|-----------------------------------------------------------------------|---------------------------|----------------------------------|
| Number of dnDSA per patient, median [Q1:Q3]                           | 2 [1: 3]                  | 1 [1: 2]                         |
| Distribution of patients according to number of dnDSA detected, n (%) |                           |                                  |
| 1                                                                     | 12 (40.0)                 | 17 (58.6)                        |
| 2                                                                     | 8 (26.6)                  | 5 (17.2)                         |
| 3                                                                     | 5 (16.7)                  | 4 (13.8)                         |
| >3                                                                    | 5 (16.7)                  | 3 (10.4)                         |
| Class of the idDSA                                                    |                           |                                  |
| I                                                                     | 8 (26.7)                  | 3 (10.3)                         |
| II                                                                    | 22 (73.3)                 | 26 (89.7)                        |
| Class of all DSA                                                      |                           |                                  |
| I                                                                     | 7 (23.3)                  | 3 (10.3)                         |
| II                                                                    | 14 (46.7)                 | 21 (72.4)                        |
| I+II                                                                  | 9 (30.0)                  | 5 (17.3)                         |
| idDSA MFI                                                             | 17,130 [4,121: 22,772]    | 11,234 [2,599: 22,549]           |
| Sum of all the DSA MFI                                                | 22,440 [4,946: 29,851]    | 11,743 [2,599: 28,756]           |

DSA; donor specific antibodies, idDSA; immunodominant donor specific antibody, dnDSA; *de novo* DSA, MFI; mean fluorescence intensity. All values are according to central reading and are expressed as n (%) or median [Q1: Q3].

\*Missing data for one patient in the standard treatment group.

DSA and in the cumulative MFI, iii) change in DSA MFI after 6 months, iv) histological changes between baseline and 12-month biopsies, including acute and chronic lesions according to the latest Banff criteria, v) changes over 12 months in proteinuria (24-h excretion and urine protein/creatinine ratio), serum creatinine and eGFR (Modification of Diet in Renal Disease (MDRD) formula), and vi) patient and graft survival at 12 months. Safety was assessed throughout treatment and during the 12-month follow-up, with a focus on hematologic, hepatic, neurologic (including neuropathy) and infectious adverse events.

## Data collection

For histopathological assessment, all available baseline/screening and 12-month kidney biopsy slides were centrally reviewed at the pathology laboratory of Hôtel Dieu de Nantes by an expert renal pathologist blinded to treatment allocation. Slides were assessed using standard histological stains, including hematoxylin–eosin–safran, periodic acid–Schiff, Masson's trichrome, and silver staining, and lesions were graded according to updated Banff classification at the time of reading. Kidney biopsies were subsequently reclassified according to the 2022 Banff criteria using an automated method [15]. DSA were centrally assessed using Luminex single antigen (LabScreen One Lambda, Thermofisher) by immunologists blinded to treatment allocation. Investigators at each participating center collected clinical and transplant-related data, including sex, weight, rank of transplantation, donor type, dialysis vintage, local histological readings, local pre- and post-transplant HLA antibody assays, and concomitant immunosuppressive therapies.

## Sample size

The sample size calculation assumed primary endpoint rates of 5% in the standard-of-care group and 25% in the bortezomib group. With a two-sided alpha of 0.05% and 80% power, the planned sample size was 50 patients per group, or 100 patients in total. The planned inclusion period was 24 months. Despite multicenter recruitment and a 30-month extension of the inclusion period, the target sample size was not reached because of slow recruitment, reflecting the limited number of patients who met all eligibility criteria and were willing to participate. Recruitment was therefore closed at the end of the extended inclusion period after 60 patients had been randomized.

## Statistical analysis

The primary endpoint was compared in the intention-to-treat (ITT) population with missing data considered as treatment failures. A sensitivity analysis was also performed in the per-protocol (PP) population. Secondary endpoints were analyzed in the ITT population for the two components of the primary endpoint, and on available data (complete-case analysis) for the other endpoints. All statistical tests were two-tailed with a significance level of 5%.

Baseline clinical and biological characteristics were described by group. Quantitative variables were expressed as mean  $\pm$  standard deviation (SD) or median [Q1:Q3] and qualitative variables as numbers and percentages (%). Comparisons of the primary endpoint and other categorical variables between the two groups were performed using Chi-squared or Fisher's exact tests, as

TABLE 4 Biopsy results at inclusion.

| Variable                                              | Bortezomib group (n = 30) | Standard-of-care group (n = 30) |
|-------------------------------------------------------|---------------------------|---------------------------------|
| <b>Histological lesions</b>                           |                           |                                 |
| <b>Glomerular inflammation (g score)</b>              |                           |                                 |
| Score $\geq 1$                                        | 19 (63.3)                 | 21 (70.0)                       |
| Score value                                           | 1.0 [0.0; 2.0]            | 1.0 [0.0; 2.0]                  |
| <b>Peritubular capillary inflammation (ptc score)</b> |                           |                                 |
| Score $\geq 1$                                        | 23 (76.7)                 | 23 (76.7)                       |
| Score value                                           | 2.0 [1.3; 3.0]            | 2.0 [2.0; 3.0]                  |
| <b>MVI score</b>                                      |                           |                                 |
| Score $\geq 2$                                        | 25 (83.3)                 | 24 (80.0)                       |
| Score value                                           | 3.0 [2.0; 4.0]            | 3.0 [2.3; 4.8]                  |
| <b>Chronic glomerulopathy (cg score)</b>              |                           |                                 |
| Score $\geq 1$                                        | 7 (23.3)                  | 7 (23.3)                        |
| Score value                                           | 0.0 [0.0; 0.0]            | 0.0 [0.0; 0.0]                  |
| C4d staining (positive)                               | 17 (56.7)                 | 15 (50.0)                       |
| <b>Vascular inflammation (v score)</b>                |                           |                                 |
| Score $\geq 1$                                        | 3/29 (10.3)               | 1/29 (3.4)                      |
| Tubular atrophy (ct score)                            | 1.0 [1.0; 1.8]            | 1.0 [1.0; 1.0]                  |
| Interstitial fibrosis (ci score)                      | 1.0 [0.0; 1.8]            | 0.0 [0.0; 1.0]                  |
| IFTA grade $\geq 1$                                   | 24/30 (80.0)              | 20/29 (69.0)                    |
| <b>Banff 2022 diagnosis*</b>                          |                           |                                 |
| <b>AMR</b>                                            |                           |                                 |
| Active                                                | 18 (60.0)                 | 18 (60.0)                       |
| Chronic active                                        | 7 (23.3)                  | 6 (20.0)                        |
| Chronic inactive                                      | 0 (0.0)                   | 1 (3.3)                         |
| C4d staining only+DSA                                 | 1 (3.3)                   | 0 (0.0)                         |
| Probable                                              | 0 (0.0)                   | 4 (13.4)                        |
| None                                                  | 4 (13.4)                  | 1 (3.3)                         |
| <b>Associated TCMR</b>                                |                           |                                 |
| Borderline Changes                                    | 4 (13.3)                  | 2 (6.7)                         |
| Acute (grade I and above)                             | 1 (3.3)                   | 2 (6.7)                         |
| Chronic active                                        | 4 (13.3)                  | 1 (3.3)                         |

MVI score: microvascular inflammation corresponding to g+ptc sum score; DSA; donor specific antibodies, IFTA; interstitial fibrosis and tubular atrophy, AMR; antibody-mediated rejection, TCMR; T-cell-mediated rejection. All values are according to central reading and are expressed as n (%) or mean  $\pm$  SD or median [Q1: Q3].

\*Reclassified according to the 2022 Banff criteria [15].

appropriate. Quantitative variables were compared using Student's t-test or Wilcoxon's rank-sum test when distributional assumptions were not met. Relative risks (RR) and 95% confidence interval (95% CI) were estimated using log-binomial regression models. Longitudinal outcomes were analyzed using linear mixed-effects models. Secondary analyses were considered exploratory and no adjustment for multiple comparisons was performed. Given the limited sample size,

secondary endpoints were analyzed on available data without imputation. All analyses were performed with R software ([www.r-project.org](http://www.r-project.org)).

## Ethical considerations

This study was conducted in accordance with the Declaration of Helsinki. Approval was obtained from the *Comité de protection des*

TABLE 5 Primary and secondary efficacy endpoint at 12 months.

| Endpoint (each one assessed at 12 months)                               | Patients         |                        | RR [95% CI]*        | Absolute difference [95% CI]* | p-value |
|-------------------------------------------------------------------------|------------------|------------------------|---------------------|-------------------------------|---------|
|                                                                         | Bortezomib group | Standard-of-care group |                     |                               |         |
| Primary endpoint                                                        |                  |                        |                     |                               |         |
| ITT analysis                                                            | 12/30 (40.0)     | 10/30 (33.3)           | 1.20 [0.61 to 2.34] | 6.7 [-17.6 to 30.5]           | 0.59    |
| PP analysis                                                             | 10/20 (50.0)     | 8/19 (42.1)            | 1.19 [0.60 to 2.36] | 7.9 [-23.1 to 37.8]           | 0.62    |
| Secondary endpoints                                                     |                  |                        |                     |                               |         |
| >50% reduction in idDSA MFI (ITT analysis)                              | 17/30 (56.7)     | 14/30 (46.7)           |                     |                               | 0.44    |
| Stabilization or improvement of histological AMR lesions (ITT analysis) | 17/30 (56.7)     | 17/30 (56.7)           |                     |                               | 1.00    |
| >50% reduction in idDSA MFI (PP analysis)                               | 13/20 (65.0)     | 11/19 (57.9)           |                     |                               | 0.65    |
| Stabilization or improvement of histological AMR lesions (PP analysis)  | 13/19 (68.4)     | 12/18 (66.7)           |                     |                               | 0.91    |
| Estimated slope between baseline and 12 months                          |                  |                        |                     |                               |         |
| Serum creatinine (μmol/L)                                               | 45.9 ± 13.5      | 29.7 ± 13.5            |                     | 16.14 [-21.5 to 53.8]         | 0.40    |
| eGFR (mL/min/1.73 m²)                                                   | -7.10 ± 2.19     | -4.68 ± 2.18           |                     | -2.42 [-8.51 to 3.67]         | 0.43    |
| Log-transformed PCR (g/g)                                               | 0.20 ± 0.23      | 0.11 ± 0.23            |                     | 0.09 [-0.55 to 0.74]          | 0.78    |
| Graft survival                                                          | 29/30 (96.7)     | 29/30 (96.7)           |                     |                               | 1.00    |
| Patient survival                                                        | 30/30 (100.0)    | 30/30 (100.0)          |                     |                               |         |

ITT; intention to treat, PP; per protocol, DSA; donor specific antibodies, idDSA; immunodominant donor specific antibodies, dnDSA; *de novo* DSA, MFI; mean fluorescence intensity, RR; risk ratio, CI; confidence interval, SD; standard deviation. All values are according to central reading and are expressed as n/N (%) or mean ± SD.

\*Standard-of-care group used as the reference.

*personnes Ile de France II* (CPP) (Reference: 2014-03-01 RBM) and the *Agence Nationale de Sécurité du Médicament et des Produits de Santé* (ANSM) (Reference: 140368A-11). This trial was registered at [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02201576) (NCT02201576) and an independent Data Safety Monitoring Board (DSMB) regularly reviewed the data to ensure patient safety and monitor the progress of the study.

## Results

### Baseline characteristics

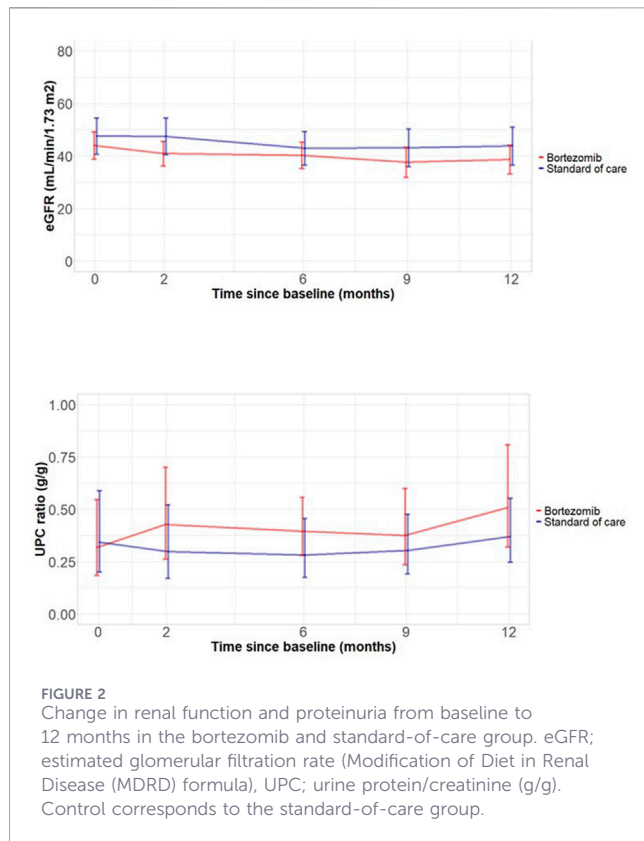
Between February 12, 2015, and July 25, 2019, 60 patients were randomized. The planned sample size of 100 patients was not reached by the end of the extended recruitment period because of slower-than-anticipated enrolment related to the restrictive eligibility criteria. For the ITT analysis, 30 patients were allocated to each group. For the PP analysis, 20 patients were included in the bortezomib group and 19 in the standard-of-care group; one patient randomized to bortezomib did not receive the intervention and was therefore analyzed in the standard-of-care group for the PP analysis (Figure 1). Baseline characteristics are presented in Table 2. Patients in the bortezomib group were more frequently women (53.3% vs. 13.3%) and had a lower mean age ( $44.4 \pm 13.8$  vs.  $51.3 \pm 13.0$  years). The median duration from transplantation to inclusion was

76 months in both groups. Notably, retransplantations occurred only in the bortezomib group (13.3%) (Table 2). A history of T-cell mediated rejection (TCMR) prior to inclusion was reported in one patient in the bortezomib group, and four in the standard-of-care group. Most patients received triple maintenance immunosuppression consisting of prednisone, ciclosporin or tacrolimus, and mycophenolate (Table 2). At inclusion, six patients in each group were not receiving a calcineurin inhibitor. AMR was more often subclinical than clinical for both groups (bortezomib group: 60.0% and standard-of-care group: 66.7%).

### Immunological and histological characteristics

At inclusion, patients in the bortezomib group more frequently had two or more dnDSA than in the standard-of-care (60.0% vs. 41.3%), with a median of 2 [1:3] DSA vs. 1 [1:2] in the standard-of-care group, and they also had a higher proportion of class I DSA than in the standard-of-care group (whether considering the idDSA or all the DSA) (Table 3). Both the idDSA MFI and the sum of all the DSA MFI per patient were higher for patients in the bortezomib group.

Concerning biopsy findings at inclusion, discrepancies were present between local and central readings of AMR lesions (Table 4). According to the central assessment, only



25 patients (83.3%) in the bortezomib group and 24 patients (80.0%) in the standard-of-care group had MVI score  $\geq 2$ , which was used as an inclusion criterion by local readings. Chronic glomerulopathy (cg score  $\geq 1$ ) was only reported in seven patients (23.3%) in each group, explaining the low median [IQR] score of 0[0:0]. According to the 2022 Banff criteria reclassification, active AMR was present in 18 patients (60.0%) in each treatment group. Chronic active AMR was present in seven patients (23.3%) in the bortezomib group and six patients (20.0%) in the standard-of-care group; one additional patient (3.3%) in the standard-of-care group had chronic inactive AMR. The remaining patients were classified as having C4d staining with DSA, probable AMR, or no AMR. Associated TCMR lesions were present in nine patients (30.0%) in the bortezomib group and five patients (16.7%) in the standard-of-care group (Table 4).

## Combined primary efficacy endpoint

In both the ITT and PP analyses, the primary endpoint rate did not significantly differ between groups. In the ITT analysis, the endpoint was achieved by 40.0% of patients in the bortezomib group and 33.3% in the standard-of-care group (RR = 1.20 [95% CI: 0.61 to 2.34],  $p = 0.59$ ). Missing primary endpoint data were considered treatment failure in four patients in the standard-of-care group and one in the bortezomib group. In the PP analysis, the corresponding rates were 50.0% and 42.1% (RR = 1.19 [95% CI: 0.60 to 2.36],  $p = 0.62$ ) (Table 5). No significant interaction was observed between treatment allocation and rejection type (clinical or subclinical;  $p = 0.86$ ).

When separately analyzed, neither component of the combined endpoint was different between groups. After 12 months, a  $>50\%$  reduction in idDSA MFI was observed in 56.7% of patients in the bortezomib group and 46.7% of patients in the standard-of-care group in the ITT analysis ( $p = 0.44$ ), and 65.0% and 57.9% in the PP analysis ( $p = 0.65$ ), respectively. Stabilization or improvement of histological lesions at month 12 was reached in 56.7% of patients in both groups in the ITT analysis ( $p = 1.00$ ), and 68.4% and 66.7% of patients in the PP analysis, respectively ( $p = 0.91$ ). Thus, 5/28 (17.8%, bortezomib) and 2/25 (8.0%, standard-of-care) of patients experienced a  $>50\%$  decrease in idDSA MFI without improving or stabilizing MVI, and 5/28 (17.8%, bortezomib) and 7/25 (28.0%, standard-of-care) improved or stabilized MVI without significantly reducing idDSA MFI.

In a *post hoc* analysis of patients with baseline idDSA MFI  $>15,000$ , the primary composite endpoint was achieved in 42.1% of patients in the bortezomib group and 8.3% in the standard-of-care group ( $p = 0.10$ ). A  $>50\%$  reduction in idDSA MFI was observed in 52.6% and 16.7% of patients, respectively ( $p = 0.065$ ) (Supplementary Table S1).

In separate sensitivity analyses adjusted individually for baseline characteristics that were imbalanced between groups despite randomization (sex, transplantation rank, idDSA class, and baseline idDSA MFI), the association between treatment allocation and the primary endpoint remained non-significant (Supplementary Table S2).

## Changes in renal function, DSA and MVI

Changes in serum creatinine, eGFR, and urine protein/creatinine ratio between baseline and 12 months did not significantly differ between patients in the bortezomib and standard-of-care groups (Table 5; Figure 2). No deaths occurred, and one patient in each group returned to dialysis.

For patients receiving bortezomib, the idDSA MFI decreased by 45.1% [-69.7: -6.1] after 6 months ( $n = 23$ ), and by 60.2% [-80.6: -11.5] after 12 months ( $n = 28$ ). Similar reductions were observed for patients in the standard-of-care group: 50.7% [-76.6: -6.6] after 6 months ( $n = 27$ ;  $p = 0.92$ ) and 60.0% [-81.3: -11.5] after 12 months ( $n = 27$ ;  $p = 0.85$ ) (Figure 3). Median relative change in the sum of all DSA MFI at 12 months was also comparable: 60.3% [-74.3: -27.6] in the bortezomib group ( $n = 28$ ) and 57.6% [-81.8: -21.5] in the standard-of-care group ( $n = 27$ ;  $p = 0.80$ ).

Patients whose idDSA MFI dropped by at least half at 12 months (responders) had lower baseline MFI values than non-responders: median 16,080 vs. 22,582 in the bortezomib group and 2,996 vs. 22,054 in the standard-of-care group.

Median scores of histological lesions remained stable between baseline and 12 months in both groups (Table 6). Histological failure was due to the progression of MVI ( $\Delta g + ptc$  score  $\geq 2$ ) in three patients in each group and of transplant glomerulopathy ( $\Delta cg$  score  $\geq 1$ ) in eight patients in the bortezomib group and five patients in the standard-of-care group (Figure 3). One patient in each group had progression of both MVI and cg scores, while most with cg progression showed no or only mild worsening of MVI. Among patients in the ITT population with an MVI score  $\geq 2$  at baseline and available 12-month histological data, nine of 23 patients



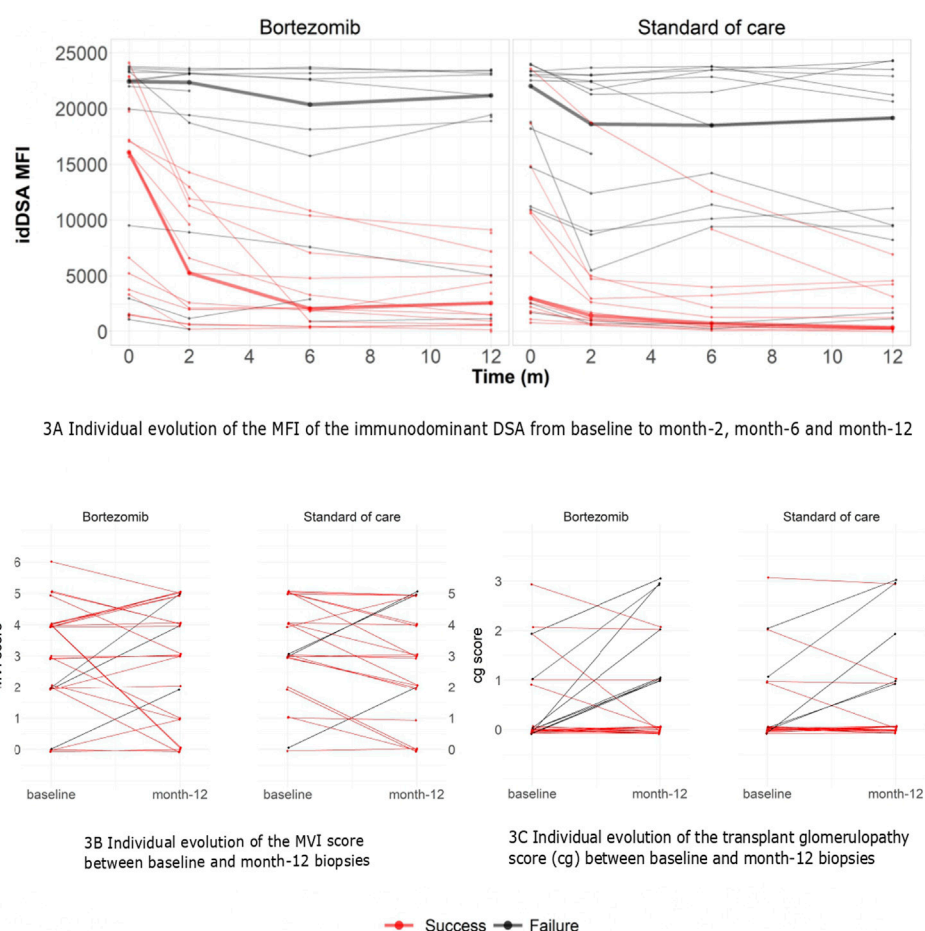


FIGURE 3

Individual changes in donor-specific antibodies (DSA) and histological lesions from baseline to 12 months for patients in the bortezomib and standard-of-care group. idDSA; immunodominant donor-specific antibodies, MFI; mean fluorescence intensity, MVI; microvascular inflammation score, cg; transplant glomerulopathy score. (A) Individual changes in idDSA MFI over 12 months. Red curves represent patients with immunological success (reduction in idDSA MFI >50%), and black curves represent patients with immunological failure. The thicker lines represent the median values of the responder and non-responder subgroups. (B) Individual changes in microvascular inflammation (MVI score) between baseline and 12 months. (C) Individual changes in transplant glomerulopathy (cg score) between baseline and 12 months. Red curves represent patients with histological success ( $\Delta$ MVI  $\leq 1$  and  $\Delta$ cg <1), and black curves represent patients with histological failure.

in the bortezomib group and 10 of 20 patients in the standard-of-care group showed a decrease in MVI score between baseline and 12 months. Complete MVI resolution, defined as an MVI score of 0 at 12 months, occurred in 3/23 patients (13.0%) and 2/20 patients (10.0%), respectively. In the PP analysis, complete MVI resolution occurred in 3/20 patients (15.0%) and 2/17 patients (11.8%), respectively. The cg score decreased in three patients in the bortezomib group and two patients in the standard-of-care group (Table 6; Figure 3).

In the bortezomib group, tubulitis with a Banff t score  $\geq 1$  was present in 29.6% of patients at baseline and 7.4% at 12 months, compared with 20.8% and 4.2%, respectively, in the standard-of-care group. Interstitial inflammation with a Banff i score  $\geq 1$  was present in 19.0% and 7.0% of patients in the bortezomib group and in 21.0% and 8.0% of patients in the standard-of-care group at baseline and 12 months, respectively. Interstitial fibrosis and tubular atrophy (IFTA) scores worsened in six patients in the bortezomib group and eight patients in the standard-of-care group, which was associated with a cg or MVI progression in two patients in each

group. According to the 2022 Banff classification at the 12-month biopsy, 19/27 patients in the bortezomib group and 19/24 patients in the standard-of-care group still had active or chronic active AMR (Table 6).

## Adverse events and safety of bortezomib

Overall, 132 adverse events were reported in the bortezomib group and 118 in the standard-of-care group, with infections, neurological and gastrointestinal events being the most frequent in both groups. In the bortezomib group, 11 patients discontinued or did not complete the two planned treatment courses due to hematological, neurological or hepatic toxicity. Twenty-three patients in the bortezomib group (76.7%) experienced at least one serious adverse event compared to 14 (46.7%) in the standard-of-care group ( $p = 0.017$ ), corresponding to 43 and 30 serious adverse events, respectively (Table 7). No significant between-group differences were observed in the proportions of patients experiencing adverse events or serious adverse events

TABLE 6 Histological lesion findings at baseline and at 12 months.

| Variable                                                       | Bortezomib group (n = 27) | Standard-of-care group (n = 24) | p-value |
|----------------------------------------------------------------|---------------------------|---------------------------------|---------|
| <b>Histological lesions</b>                                    |                           |                                 |         |
| <b>Glomerular inflammation (g score)</b>                       |                           |                                 |         |
| Baseline                                                       | 1.0 [0.0: 2.0]            | 1.0 [0.0: 2.0]                  | 0.98    |
| 12 months                                                      | 1.0 [0.0: 2.0]            | 1.0 [0.0: 2.0]                  |         |
| ≥1 at 12 months                                                | 17 (63.0)                 | 15 (62.5)                       | 0.97    |
| Change between baseline and 12 months                          | 0.0 [0.0: 0.0]            | 0.0 [-1.0: 0.0]                 | 0.57    |
| <b>Peritubular capillary inflammation (ptc score)</b>          |                           |                                 |         |
| Baseline                                                       | 2.0 [1.5: 3.0]            | 2.0 [2.0: 3.0]                  | 0.87    |
| 12 months                                                      | 2.0 [1.0: 3.0]            | 2.0 [1.5: 3.0]                  |         |
| ≥1 at 12 months                                                | 21 (77.8)                 | 18 (75.0)                       | 0.82    |
| Change between baseline and 12 months                          | 0.0 [-0.5: 1.0]           | 0.0 [-0.3: 0.0]                 | 0.41    |
| <b>MVI score</b>                                               |                           |                                 |         |
| Baseline                                                       | 3.0 [2.0: 4.0]            | 3.0 [2.8: 4.3]                  | 0.91    |
| 12 months                                                      | 3.0 [1.0: 4.5]            | 3.0 [2.0: 4.3]                  |         |
| ≥2 at 12 months                                                | 19 (70.4)                 | 19 (79.2)                       | 0.47    |
| Change between baseline and 12 months                          | 0.0 [-1.0: 1.0]           | 0.0 [-1.0: 0.0]                 | 0.35    |
| Decrease in the MVI score at 12 months*, n/N (%)               | 9/23 (39.1)               | 10/20 (50.0)                    |         |
| MVI = 0 at 12 months (ITT population), n/N (%)                 | 3/23 (13.0)               | 2/20 (10.0)                     |         |
| MVI = 0 at 12 months (PP population), n/N (%)                  | 3/20 (15.0)               | 2/17 (11.8)                     |         |
| <b>Chronic glomerulopathy (cg score)</b>                       |                           |                                 |         |
| Baseline                                                       | 0.0 [0.0: 0.5]            | 0.0 [0.0: 0.3]                  | 0.62    |
| 12 months                                                      | 0.0 [0.0: 1.0]            | 0.0 [0.0: 1.0]                  |         |
| ≥1 at 12 months                                                | 11 (40.7)                 | 8 (33.3)                        | 0.58    |
| Change between baseline and 12 months                          | 0.0 [0.0: 1.0]            | 0.0 [0.0: 0.0]                  | 0.69    |
| Decrease in the cg score for patients with cg > 0 at inclusion | 3/7                       | 2/6                             |         |
| <b>C4d staining (positive)</b>                                 |                           |                                 |         |
| ≥1 at baseline                                                 | 16 (59.3)                 | 12 (50.0)                       | 0.80    |
| ≥1 at 12 months                                                | 7 (25.9)                  | 7 (29.2)                        |         |
| Change between baseline and 12 months                          | 0.0 [-2.0: 0.0]           | 0.0 [-1.0: 0.0]                 | 0.29    |
| <b>Vascular inflammation (v score)</b>                         |                           |                                 |         |
| ≥1 at baseline                                                 | 3/25 (12.0)               | 1/23 (4.3)                      | 1       |
| ≥1 at 12 months                                                | 1/25 (4.0)                | 0/23 (0.0)                      |         |
| Change between baseline and 12 months                          | 0.0 [0.0: 0.0]            | 0.0 [0.0: 0.0]                  | 0.73    |
| <b>Tubular atrophy (ct score)</b>                              |                           |                                 |         |
| Baseline                                                       | 1.0 [1.0: 1.0]            | 1.0 [1.0: 1.0]                  | 0.31    |
| 12 months                                                      | 1.0 [1.0: 1.5]            | 1.0 [1.0: 1.3]                  |         |
| Change between baseline and 12 months                          | 0.0 [0.0: 0.5]            | 0.0 [0.0: 0.0]                  | 0.74    |
| <b>Interstitial fibrosis (ci score)</b>                        |                           |                                 |         |
| Baseline                                                       | 1.0 [0.0: 1.0]            | 0.0 [0.0: 1.0]                  | 0.98    |
| 12 months                                                      | 1.0 [0.0: 1.5]            | 1.0 [0.0: 2.0]                  |         |

(Continued)

TABLE 6 Continued

| Variable                              | Bortezomib group (n = 27) | Standard-of-care group (n = 24) | p-value |
|---------------------------------------|---------------------------|---------------------------------|---------|
| Change between baseline and 12 months | 0.0 [-1.0: 0.5]           | 0.0 [0.0: 1.0]                  | 0.39    |
| IFTA grade≥1                          |                           |                                 |         |
| ≥1 at baseline                        | 21 (77.8)                 | 16/23 (69.6)                    | 0.83    |
| ≥1 at 12 months                       | 18 (66.7)                 | 16/23 (69.6)                    |         |
| Change between baseline and 12 months | −1.0 [-1.5: 0.0]          | 0.0 [-2.0: 1.0]                 |         |
| Banff 2022 diagnosis†                 |                           |                                 |         |
| AMR, n (%)                            |                           |                                 |         |
| Active                                | 8 (29.6)                  | 11 (45.8)                       |         |
| Chronic active                        | 11 (40.7)                 | 8 (33.3)                        |         |
| Chronic inactive                      | 8 (29.6)                  | 5 (20.8)                        |         |
| Associated TCMR, n (%)                |                           |                                 |         |
| Borderline Changes                    | 0 (0.0)                   | 1 (4.2)                         |         |
| Acute (grade I and above)             | 1 (3.7)                   | 0 (0.0)                         |         |
| Chronic active                        | 0 (0.0)                   | 0 (0.0)                         |         |

MVI score: microvascular inflammation corresponding to g+ptc sum score; IFTA; interstitial fibrosis and tubular atrophy, AMR; antibody-mediated rejection, TCMR; T-cell-mediated rejection. All values are according to central reading and are expressed as n (%) or median [Q1: Q3]. Comparisons were performed using the Wilcoxon or Chi-squared tests as appropriate.

\*Among patients with MVI score  $\geq$ 2 at baseline.

<sup>†</sup>Reclassified according to the 2022 Banff criteria (Yoo et al., 2023).

within individual System Organ Class categories. Infections, including urinary tract and viral infections were the most common serious adverse events in both groups.

## Discussion

In this multicenter trial, we found that the addition of bortezomib to standard-of-care did not improve efficacy compared with standard-of-care alone in patients with active AMR associated with dnDSA. No significant differences were observed between groups in the individual immunological and histological efficacy endpoints or renal function parameters. This study focused on late rejection, with a median time from transplantation to inclusion of 76 months.

Randomized trials in AMR are rare [16–19]. A major methodological strength of our study was the blinded central assessment of both DSA and histological lesions. The only difference between the two treatment groups was the addition of bortezomib. We selected this design because proteasome inhibitors target antibody-secreting cells, including plasmablasts and plasma cells, thereby reducing antibody production. In the context of AMR, they may need to be combined with strategies that rapidly remove or neutralize circulating pathogenic antibodies, such as plasma exchange and intravenous immunoglobulins. Corticosteroids were also included because they may enhance the pro-apoptotic effects of proteasome inhibitors on plasma cells. Given the poor prognosis of late AMR and the relatively recent occurrence of dnDSA in most patients in our study, the combination of corticosteroids, plasma exchange, and IVIg was considered standard-of-care at the time of the study and was subsequently

recommended by the Transplantation Society Working Group consensus in 2020 [20], although some experts now recommend avoiding these treatments in late AMR, in favor of anti-CD38 monoclonal antibodies alone [16].

Previous uncontrolled studies have reported contradictory findings regarding the effect of bortezomib in patients with AMR and DSA. When used alone, bortezomib appeared to have little or no effect on DSA [12, 13], whereas a transient but significant reduction in DSA was observed when bortezomib was combined with IVIg, corticosteroids, plasma exchange, and sometimes rituximab [10, 11]. However, the effects on AMR outcomes were not assessed in these studies.

In the BORTEJECT study, 44 patients with DSA and late AMR were randomized to receive bortezomib or placebo, with no benefit in preserving eGFR [14, 17]. Despite these negative results, there remained a rationale for evaluating bortezomib in combination with other therapies and in a more homogeneous population of patients. The BORTEJECT study included patients with both preformed DSA and dnDSA. In contrast, our study specifically focused on patients with dnDSA, and combined bortezomib with corticosteroids, plasma exchange, and IVIg to achieve a rapid and sustained effect on DSA, while maximizing the effect of proteasome inhibition on plasma cells. Direct comparison between the two studies is difficult because of differences in patient populations, baseline DSA characteristics, treatment regimens, and follow-up duration. Baseline idDSA MFI was substantially lower in the BORTEJECT study than in the two groups of the TRIBUTE study, and the extent of MFI reduction appeared to be lower in BORTEJECT. Although median MVI scores on biopsies performed at 2 years remained stable, more patients developed cg lesions in BORTEJECT than in TRIBUTE (69% versus 40%).

TABLE 7 Serious adverse events and adverse events in patients receiving bortezomib and standard-of-care treatment.

| Type of adverse events                                          | Bortezomib group, n = 30 | Standard-of-care group, n = 30 | p-value |
|-----------------------------------------------------------------|--------------------------|--------------------------------|---------|
| Patients with at least one serious adverse event, n (%)         | 23 (76.7)                | 14 (46.7)                      | 0.017   |
| Patients with at least one adverse event, n (%)                 | 24 (80.0)                | 26 (86.7)                      | 0.49    |
| Patients with at least one serious adverse event, by SOC, n (%) |                          |                                |         |
| Infections                                                      | 8 (26.7)                 | 7 (23.3)                       | 0.77    |
| Urinary tract infection                                         | 4                        | 1                              |         |
| Respiratory infection                                           | 1                        | 0                              |         |
| Viral infection                                                 | 4                        | 2                              |         |
| Bacterial infection                                             | 1                        | 5                              |         |
| Cardiac                                                         | 0 (0)                    | 4 (13.3)                       | 0.11    |
| Heart failure                                                   | 0                        | 4                              |         |
| Blood and lymphatic disorders                                   | 6 (20.0)                 | 1 (3.3)                        | 0.10    |
| Thrombocytopenia                                                | 4                        | 0                              |         |
| Neutropenia                                                     | 2                        | 0                              |         |
| Anemia                                                          | 0                        | 1                              |         |
| Renal                                                           | 5 (16.7)                 | 1 (3.3)                        | 0.19    |
| Acute kidney injury                                             | 4                        | 1                              |         |
| Gastro-intestinal                                               | 3 (10)                   | 0 (0)                          | 0.24    |
| Hepatobiliary                                                   | 5 (16.7)                 | 0 (0)                          | 0.052   |
| Nervous system disorders                                        | 3 (10)                   | 0 (0)                          | 0.24    |
| Metabolic                                                       | 2 (6.7)                  | 2 (6.7)                        | >0.99   |
| Patients with at least one adverse event, by SOC, n (%)         |                          |                                |         |
| Infections                                                      | 16 (53.3)                | 11 (36.7)                      | 0.19    |
| Urinary tract infection                                         | 3                        | 4                              |         |
| Viral infection                                                 | 4                        | 1                              |         |
| Cardiac                                                         | 2 (6.7)                  | 4 (13.3)                       | 0.67    |
| Hypertension                                                    | 2                        | 0                              |         |
| Cardiac failure                                                 | 0                        | 1                              |         |
| Hematologic                                                     | 7 (23.3)                 | 8 (26.7)                       | 0.77    |
| Anemia                                                          | 5                        | 3                              |         |
| Thrombocytopenia                                                | 0                        | 3                              |         |
| Renal                                                           | 7 (23.3)                 | 4 (13.3)                       | 0.32    |
| Acute kidney injury                                             | 3                        | 2                              |         |
| Proteinuria                                                     | 3                        | 0                              |         |
| Gastro-intestinal                                               | 13 (43.3)                | 8 (26.7)                       | 0.18    |
| Diarrhea                                                        | 6                        | 5                              |         |
| Nausea, vomiting                                                | 10                       | 3                              |         |
| Hepatobiliary                                                   | 2 (6.7)                  | 2 (6.7)                        | >0.99   |
| Nervous system                                                  | 15 (50.0)                | 14 (46.7)                      | 0.80    |
| Paresthesia                                                     | 8                        | 6                              |         |
| Headache/migraine                                               | 7                        | 4                              |         |
| Metabolism                                                      | 7 (23.3)                 | 5 (16.7)                       | 0.52    |

(Continued)

TABLE 7 Continued

| Type of adverse events  | Bortezomib group, n = 30 | Standard-of-care group, n = 30 | p-value |
|-------------------------|--------------------------|--------------------------------|---------|
| Gout, hyperuricemia     | 2                        | 4                              |         |
| Diabetes, hyperglycemia | 2                        | 2                              |         |
| General disorders       | 5 (16.7)                 | 7 (23.3)                       | 0.52    |
| Asthenia                | 4                        | 2                              |         |

SOC, System Organ Class

Few controlled trials have recently been published in late AMR. Anti-CD38 monoclonal antibodies are of interest because they target plasma cells, like bortezomib, but also NK cells involved in AMR lesions through antibody-dependent cellular cytotoxicity. Mayer et al. recently reported a randomized trial evaluating the anti-CD38 antibody felzartamab versus placebo in late AMR. Felzartamab significantly reduced MVI but produced only a slight reduction in DSA MFI, suggesting a direct effect on inflammatory cells involved in MVI lesions [21]. Effects on circulating NK cells and NK cell-related AMR genes were also demonstrated in an ancillary study [22]. At 1 year, 4/11 patients (36%) treated with felzartamab had an MVI score of 0, compared with 13% of patients treated with bortezomib in the TRIBUTE study.

Anti-IL-6 antibodies have also generated considerable interest because of their expected inhibitory effects on T cells, B cells, and plasma cells. In a phase 2 placebo-controlled trial, clazakizumab showed effects on DSA levels, molecular AMR activity on biopsy, and the slope of eGFR decline in patients with late chronic active AMR, but no significant effect on MVI. Safety concerns were observed because several patients discontinued treatment due to serious infections or diverticulitis [23]. Another phase 2 trial of clazakizumab showed similar effects without the same safety concerns, but the subsequent phase 3 IMAGINE study was terminated early because of insufficient efficacy on renal function [24].

In the VIPAR study, patients with chronic active AMR were randomized to receive six monthly IVIg infusions or no IVIg. Patients in the IVIg group showed stabilization of chronic histological lesions and eGFR at 1 year, whereas those in the no-IVIg group experienced worsening renal function and histological injury. Compared with the TRIBUTE study, there was no substantial change in MVI score during the 12-month study period in either group, despite a reduction in mean idDSA MFI with IVIg between inclusion and month-12 [25].

Taken together, these studies demonstrate a dissociation between changes in DSA, MVI, and graft function in patients treated for late AMR. In our study, some patients showed improvements in both MVI and DSA MFI, whereas others showed discordant immunological and histological responses. This raises the important question of whether treatments targeting antibodies and MVI should be combined. Anti-CD38 antibodies appear to have a marked but potentially transient effect on MVI, possibly because high-level DSA may continue to circulate and stimulate antibody-dependent cellular cytotoxicity.

The observed discordance also raises questions regarding the sensitivity of the chosen composite primary endpoint.

Although the endpoint was prespecified to capture both immunological and histological responses, combining these biologically distinct components may have reduced its ability to detect a treatment effect. Nevertheless, monitoring DSA remains clinically relevant when DSA are considered to contribute to MVI, particularly when treatment includes antibody removal, as in our trial [16]. In addition, transplant glomerulopathy generally evolves slowly, and including stabilization of the cg score within a 12-month endpoint may have further limited its sensitivity, although changes in cg score were observed in some patients.

Our study had several limitations. First, the study was underpowered because the planned sample size was not reached, and a modest treatment effect may therefore have been missed. In the exploratory *post hoc* analysis, patients with a very high baseline idDSA MFI (>15,000) more frequently achieved the composite primary endpoint and a >50% reduction in idDSA MFI in the bortezomib group than in the standard-of-care group. However, these differences were not statistically significant, possibly because of the small subgroup size and limited statistical power. This analysis was not prespecified and should therefore be interpreted cautiously.

The lack of efficacy may also reflect compensatory humoral mechanisms whereby bortezomib-induced plasma-cell depletion is followed by expansion of germinal-center B cells and follicular helper T cells in lymph nodes, as reported by Kwun et al. [26]. Additionally, Woodle et al. observed that subsets of bone-marrow plasma cells may develop resistance to proteasome inhibitors through increased immunoproteasome activity [27].

Despite randomization, the two groups were imbalanced, and patients in the bortezomib group had a higher immunological risk, with higher proportions of women and retransplantations, higher baseline idDSA MFI, and more class I DSA. However, in separate sensitivity analyses adjusted individually for these characteristics, the association between treatment allocation and the primary endpoint remained non-significant. The open-label design may also have introduced reporting bias, particularly for subjective adverse events, although the central assessments of DSA and histological outcomes were performed blinded to treatment allocation.

Treatment exposure was limited by toxicity, with 11 of 30 patients discontinuing or not completing the planned bortezomib regimen. This safety profile, together with the absence of a significant efficacy benefit, limits the clinical applicability of bortezomib in late active AMR and supports careful patient selection in any future evaluation, particularly among patients with comorbidities or increased susceptibility to infectious, hematological, neurological, or hepatic toxicity.



Finally, central review of the immunological and histological endpoints identified a substantial number of patients who no longer fulfilled the histological inclusion criteria: six in the bortezomib group and eight in the standard-of-care group. This further decreased the already limited number of evaluable patients in the PP population.

In conclusion, adding bortezomib to standard-of-care did not demonstrate a significant efficacy benefit in patients with late active AMR associated with dnDSA and was associated with a higher incidence of serious adverse events. Although the exploratory findings in patients with high baseline idDSA MFI warrant further investigation, the present results do not support the routine addition of bortezomib in this setting. Future therapeutic strategies should aim to address both circulating DSA and tissue-level inflammation while maintaining an acceptable safety profile.

## Data availability statement

The datasets presented in this article are not readily available because of French data protection regulations governed by the Commission nationale de l'informatique et des libertés (CNIL). Biological samples collected during the study will be stored for up to 30 years at the Tissuthèque DNA/RNA biobank located in the Department of Pathology at Necker Children's Hospital (Paris, France), under the responsibility of the coordinating investigator and site manager. Requests to access the datasets should be directed to Assistance Publique-Hôpitaux de Paris (AP-HP) and the Delegation for Clinical Research and Innovation (DRCI), [secretariat-direction.drc@aphp.fr](mailto:secretariat-direction.drc@aphp.fr).

## Ethics statement

The studies involving humans were approved by Comité de protection des personnes Île-de-France II (reference: 2014-03-01 RBM) and by the Agence Nationale de Sécurité du Médicament et des Produits de Santé (reference: 140368A-11). 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

RS: Conceptualization, Funding acquisition, Formal analysis, Investigation, Supervision, Writing – original draft. FM: Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. KR: Investigation, Visualization, Writing – original draft. VD: Investigation, Visualization, Writing – review and editing. AK: Investigation, Project administration, Writing – review and editing. NA: Investigation, Project administration, Writing – review and editing. SC: Investigation, Project administration, Writing – review and editing. MG: Conceptualization, Funding acquisition, Investigation, Project administration, Writing –

review and editing. AJ: Investigation, Project administration, Writing – review and editing. HM: Investigation, Project administration, Writing – review and editing. NK: Investigation, Project administration, Writing – review and editing. VP: Investigation, Project administration, Writing – review and editing. MM: Investigation, Project administration, Writing – review and editing. NO: Investigation, Project administration, Writing – review and editing. DA: Investigation, Project administration, Writing – review and editing. CE: Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing – original draft. 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.2026.16702/full#supplementary-material>

**SUPPLEMENTARY DATA SHEET 1**  
Tribute study protocol Version n°10 2019-08-08.

## References

1. Everly MJ, Rebellato LM, Haisch CE, Ozawa M, Parker K, Briley KP, et al. Incidence and Impact of *de novo* Donor-Specific Alloantibody in Primary Renal Allografts. *Transplantation* (2013) 95:410–7. doi: 10.1097/TP.0b013e31827d62e3
2. Wiebe C, Gibson IW, Blydt-Hansen TD, Karpinski M, Ho J, Storsley LJ, et al. Evolution and Clinical Pathologic Correlations of *de novo* Donor-Specific HLA Antibody Post Kidney Transplant. *Am J Transplant* (2012) 12:1157–67. doi: 10.1111/j.1600-6143.2012.04013.x
3. Aubert O, Loupy A, Hidalgo L, Duong Van Huyen J-P, Higgins S, Viglietti D, et al. Antibody-Mediated Rejection Due to Preexisting *versus de novo* Donor-Specific Antibodies in Kidney Allograft Recipients. *J Am Soc Nephrol* (2017) 28:1912–23. doi: 10.1681/ASN.2016070797
4. Gaston RS, Cecka JM, Kasiske BL, Fieberg AM, Leduc R, Cosio FC, et al. Evidence for antibody-mediated injury as a major determinant of late kidney allograft failure. *Transplantation* (2010) 90:68–74. doi: 10.1097/TP.0b013e3181e065de
5. Sellarés J, De Freitas DG, Mengel M, Reeve J, Einecke G, Sis B, et al. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant* (2012) 12:388–99. doi: 10.1111/j.1600-6143.2011.03840.x
6. Aubert O, Suberbielle C, Gauthier R, Francois H, Obada EN, Durrbach A. Effect of a proteasome inhibitor plus steroids on HLA antibodies in sensitized patients awaiting a renal transplant. *Transplantation* (2014) 97:946–52. doi: 10.1097/01.TP.0000438207.42465.40
7. Jeong JC, Jambaldorj E, Kwon HY, Kim M-G, Im HJ, Jeon HJ, et al. Desensitization using bortezomib and high-dose immunoglobulin increases rate of deceased donor kidney transplantation. *Medicine* (2016) 95:e2635. doi: 10.1097/MD.0000000000002635
8. Moreno Gonzales MA, Gandhi MJ, Schinstock CA, Moore NA, Smith BH, Braaten NY, et al. 32 doses of bortezomib for desensitization is not well tolerated and is associated with only modest reductions in Anti-HLA antibody. *Transplantation* (2017) 101:1222–7. doi: 10.1097/TP.0000000000001330
9. Philogene MC, Sikorski P, Montgomery RA, Leffell MS, Zachary AA. Differential effect of bortezomib on HLA class I and class II antibody. *Transplantation* (2014) 98:660–5. doi: 10.1097/TP.0000000000000132
10. Flechner SM, Fatica R, Askar M, Stephany BR, Poggio E, Koo A, et al. The role of proteasome inhibition with Bortezomib in the treatment of antibody-mediated rejection after kidney-only or kidney-combined organ transplantation. *Transplantation* (2010) 90:1486–92. doi: 10.1097/TP.0b013e3181fdd9b0
11. Walsh RC, Brailey P, Girnita A, Alloway RR, Shields AR, Wall GE, et al. Early and late acute antibody-mediated rejection differ immunologically and in response to proteasome inhibition. *Transplantation* (2011) 91:1218–26. doi: 10.1097/TP.0b013e318218e901
12. Sberro-Soussan R, Zuber J, Suberbielle-Boissel C, Candon S, Martinez F, Snanoudj R, et al. Bortezomib as the sole post-renal transplantation desensitization agent does not decrease donor-specific Anti-HLA antibodies. *Am J Transplant* (2010) 10:681–6. doi: 10.1111/j.1600-6143.2009.02968.x
13. Touzot M, Couvrat-Desvergnès G, Castagnet S, Cesbron A, Renaudin K, Cantarovich D, et al. Differential modulation of donor-specific antibodies after B-Cell depleting therapies to cure chronic antibody mediated rejection. *Transplantation* (2015) 99:63–8. doi: 10.1097/TP.0000000000000285
14. Eskandary F, Regele H, Baumann L, Bond G, Kozakowski N, Wahrmann M, et al. A randomized trial of bortezomib in late antibody-mediated kidney transplant rejection. *J Am Soc Nephrol* (2018) 29:591–605. doi: 10.1681/ASN.2017070818
15. Yoo D, Goutaudier V, Divard G, Gueguen J, Astor BC, Aubert O, et al. An automated histological classification system for precision diagnostics of kidney allografts. *Nat Med* (2023) 29:1211–20. doi: 10.1038/s41591-023-02323-6
16. Böhmig GA, Naesens M, Viklicky O, Thauinat O, Diebold M, Rostaing L, et al. Antibody-mediated rejection—treatment standard. *Nephrol Dial Transplant* (2025) 40:1615–27. doi: 10.1093/ndt/gfaf097
17. Eskandary F, Bond G, Schwaiger E, Kikic Z, Winzer C, Wahrmann M, et al. Bortezomib in late antibody-mediated kidney transplant rejection (BORTEJECT study): study protocol for a randomized controlled trial. *Trials* (2014) 15:107. doi: 10.1186/1745-6215-15-107
18. Moreso F, Crespo M, Ruiz JC, Torres A, Gutierrez-Dalmau A, Osuna A, et al. Treatment of chronic antibody mediated rejection with intravenous immunoglobulins and rituximab: a multicenter, prospective, randomized, double-blind clinical trial. *Am J Transplant* (2018) 18:927–35. doi: 10.1111/ajt.14520
19. Sautenet B, Blanche G, Büchler M, Morelon E, Toupance O, Barrou B, et al. One-year results of the effects of rituximab on acute antibody-mediated rejection in renal transplantation: RITUX ERAH, a multicenter double-blind randomized placebo-controlled trial. *Transplantation* (2016) 100:391–9. doi: 10.1097/TP.0000000000000958
20. Schinstock CA, Mannon RB, Budde K, Chong AS, Haas M, Knechtle S, et al. Recommended treatment for antibody-mediated rejection after kidney transplantation: the 2019 expert consensus from the transplantation society working group. *Transplantation* (2020) 104:911–22. doi: 10.1097/TP.0000000000003095
21. Mayer KA, Schrezenmeier E, Diebold M, Halloran PF, Schatzl M, Schranz S, et al. A randomized phase 2 trial of Felzartamab in antibody-mediated rejection. *N Engl J Med* (2024) 391:122–32. doi: 10.1056/NEJMoa2400763
22. Diebold M, Gauthier PT, Mayer KA, Mackova M, Hinze C, Chang J, et al. Effect of felzartamab on the molecular phenotype of antibody-mediated rejection in kidney transplant biopsies. *Nat Med* (2025) 31:1668–76. doi: 10.1038/s41591-025-03653-3
23. Doberer K, Duerr M, Halloran PF, Eskandary F, Budde K, Regele H, et al. A randomized clinical trial of Anti-IL-6 antibody clazakizumab in late antibody-mediated kidney transplant rejection. *J Am Soc Nephrol* (2021) 32:708–22. doi: 10.1681/ASN.2020071106
24. Jordan SC, Ammerman N, Choi J, Huang E, Najjar R, Peng A, et al. Evaluation of Clazakizumab (Anti-Interleukin-6) in patients with treatment-resistant chronic active antibody-mediated rejection of kidney allografts. *Kidney Int Rep* (2022) 7:720–31. doi: 10.1016/j.ekir.2022.01.1074
25. Mulley WR, Tharmaraj D, Polkinghorne KR, Tesch GH, Dayan SK, Kwan E, et al. A randomized controlled trial of intravenous immunoglobulin vs standard of care for the treatment of chronic active antibody-mediated rejection in kidney transplant recipients. *Kidney Int* (2025) 108:470–80. doi: 10.1016/j.kint.2025.04.023
26. Kwun J, Burghuber C, Manook M, Iwakoshi N, Gibby A, Hong JJ, et al. Humoral compensation after Bortezomib treatment of allosensitized recipients. *J Am Soc Nephrol* (2017) 28:1991–6. doi: 10.1681/ASN.2016070727
27. Woodlee ES, Tremblay S, Brailey P, Girnita A, Alloway RR, Aronow B, et al. Proteasomal adaptations underlying carfilzomib-resistance in human bone marrow plasma cells. *Am J Transplant* (2020) 20:399–410. doi: 10.1111/ajt.15634



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# Abatacept as maintenance therapy in kidney transplantation: a retrospective comparative study

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Belatacept use is increasing in kidney transplantation, as it has demonstrated improved graft survival compared with calcineurin inhibitors. However, its intravenous administration and recurrent supply shortages may limit its use. Abatacept has a similar mechanism of action. Although widely used in rheumatology, its use in kidney transplantation remains poorly documented. This study aimed to compare the efficacy and safety of abatacept and belatacept as maintenance immunosuppressive therapy after kidney transplantation. We conducted a retrospective study including adult kidney transplant recipients who were switched to abatacept or belatacept between 2017 and 2023. The primary endpoint was the occurrence of rejection within the first year after conversion. A total of 74 patients were included: 21 in the abatacept group and 53 in the belatacept group. No significant difference was observed in rejection rates at 1 year (respectively 9.5% vs. 9.4%;  $p > 0.999$ ). Rates of viral and opportunistic infections were also comparable. These findings, which warrant confirmation in larger studies, suggest that abatacept is associated with acceptable safety outcomes relative to belatacept. Abatacept may therefore be considered a potential alternative in selected situations, including drug shortage, limited venous access, pregnancy or the need for greater treatment autonomy.

### KEYWORDS

abatacept, belatacept, calcineurin inhibitor toxicity, immunosuppressive therapy, kidney transplantation

## Introduction

Abatacept is an immunosuppressive treatment widely used in rheumatology, especially in the management of rheumatoid arthritis [1]. This fusion protein is composed of the constant region of the human IgG1 heavy chain, linked to the extracellular domain of human cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). This structure allows selective inhibition of T-cell activation by blocking the CD28-CD80/86 costimulatory pathway. Abatacept can be administered either subcutaneously or intravenously.

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### Methods :

74 kidney transplant recipients

→ Subcutaneous abatacept  
125 mg per week (n=21)

→ Intravenous belatacept  
5 or 6 mg/kg per month  
(n=53)



**No significant difference in rejection rates at one year**  
(9,5% in the Abatacept group vs 9,4% in the Belatacept group,  $p>0,999$ )



**No significant difference in DSA de novo appearance**  
(4,8 % in Abatacept group vs 3,8% in belatacept group,  $p>0,999$ )



**No significant difference in viral infection**  
(9,5% in the Abatacept group vs 15,1% in the Belatacept group,  $p=0,715$ )

Abatacept is associated with acceptable safety outcomes relative to Belatacept and may therefore be considered a potential alternative in selected situations, including drug shortage, limited venous access, pregnancy or the need for greater treatment autonomy.



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GRAPHICAL ABSTRACT

Belatacept has a mechanism of action similar to that of Abatacept. Its composition differs by only 2 amino acids, making it more affine to its ligand. It was developed as an alternative to calcineurin inhibitors (CNIs) in kidney transplantation, as CNI-associated vascular toxicity contributes to accelerated kidney graft ageing [2]. Following its approval by the U.S. Food and Drug Administration and the European Medicines Agency in 2011, belatacept use has become increasingly widespread, as it has demonstrated superior outcomes in renal transplant (RT) survival and improved long-term renal function, both in induction therapy and as an alternative to CNI treatment [3–5]. More recently, some studies have highlighted the potential benefit of using belatacept, in some cases in combination with low-dose CNIs, as maintenance therapy for patients with immunological challenges, such as those related to the presence of donor-specific antibodies (DSAs) or chronic antibody-mediated rejection (cAMR) [6–11].

However, there are certain considerations regarding the use of belatacept. It is administered exclusively intravenously, which can be challenging for patients with chronic kidney disease who often have limited peripheral venous access. Moreover, belatacept has at times become unavailable because of production shortages. There is therefore considerable interest in finding a therapeutic alternative.

Despite significant similarities between belatacept and abatacept, the latter has rarely been studied in the context of renal transplantation. To date, there are 3 case series concerning its use in this context [12–14], whose findings are generally encouraging in terms of safety and efficacy.

This study aimed to assess the safety and efficacy of abatacept in comparison with belatacept over the course of the first year following treatment conversion.

## Materials and methods

### Population and general data

We conducted a retrospective observational study in 3 centres in the Auvergne Region (Hôpital Universitaire de Clermont-Ferrand, Hôpital de Vichy, Hôpital du Puy-en-Velay). We included all kidney transplant patients over 18 years old who were switched to abatacept or belatacept between January 2017 and June 2023. Patient characteristics and biological data were collected from electronic medical records.

This study was approved by the local Ethics Committee (IRB00013412, “CHU de Clermont Ferrand IRB #1”, IRB 2024-CF273) and complied with the French policy of individual data protection.

### Definition of groups

Patients in the belatacept group received intravenous (IV) belatacept (5 or 6 mg/kg according to the pharmaceutical company's recommendations at the time of treatment); they received one injection every 2 weeks for 2 months during the transition phase, followed by monthly maintenance infusions. The initial infusions were administered in a hospital setting, while the maintenance infusions were given either in the hospital or at the patients' homes.

Patients in the abatacept group received subcutaneous abatacept (125 mg per week). The first injection was administered in a hospital setting, after which the treatment was continued at home. Two patients had been receiving belatacept therapy prior to conversion to abatacept—one for 18 months and the other for 7 years. The first

TABLE 1 Patient characteristics, transplantation details, and parameters related to conversion to costimulation inhibitors.

| Characteristics                          | Overall<br>N = 74 | Abatacept<br>N = 21 | Belatacept<br>N = 53 | p value |
|------------------------------------------|-------------------|---------------------|----------------------|---------|
| Age at transplantation                   | 49.1 ± 18.2       | 50.0 ± 20.7         | 48.8 ± 17.2          | 0.640   |
| Male sex                                 | 53 (71.6%)        | 15 (71.4%)          | 38 (71.7%)           | 0.982   |
| <b>Causal nephropathy</b>                |                   |                     |                      |         |
| Glomerulonephritis                       | 22 (29.7%)        | 5 (23.8%)           | 17 (32.1%)           | 0.483   |
| Genetic cause                            | 19 (25.7%)        | 7 (33.3%)           | 12 (22.6%)           | 0.384   |
| Diabetes                                 | 8 (10.8%)         | 3 (14.3%)           | 5 (9.4%)             | 0.680   |
| Vascular nephropathy                     | 7 (9.5%)          | 2 (9.5%)            | 5 (9.4%)             | >0.999  |
| Interstitial nephritis                   | 8 (10.8%)         | 1 (4.8%)            | 7 (13.2%)            | 0.427   |
| Unknown                                  | 7 (9.5%)          | 2 (9.5%)            | 5 (9.4%)             | >0.999  |
| Others                                   | 3 (4.1%)          | 1 (4.8%)            | 2 (3.8%)             | >0.999  |
| Retransplantation                        | 9 (12.2%)         | 2 (9.5%)            | 7 (13.2%)            | >0.999  |
| Induction therapy                        |                   |                     |                      | 0.412   |
| Basiliximab                              | 51 (68.9%)        | 13 (61.9%)          | 38 (71.7%)           |         |
| Antithymocyte globulin                   | 23 (31.1%)        | 8 (38.1%)           | 15 (28.3%)           |         |
| <b>CMV status</b>                        |                   |                     |                      |         |
| CMV donor -/recipient -                  | 12 (16.2%)        | 5 (23.8%)           | 7 (13.2%)            | 0.303   |
| CMV donor +/-recipient -                 | 21 (28.4%)        | 8 (38.1%)           | 13 (24.5%)           | 0.243   |
| CMV recipient +                          | 39 (52.7%)        | 8 (38.1%)           | 31 (58.5%)           | 0.113   |
| DSA at transplantation                   | 12 (16.2%)        | 4 (19.0%)           | 8 (15.1%)            | 0.731   |
| cPRA                                     |                   |                     |                      | 0.817   |
| <5                                       | 57 (77.0%)        | 16 (76.2%)          | 41 (77.4%)           |         |
| 5–85                                     | 8 (10.8%)         | 3 (14.3%)           | 5 (9.4%)             |         |
| >85                                      | 9 (12.2%)         | 2 (9.5%)            | 7 (13.2%)            |         |
| Time of conversion after transplantation | 34.1 [10.6, 83.2] | 44.0 [13.0, 92.5]   | 32.6 [9.3, 78.7]     | 0.465   |
| <b>Conversion indication</b>             |                   |                     |                      |         |
| CNI toxicity/marginal graft              | 51 (68.9%)        | 14 (66.7%)          | 37 (69.8%)           | 0.891   |
| CAMR                                     | 17 (23.0%)        | 6 (28.6%)           | 11 (20.8%)           | 0.763   |
| <i>De novo</i> DSA                       | 2 (2.7%)          | 0 (0.0%)            | 2 (3.8%)             | >0.999  |
| Gastroparesis                            | 2 (2.7%)          | 1 (4.8%)            | 1 (1.9%)             | 0.490   |
| Medication nonadherence                  | 1 (1.4%)          | 0 (0.0%)            | 1 (1.9%)             | >0.999  |
| Multiple medication intolerance          | 1 (1.4%)          | 0 (0.0%)            | 1 (1.9%)             | >0.999  |
| <b>Reason for choosing abatacept</b>     |                   |                     |                      |         |
| Belatacept unavailability                | —                 | 13 (61.9%)          | —                    | —       |
| Difficult venous access                  | —                 | 6 (28.6%)           | —                    | —       |
| COVID-19 pandemic                        | —                 | 2 (9.5%)            | —                    | —       |
| <b>Associated treatment</b>              |                   |                     |                      |         |
| Tacrolimus                               | 15 (20.3%)        | 7 (33.3%)           | 8 (15.1%)            | 0.109   |
| MMF                                      | 59 (79.7%)        | 15 (71.4%)          | 44 (83.0%)           | 0.338   |
| mTORi                                    | 7 (9.5%)          | 3 (14.3%)           | 4 (7.5%)             | 0.397   |

(Continued)



TABLE 1 Continued

| Characteristics | Overall<br>N = 74 | Abatacept<br>N = 21 | Belatacept<br>N = 53 | p value |
|-----------------|-------------------|---------------------|----------------------|---------|
| Corticosteroids | 64 (86.5%)        | 14 (66.7%)          | 50 (94.3%)           | 0.004   |
| Azathioprin     | 4 (5.4%)          | 2 (9.5%)            | 2 (3.8%)             | 0.318   |

Data are presented as the numbers (percentages) of patients, means  $\pm$  standard deviations or medians [25th; 75th percentiles]. CAMR: chronic antibody-mediated rejection, CMV: cytomegalovirus, CNI: calcineurin inhibitor, cPRA: calculated panel reactive antibody, DSA: donor-specific antibody, MMF: mycophenolate mofetil, mTORi: mammalian target of rapamycin inhibitor.

dose of abatacept was administered 1 month after the last infusion of belatacept.

## End points and event definition

The primary endpoint was the occurrence of rejection within the first year post-conversion. Rejection was defined as either T-cell-mediated rejection (TCMR) or antibody-mediated rejection according to the Banff criteria. No protocol biopsies were performed following conversion to CTLA4-Ig; indication biopsies were carried out at the clinician's discretion, typically in cases of graft dysfunction, new-onset proteinuria, or the appearance or an increased level of DSAs.

Secondary endpoints included DSA appearance and an increase in DSA levels. Anti-HLA antibody levels were measured using a LUMINEX-based assay at 3, 6, and 12 months after conversion to belatacept or abatacept or in cases of graft dysfunction. A mean fluorescence intensity (MFI) threshold of 1000 was used to define DSA positivity; a significant increase in the DSA level was defined as a >50% increase in the MFI compared with the preconversion value.

## Statistical analysis

Statistical analyses were performed using R software (version 4.3.1; R Core Team, 2023; R Foundation for Statistical Computing, Vienna, Austria), considering a two-sided type I error rate of 5%. Categorical variables are presented as counts and associated proportions, and quantitative variables are presented as the mean  $\pm$  standard deviation or median [Q1, Q3], depending on their distribution. Data between groups (abatacept vs. belatacept) were compared using Student's t-test or the Mann-Whitney test for nonnormally distributed quantitative variables and the chi-square test or Fisher's exact test for categorical variables, as appropriate.

## Results

### Patient characteristics

A total of 74 patients were included in our study: 21 were in the abatacept group, and 53 were in the belatacept group. Patient characteristics, transplantation details, and parameters related to conversion to costimulation inhibitors are given in Table 1. Our cohort consisted of 53 men (71.6%) with a mean age of 49.1 years. The majority of patients received induction therapy with basiliximab: 13 patients (61.9%) in the abatacept group and

38 patients (71.7%) in the belatacept group ( $p = 0.412$ ). Twelve patients (16.2%) had a preformed DSA at the time of transplantation.

Conversion to CTLA4-Ig occurred at a mean of 34.1 months (range: 10.6–83.2) post-transplantation—44 months (13–92.5) in the abatacept group and 32.6 months (9.3–78.7) in the belatacept group ( $p = 0.465$ ). Conversion was prompted by CNI toxicity or a marginal graft (68.9% of cases), cAMR (23% of cases), the emergence of a DSA (2.7% of cases), gastroparesis (2.7% of cases), nonadherence to oral immunosuppressive therapy (1.4% of cases), or intolerance to multiple immunosuppressive agents (1.4% of cases). The reasons for conversion did not differ significantly between the two groups.

Abatacept was selected over belatacept mainly because of a shortage of belatacept ( $n = 13$ , 61.9%). In 28.6% of the cases ( $n = 6$ ), the choice was driven by issues related to vascular access, whereas in 2 patients (9.5%), abatacept was preferred to minimize hospital visits during the COVID-19 pandemic.

Regarding the immunosuppressive regimen administered in combination with CTLA4-Ig, 15 patients (20.3%) were maintained on tacrolimus, aiming for a target trough concentration between 2 and 5 ng/mL. A greater proportion of patients in the abatacept group were on maintenance tacrolimus, although this difference was not statistically significant (33.3% in the abatacept group vs. 15.1% in the belatacept group;  $p = 0.109$ ). Corticosteroids were more frequently prescribed in the belatacept group (94.3%) than in the abatacept group (66.7%) ( $p = 0.004$ ). Overall, 59 patients (79.7%) received mycophenolate mofetil (MMF), and 7 patients (9.5%) were treated with a mammalian target of rapamycin inhibitor (mTORi). The distributions of these two agents were comparable between the two groups.

## Rejection and DSA

The rejection rates within the first year following conversion to abatacept or belatacept were similar between the two groups: 2 episodes (9.5%) in the abatacept group and 5 episodes (9.4%) in the belatacept group ( $p > 0.999$ ) (Table 2). The characteristics of patients who experienced rejection, as well as details of the rejection episodes, are presented in Table 3. Only one patient, in the belatacept group, experienced rejection with microvascular inflammation in the absence of DSA; all other episodes were TCMR.

DSAs appeared in 3 patients within the year following conversion: one patient in the abatacept group developed, 12 months after conversion, an anti-B8 DSA with a MFI of 1700, without histological signs of rejection on graft biopsy, and 2 patients in the belatacept group developed DSAs—one with anti-Cw2 (MFI

TABLE 2 Occurrence of rejection within the first year post-conversion and secondary endpoints.

| Endpoints                  | Total n = 74 | Abatacept n = 21 | Belatacept n = 53 | p value |
|----------------------------|--------------|------------------|-------------------|---------|
| Rejection                  | 7 (9.5%)     | 2 (9.5%)         | 5 (9.4%)          | >0.999  |
| TCMR                       | 6            | 2                | 4                 |         |
| ABMR                       | 1            | 0                | 1                 |         |
| <b>Secondary endpoints</b> |              |                  |                   |         |
| DSA <i>de novo</i>         | 3 (4.1%)     | 1 (4.8%)         | 2 (3.8%)          | >0.999  |
| Increased in DSA levels    | 2 (2.7%)     | 2 (9.5%)         | 0 (0.0%)          | 0.078   |
| Viral complication         | 10 (13.5%)   | 2 (9.5%)         | 8 (15.1%)         | 0.715   |
| - BK virus replication     | 1 (1.4%)     | 0 (0.0%)         | 1 (12.5%)         |         |
| - Severe COVID-19          | 1 (1.4%)     | 0 (0.0%)         | 1 (12.5%)         |         |
| - CMV disease              | 6 (60.0%)    | 1 (50.0%)        | 5 (62.5%)         |         |
| - Norovirus infection      | 1 (1.4%)     | 0 (0.0%)         | 1 (12.5%)         |         |
| - Herpes zoster            | 1 (1.4%)     | 1 (50.0%)        | 0 (0.0%)          |         |

Data are presented as the numbers (percentages) of patients. CMV: cytomegalovirus, DSA: donor-specific antibody.

1200) and the other with anti-A11 (MFI 1000) at 3 months and 12 months, respectively, after conversion.

A significant increase in DSA was observed in two patients in the abatacept group. In both patients, the increase involved an anti-DQ antibody. The MFI rose from 1,400 to 2,100 in the first patient and from 2,000 to 8,000 in the second. The second patient was also the one in whom the emergence of an anti-B8 antibody was observed.

## Viral complications and opportunistic infection

Ten patients (13.5%) experienced viral complications: 2 patients (9.5%) in the abatacept group and 8 patients (15.1%) in the belatacept group ( $p > 0.999$ ). Details of the infections are presented in Table 2.

In the abatacept group, one patient experienced CMV colitis with a viral load of 6 log copies/mL 5 months after the initiation of abatacept in a 76-year-old patient with positive CMV serology, in whom CMV prophylaxis had been discontinued. The second infection was a case of zoster reactivation occurring 1 month after starting abatacept in a 57-year-old patient, who achieved a favourable outcome under valaciclovir treatment.

Within the cohort, only one case of nonviral opportunistic infection was reported, occurring in a patient from the belatacept group who presented with cryptosporidium-induced diarrhoea 4 months after conversion. All patients received cotrimoxazole after CTLA4-Ig conversion.

## Changes in the estimated glomerular filtration rate (eGFR) at 12 months

A global increase in the eGFR was observed in both groups following the introduction of abatacept or belatacept. In the abatacept group, the increase was 2.3 ( $\pm 11.6$ ) mL/min/1.73 m<sup>2</sup> at 3 months and 2.9 ( $\pm 12.9$ ) mL/min/1.73 m<sup>2</sup> at 1 year, whereas in the belatacept group, it was 3.8 ( $\pm 10.3$ ) mL/min/1.73 m<sup>2</sup> at 3 months and 4.5 ( $\pm 15.2$ ) mL/min/1.73 m<sup>2</sup> at 1 year. There was no statistically

significant difference in eGFR evolution between the two groups. No patient experienced graft loss.

## Discussion

To our knowledge, this is the first study directly comparing abatacept and belatacept as maintenance immunosuppressive therapy following kidney transplantation. Our findings provide reassuring evidence indicating the absence of an increased risk of rejection following conversion to abatacept. The observed acute rejection rate of 9.5% within the first year post-conversion was comparable to that observed following belatacept conversion in our cohort, as well as to rates previously reported in the literature. For instance, in a prospective study, Budde et al. reported an 8% incidence of acute cellular rejection at 24 months following late conversion to belatacept (>6 months post-transplant) [4]. Similarly, two European multicentre retrospective studies, separately authored by Bertrand et al. and Darres et al., reported rejection rates of 6.1%—predominantly occurring within the first 3 months post-conversion—and 8.2%, respectively [4, 5, 15]. Across all these studies, most rejection episodes occurred within the first year after conversion, supporting our decision to use this timeframe as the primary endpoint. In our cohort, both rejection episodes following conversion to abatacept were TCMR. One of these patients had been converted early, at 3 months post-transplant, as suggested in the literature to potentially improve graft survival—although this timing is also considered a risk factor for rejection [15, 16]. In both cases, follow-up biopsies performed 3 months after rejection and subsequent treatment showed complete resolution of inflammation, allowing continuation of abatacept therapy [15]. In our cohort, tacrolimus was maintained in 7 of 21 patients (33.3%) receiving abatacept, with low target trough levels (2–4 ng/mL) [17]. This combination may have contributed to the low rejection rate. In 5 of these 7 patients, the decision to maintain tacrolimus was motivated by a history of cAMR prior to the initiation of abatacept [6–11]. In

TABLE 3 Patients who experienced rejection: characteristics and details.

| Characteristics                            | Abatacept                                                                   |                                                                                                                 |                                                                             |                                                                                                          | Belatacept                                                                                                          |                                                                                                      |                                                                               |
|--------------------------------------------|-----------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
|                                            | Patient 1                                                                   | Patient 2                                                                                                       | Patient 3                                                                   | Patient 4                                                                                                | Patient 5                                                                                                           | Patient 6                                                                                            | Patient 7                                                                     |
| Age at Tx (years)                          | 72                                                                          | 48                                                                                                              | 72                                                                          | 46                                                                                                       | 71                                                                                                                  | 44                                                                                                   | 68                                                                            |
| Sex                                        | F                                                                           | M                                                                                                               | F                                                                           | M                                                                                                        | F                                                                                                                   | F                                                                                                    | M                                                                             |
| Induction therapy                          | Basiliximab                                                                 | ATG                                                                                                             | Basiliximab                                                                 | Basiliximab                                                                                              | ATG                                                                                                                 | Basiliximab                                                                                          | Basiliximab                                                                   |
| Conversion indication                      | Marginal graft                                                              | CNI toxicity                                                                                                    | Marginal graft                                                              | CNI toxicity                                                                                             | CNI toxicity                                                                                                        | CNI toxicity                                                                                         | CNI toxicity                                                                  |
| Time of conversion after Tx                | 1 month                                                                     | 16 months                                                                                                       | Induction treatment                                                         | 6 months                                                                                                 | 11 months                                                                                                           | 15 months                                                                                            | 3 months                                                                      |
| Delay CNI discontinuation after conversion | 2 weeks                                                                     | 1 month                                                                                                         | No CNI                                                                      | 1 month                                                                                                  | 3 weeks                                                                                                             | 1 month                                                                                              | 3 weeks                                                                       |
| Cause of graft biopsy                      | 3 months protocol biopsy                                                    | Graft dysfunction                                                                                               | 3 months protocol biopsy                                                    | Graft dysfunction                                                                                        | Graft dysfunction                                                                                                   | Graft dysfunction                                                                                    | Graft dysfunction                                                             |
| Rejection type                             | Cellular 1B                                                                 | Cellular 1A                                                                                                     | Cellular 1B                                                                 | Cellular 2B                                                                                              | MVI without DSA<br>Suspicious AMR                                                                                   | Borderline                                                                                           | Cellular 1A                                                                   |
| Time from conversion to rejection          | 2 months                                                                    | 8 months                                                                                                        | 3 months                                                                    | 3 months                                                                                                 | 2 months                                                                                                            | 3 months                                                                                             | 3 months                                                                      |
| Associated treatment at time of rejection  | MMF 750 mg/d<br>Prednisone 7.5 mg/d                                         | mTORi<br>Prednisone 5 mg/d                                                                                      | MMF 1500 mg/d<br>Prednisone 5 mg/d                                          | MMF 2 g/d,<br>prednisone 5 mg/d                                                                          | MMF 750 mg/d                                                                                                        | AZA 100 mg/d,<br>prednisone 5 mg/d                                                                   | MMF 2 g/d,<br>prednisone 5 mg/d                                               |
| Treatment                                  | Pulse steroids followed by oral steroids at 1 mg/kg/d tapered over 3 months | - Pulse steroids followed by oral steroids at 1 mg/kg/d tapered over 3 months<br>- Switch mTORi to low dose CNI | Pulse steroids followed by oral steroids at 1 mg/kg/d tapered over 3 months | - ATG<br>- Pulse steroids followed by oral steroids at 1 mg/kg/d tapered over 3 months<br>- Low dose CNI | - Plasmapheresis<br>- Pulse steroids followed by oral steroids at 1 mg/kg/d tapered over 3 months<br>- Low dose CNI | - Pulse steroids followed by oral steroids at 1 mg/kg/d tapered over 3 months<br>- Switch AZA to MMF | - Pulse steroids followed by oral steroids at 1 mg/kg/d tapered over 3 months |
| Re-evaluation graft biopsy                 | Resolution of graft inflammation at 3 months                                | Resolution of graft inflammation at 3 months                                                                    | No re-evaluation graft biopsy                                               | No re-evaluation graft biopsy                                                                            | Advanced renal fibrosis                                                                                             | No re-evaluation graft biopsy                                                                        | No re-evaluation graft biopsy                                                 |
| Evolution of graft function                | SCr 70 $\mu$ mol/L at biopsy; stable at 3 and 12 months                     | SCr 300 $\mu$ mol/L at biopsy (baseline 200) 250 at 3 months and 1 year                                         | SCr 80 $\mu$ mol/L at biopsy; stable at 3 and 12 months                     | SCr 380 $\mu$ mol/L, 200 at 3 months and 1 year                                                          | SCr 800 $\mu$ mol/L at biopsy, dialysis requirement at 4 months                                                     | SCr 350 $\mu$ mol/L at biopsy, 200 at 3 months                                                       | SCr 250 $\mu$ mol/L at biopsy, 140 at 2 weeks                                 |

ATG: antithymocyte globulin, AZA: azathioprine, SCr: serum creatinine, CNI: calcineurin inhibitor, DSA: donor-specific antibody F: female, M: male, MMF: mycophenolate mofetil, mTORi: mammalian target of rapamycin inhibitor, MVI: microvascular inflammation, Tx: transplantation.

addition, it should be noted that, as in most studies and case series addressing conversion to a costimulation inhibitor [3–5, 7, 18], no systematic biopsies were performed in either the abatacept or belatacept groups, raising the possibility of undiagnosed subclinical rejection. However, the observed stability of renal function at 1-year-follow-up in patients who did not undergo biopsy may be considered a reassuring finding.

To date, only three case series have documented the use of abatacept in kidney transplantation. Badell et al. described the use

of IV abatacept (10 mg/kg/month) in 9 recipients with a median follow-up of 82 months [14]. Only one patient experienced favourable-outcome cellular rejection when treated with corticosteroids, while renal function and panel reactive antibody (PRA) levels remained stable in all patients. We previously reported the first case series of five patients who converted to subcutaneous abatacept, none of whom experienced rejection [12]. The largest series to date, published by Bertrand et al., involved 171 patients who received abatacept

for 3 months during the COVID-19 pandemic to avoid in-hospital belatacept infusions, which were not permitted in outpatient settings at the time [13]. The authors reported reassuring results, with stable graft function in 97.2% of the recipients. It is noteworthy that, in the three aforementioned series, the indication for conversion to CTLA-4 inhibitors was exclusively [14] or predominantly [12, 13] intolerance to calcineurin inhibitors, encompassing both CNI-related toxicity and the use of marginal grafts. In contrast, indications for conversion in our study were more heterogeneous, resulting in a more diverse cohort. Nevertheless, this heterogeneity may strengthen the external validity of our findings, as it more accurately reflects the contemporary, diverse use of CTLA-4 inhibitors in clinical practice [6–11].

While the risk of opportunistic infections following conversion to belatacept has been frequently reported in the literature [19, 20], few infectious events were observed in our cohort, whether in the abatacept or belatacept groups. In terms of its primary indication, rheumatoid arthritis, the reported infectious risk of abatacept remains modest [21]; however, extrapolation to transplant recipients must be made with caution, given the concomitant use of immunosuppressive therapy. A lower infection risk with abatacept than with belatacept could be hypothesized, given its lower binding affinity for CD80/CD86, but this requires confirmation in larger studies with extended follow-up.

Several practical considerations motivated the use of abatacept in our centre. Following the COVID-19 pandemic and in the context of promoting outpatient care for patients with significant CNI-induced nephrotoxicity, the most frequent reason for abatacept use in our cohort was the national shortage of belatacept in France from January to September 2023. An especially valuable indication may be its suitability for patients with poor venous access, for whom subcutaneous administration offers clear advantages. While belatacept was administered intravenously by a nurse for all patients in that treatment group, abatacept was self-administered via subcutaneous injections by all patients in that group, offering the patients greater autonomy. This benefit was also highlighted in a study by Bertrand et al., in which 47% of 177 patients who converted to abatacept during the COVID-19 pandemic reported that treatment was less burdensome than belatacept was, and 38% expressed a preference to continue abatacept therapy [13]. Finally, although recent data have provided reassuring evidence regarding the use of belatacept during pregnancy [22], more extensive and longer-term safety data are currently available for abatacept in this setting [23, 24].

In conclusion, this real-world study suggests that abatacept is associated with acceptable safety outcomes compared with belatacept, without evidence of an increased risk of rejection. These findings support its consideration as a potential alternative, particularly in the context of drug shortages, for patients with difficult venous access, during pregnancy, or for those seeking greater treatment autonomy. Additional data on the safety and

efficacy of this strategy are expected from an ongoing randomized prospective trial [25].

## 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 local Ethics Committee (IRB00013412, “CHU de Clermont Ferrand IRB #1”, IRB 2024-CF273). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

## Author contributions

CU-C and CG participated in research design, CU-C, AT, and MF contributed to data collection, CU-C, CG, and IR participated in data analysis and in the writing of the paper. 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

The author(s) declared that generative AI was not used in the creation of this manuscript.

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## References

1. Blair HA, Deeks ED. Abatacept: a review in rheumatoid arthritis. *Drugs* (2017) 77(11):1221–33. doi: 10.1007/s40265-017-0775-4
2. Karolin A, Genitsch V, Sidler D. Calcineurin inhibitor toxicity in solid organ transplantation. *Pharmacology* (2021) 106(7-8):347–55. doi: 10.1159/000515933

3. Vincenti F, Rostaing L, Grinyo J, Rice K, Steinberg S, Gaité L, et al. Belatacept and long-term outcomes in kidney transplantation. *N Engl J Med* (2016) 374(4):333–43. doi: 10.1056/NEJMoa1506027
4. Budde K, Prashar R, Haller H, Rial M, Kamar N, Agarwal A, et al. Conversion from calcineurin inhibitor to belatacept-based maintenance immunosuppression in renal transplant recipients: a randomized phase 3b trial. *J Am Soc Nephrol JASN* (2021) 32:3252–64. doi: 10.1681/ASN.2021050628
5. Darres A, Ulloa C, Brakemeier S, Garrouste C, Bestard O, Del Bello A, et al. Conversion to belatacept in maintenance kidney transplant patients: a retrospective multicenter european study. *Transplantation* (2018) 102(9):1545–52. doi: 10.1097/TP.0000000000002192
6. Bray RA, Gebel HM, Townsend R, Roberts ME, Polinsky M, Yang L, et al. *De novo* donor-specific antibodies in belatacept-treated vs cyclosporine-treated kidney-transplant recipients: post hoc analyses of the randomized phase III BENEFIT and BENEFIT-EXT studies. *Am J Transplant Off J Am Soc Transplant. Am Soc Transpl Surg* (2018) 18(7):1783–9. doi: 10.1111/ajt.14721
7. Santeusano AD, Bhansali A, Weinberg A, Shapiro R, Delaney V, Florman S, et al. Conversion to belatacept within 1-year of renal transplantation in a diverse cohort including patients with donor-specific antibodies. *Clin Transpl* (2020) 34(4):e13823. doi: 10.1111/ctr.13823
8. Johnson AC, Zhang J, Karadkhele G, Gragert L, Hertzberg V, Larsen CP. Belatacept with time-limited tacrolimus coimmunosuppression modifies the 3-year risk of eplet mismatch in kidney transplantation. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2024) 24(2):260–70. doi: 10.1016/j.ajt.2023.09.011
9. Kumar D, Raynaud M, Chang J, Reeve J, Yakubu I, Kamal L, et al. Impact of belatacept conversion on renal function, histology, and gene expression in kidney transplant patients with chronic active antibody-mediated rejection. *Transplantation* (2021) 105(3):660–7. doi: 10.1097/TP.0000000000003278
10. Gallo E, Abbasciano I, Mingozzi S, Lavacca A, Presta R, Bruno S, et al. Prevention of acute rejection after rescue with belatacept by association of low-dose tacrolimus maintenance in medically complex kidney transplant recipients with early or late graft dysfunction. *PLoS One* (2020) 15(10):e0240335. doi: 10.1371/journal.pone.0240335
11. Kitchens WH, Larsen CP, Badell IR. Costimulatory blockade and solid organ transplantation: the past, present, and future. *Kidney Int Rep* (2023) 8(12):2529–45. doi: 10.1016/j.ekir.2023.08.037
12. Uro-Coste C, Atenza A, Heng AE, Rouzaire PO, Garrouste C. Abatacept rescue therapy in kidney transplant recipients: a case series of five patients. *Transpl Int Off J Eur Soc Organ Transpl* (2022) 35:10681. doi: 10.3389/ti.2022.10681
13. Bertrand D, Brunel M, Lebourg L, Scemla A, Lemoine M, Amrouche L, et al. Conversion from intravenous In-Hospital belatacept injection to subcutaneous abatacept injection in kidney transplant recipients during the first COVID-19 stay-at-home order in France. *Transpl Int Off J Eur Soc Organ Transpl* (2023) 36:11328. doi: 10.3389/ti.2023.11328
14. Badell IR, Karadkhele GM, Vasanth P, Farris AB, Robertson JM, Larsen CP. Abatacept as rescue immunosuppression after calcineurin inhibitor treatment failure in renal transplantation. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2019) 19(8):2342–9. doi: 10.1111/ajt.15319
15. Bertrand D, Chavarot N, Olagne J, Greze C, Gatault P, Danthu C, et al. Biopsy-proven T-Cell mediated rejection after belatacept rescue conversion: a multicenter retrospective study. *Transpl Int* (2024) 37:13544. doi: 10.3389/ti.2024.13544
16. Noble J, Leon J, Del Bello A, Anglicheau D, Blanco G, Ville S, et al. Belatacept in kidney transplantation: reflecting on the past, shaping the future. *Transpl Int 20 Mai* (2025) 38:14412. doi: 10.3389/ti.2025.14412
17. Adams AB, Goldstein J, Garrett C, Zhang R, Patzer RE, Newell KA, et al. Belatacept combined with transient calcineurin inhibitor therapy prevents rejection and promotes improved long-term renal allograft function. *Am J Transpl* (2017) 17(11):2922–36. doi: 10.1111/ajt.14353
18. Bertrand D, Matignon M, Morel A, Ludvine L, Lemoine M, Hanoy M, et al. Belatacept rescue conversion in kidney transplant recipients with vascular lesions (banff cv score > 2): a retrospective cohort study. *Nephrol Dial Transpl* (2023) 38(2):481–90. doi: 10.1093/ndt/gfac178
19. Bertrand D, Terrec F, Etienne I, Chavarot N, Sberro R, Gatault P, et al. Opportunistic infections and efficacy following conversion to belatacept-based therapy after kidney transplantation: a French multicenter cohort. *J Clin Med* (2020) 9(11):3479. doi: 10.3390/jcm9113479
20. Brakemeier S, Kannenkeril D, Dürr M, Braun T, Bachmann F, Schmidt D, et al. Experience with belatacept rescue therapy in kidney transplant recipients. *Transpl Int* (2016) 29(11):1184–95. doi: 10.1111/tri.12822
21. Sibilia J, Westhovens R. Safety of T-cell co-stimulation modulation with abatacept in patients with rheumatoid arthritis. *Clin Exp Rheumatol* (2007) 25(5):S46–56.
22. Coscia L, Cohen D, Dube GK, Hofmann RM, Moritz MJ, Gattis S, et al. Outcomes with belatacept exposure during pregnancy in kidney transplant recipients: a case series. *Transplantation* (2023) 107(9):2047–54. doi: 10.1097/TP.0000000000004634
23. Kumar M, Ray L, Vemuri S, Simon TA. Pregnancy outcomes following exposure to abatacept during pregnancy. *Semin Arthritis Rheum* (2015) 45(3):351–6. doi: 10.1016/j.semarthrit.2015.06.016
24. Russell MD, Dey M, Flint J, Davie P, Allen A, Crossley A, et al. British society for rheumatology guideline on prescribing drugs in pregnancy and breastfeeding: immunomodulatory anti-rheumatic drugs and corticosteroids. *Rheumatol Oxf Engl* (2022) 62(4):e48–88. doi: 10.1093/rheumatology/keac551
25. Badell I. *Late Abatacept Conversion in Kidney Transplant Recipients Receiving Belatacept: A Prospective, Randomized Controlled Non-inferiority Trial.* (2022) doi: 10.1016/j.ajt.2025.07.592





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# Evolution and interrelation of physical frailty and mild cognitive impairment up to two years after kidney transplantation: a multi-center prospective cohort study

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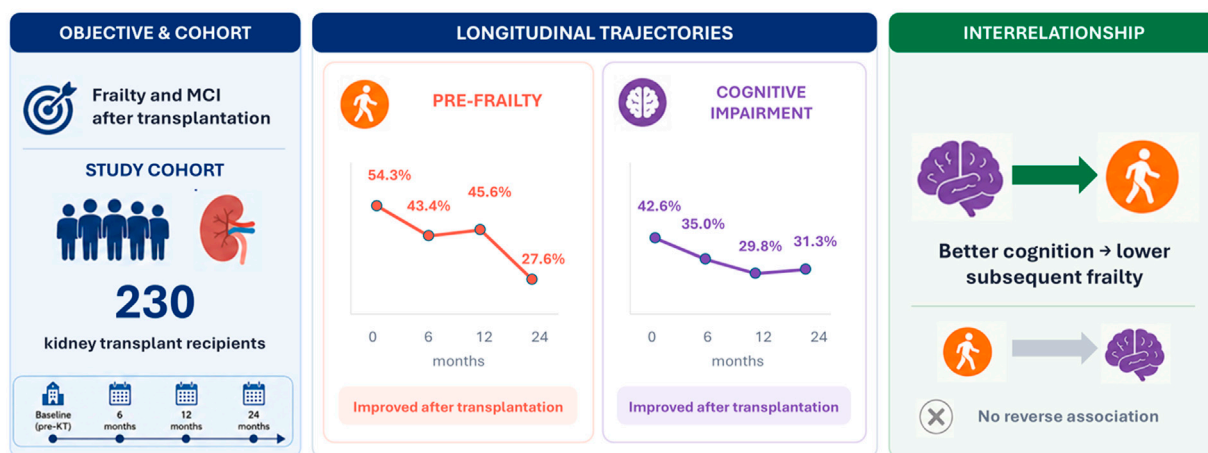
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Kidney transplantation (KT) is the preferred treatment for kidney failure, providing superior survival and quality of life compared with haemodialysis. However, the demand for transplantation exceeds organ availability, underscoring the need to optimize pre- and post-transplant care. Physical frailty and mild cognitive impairment (MCI) are important predictors of post-transplant outcomes, yet their long-term trajectories remain insufficiently understood. This prospective multicentre longitudinal study, nested within the Swiss Transplant Cohort Study, included 230 adult KT recipients followed for up to 2 years after transplantation. Physical frailty was assessed using the adapted Fried Frailty Phenotype and MCI using the Montreal Cognitive Assessment. Physical frailty declined from 8.3% before transplantation to below 2% during follow-up, while pre-frailty decreased from 54.3% to 27.6% at 2 years. MCI prevalence declined from 42.6% to 31.3%. Longitudinal analyses demonstrated that physical frailty was more dynamic than cognitive function. Better cognitive function at earlier time points predicted lower subsequent frailty, whereas frailty did not significantly predict later cognitive function. These findings suggest that cognitive assessment may help identify patients at risk of persistent frailty and support the routine assessment of both physical frailty and cognitive function before and after KT to improve risk stratification and guide individualized patient care.

### KEYWORDS

chronic kidney disease, end stage renal disease, frailty, kidney transplantation, mild cognitive impairment

# Evolution and interrelation of physical frailty and mild cognitive impairment up to two years after kidney transplantation: A multi-center prospective cohort study.



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GRAPHICAL ABSTRACT

## Introduction

Population ageing and an increasing incidence of chronic disease have led to a growing number of patients with kidney failure being considered for kidney transplantation (KT) [1, 2]. KT is recognised as the most effective treatment option for kidney failure patients and offers significant advantages over haemodialysis treatment in terms of mortality, graft function and quality of life [3–5]. As a result, the demand for grafts is steadily increasing worldwide while the number of available donor organs has been stagnating [6, 7]. Consequently, the demand for kidney transplants considerably exceeds the number of available grafts, which underlines the importance of optimising pre- and post-KT procedures and outcomes. Age significantly impacts transplant results, were individuals of similar age may present with different physical and cognitive conditions. Thus, chronological age (counted in years) alone is an inaccurate representation of patients' functional abilities [8, 9]. Physical frailty, a better predictor of biological age, and MCI were both found to be strong independent predictors of adverse health outcomes and functional decline in ESRD as well as KT recipients [5, 7, 9]. Nevertheless, there is limited evidence on how these two conditions develop long term and how they interact after KT. Therefore, studies that investigate the prevalence, the evolution, and the interrelation of both

conditions over time represent an important area of research to contribute informing clinical and policy decision making.

Physical frailty is defined as a state of vulnerability and a decline in functioning across multiple physiological body systems [10, 11]. The Fried Frailty phenotype (FFP) offers the most common way for determining physical frailty in transplantation [12]. This phenotype is based on the five components weakness, slowed walking speed, low level of physical activity, exhaustion, and chronic undernutrition [10, 11]. Depending on the presence of these components, a patient is classified as non-frail; pre-frail; or frail. Although the FFP was initially designed to identify older adults at increased risk for adverse health outcomes, it is presently utilized as a predictive tool for such detrimental health effects across diverse patient populations [10, 13]. For improving its predictive validity, the FFP components have been adapted for KT patients (e.g., chronic undernutrition is measured by asking for loss of appetite instead of asking for weight loss) [14, 15].

In addition to physical frailty, MCI has been shown to significantly influence KT outcomes; MCI is characterized as a syndrome involving cognitive decline that exceeds normative expectations for an individual's age and educational background, yet does not substantially impair activities of daily living [12, 16]. Physical frailty and MCI are common in patients with chronic kidney disease (CKD) [17–22]. Studies have demonstrated that up to 78% of patients with kidney failure and as many as 58% of KT candidates at the time of transplantation exhibit signs of frailty [18, 19, 22–25]. Both conditions are strong and independent predictors of adverse health outcomes such as postoperative complications, increased rate of early hospital readmission or mortality in chronically ill adults of all ages [22, 26–30]. A study on changes in frailty after kidney transplantation revealed that the prevalence of physical frailty initially increased from 20% immediately after

**Abbreviations:** CKD, Chronic kidney disease; ESRD, End stage renal disease; KT, Kidney transplantation; FFP, Fried frailty phenotype; MCI, Mild cognitive impairment; STCS, Swiss transplant cohort study; MoCA, Montreal cognitive assessment; SD, Standard deviation; HD, Haemodialysis; PD, Peritonealdialysis; LOS, Length of stay.

transplantation to 33% 1 month post-transplantation, but subsequently decreased to 27% at 2 months and 18% at 3 months. The findings indicate that physical frailty in kidney transplantation patients is not a stagnant condition, but rather a dynamic physiological state [31].

MCI has in parallel been found to be a strong and independent predictor of increased rates of hospitalization and mortality in different populations [20, 21, 26, 30, 32]. It also adversely affects patients' decision-making, the adoption of healthy lifestyle behaviours and adherence to medication regimens and can in turn negatively influence health outcomes in the KT population [20, 27, 33]. The prevalence of MCI in CKD patients is high, with figures up to 75%. Therefore, several studies suggest regular screening for MCI in the CKD population to allow early detection [20, 21, 26, 30, 34]. Moreover, physical frailty and mild cognitive impairment appear to contribute to a cycle of physical and cognitive decline and reduced quality of life [20, 35]. Dysregulation of multiple biological and stress-response systems has been implicated in the development of both frailty and cognitive impairment. The coexistence of these conditions may further increase vulnerability and accelerate functional decline [36, 37]. Additionally, physical frailty has been associated with lower cognitive function and poorer cognitive outcomes following the initiation of haemodialysis and after KT [24, 38, 39]. However, despite increasing recognition of the clinical importance of both conditions, little is known about their longitudinal evolution after kidney transplantation or the directionality of their relationship. Understanding whether frailty and cognitive impairment improve, worsen, or influence one another over time may provide important insights into patient recovery following transplantation and may help identify potential targets for intervention. Consequently, the identification and management of frailty and cognitive impairment have become increasingly important in kidney transplantation [19, 20]. While the associations between frailty, MCI, and adverse transplant outcomes have been well documented, substantially less is known about how these conditions evolve following transplantation and whether they influence one another over time. A better understanding of their longitudinal trajectories and interrelationship may help identify mechanisms underlying post-transplant recovery and inform the development of targeted interventions aimed at improving patient outcomes.

The objective of this study was to assess the prevalence of physical frailty and MCI among adult KT recipients at the time of transplantation and to describe their longitudinal evolution and interrelationship during the first 2 years following KT. Given the established associations of both conditions with adverse transplant outcomes, a better understanding of their trajectories may support risk stratification, guide the development of targeted interventions, and inform future studies investigating their impact on long-term transplant outcomes.

## Materials and methods

The current study presents results of the multicenter, prospective cohort study GERAS (ExplorinG frailty and mild cognitive impairmentEnt in adult kidney tRansplant recipients to enhance risk prediction for biochemicAl, psychosocial, and health cost outcomeS) [40]. GERAS is nested in the Swiss Transplant Cohort Study (STCS), a long-term open

prospective cohort study that has enrolled more than 95% of all KT recipients in Switzerland since 2008 [41, 42]. The GERAS study was approved as a multicenter study by the responsible ethics committee (EKNZ: 2015-235). Figure 1 illustrates the study design, data resources and variables of the GERAS Study. The GERAS study protocol has been published elsewhere [40].

## Setting and sample

We included a convenience sample of deceased- and living-donor KT recipients aged  $\geq 20$  years who are enrolled in the STCS. Recruitment occurred from March 2016 to July 2019 across four university KT-hospitals and one cantonal KT-hospital in Switzerland. Patients receiving a first or re-implant KT were eligible. We excluded patients that received more than one organ transplant; were not capable to give or understand informed consent; had insufficient knowledge of German, French, English or Italian; or had severe functional impairments (e.g., blindness or wheelchair bound) precluding study assessments. Enrolled participants did not differ based on age and sex from those who were not included in the cohort. With a follow-up time of 2 years, data collection was completed in August 2021.

## Data collection and data management

Potential participants for this study were screened during a routine clinic visit prior to living-donor KT, specifically within a maximum of 3 days before the procedure. For deceased-donor KT recipients, screening occurred upon hospital admission as part of the integrated routine clinical care. Trained data collectors performed informed consent procedures using methods in concordance with the Swiss Ethics regulations. Data collectors were research team members as well as KT center nursing and medical staff. All individuals collecting data were trained in their native language through face-to-face sessions at the respective KT center. Co-investigators of the GERAS project complied with applicable privacy laws, and obtained data were considered confidential with no third-party disclosure. KT recipients were assessed for physical frailty and MCI immediately pre-KT with follow-up evaluations at 6 months, 12 and 24 months post-KT.

To ensure structured and comprehensive patient follow-up, the Secu-Trial™ database (a secure, web-based data platform) had been established. Linking and coding of data from various sources was performed within Secu-Trial™ in collaboration with the STCS data centre and the Clinical Trial Unit, University Hospital Basel [40].

The KT centers received data collection packages including a step-by-step data collection manual [40]. Professional native speakers translated all assessments and questionnaires, i.e., adapted Fried frailty assessment and Montreal Cognitive Assessment (MoCA) from their original language to the three target languages French, German, and Italian [10, 43]. Native speaking members of the research team performed the back translation. Its comparability with the original was discussed in the research team. We pilot-tested the study documents in a convenience sample of four hemodialysis patients, KT candidates

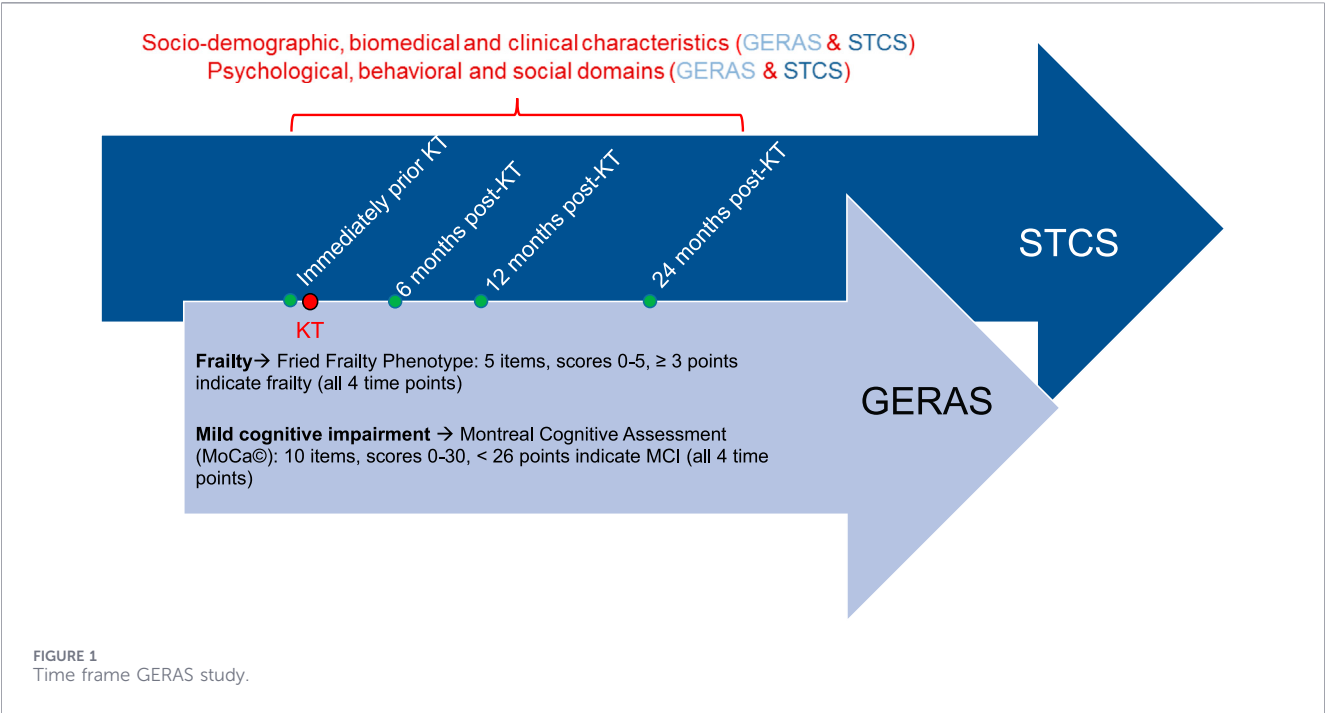


TABLE 1 Fried frailty assessment.

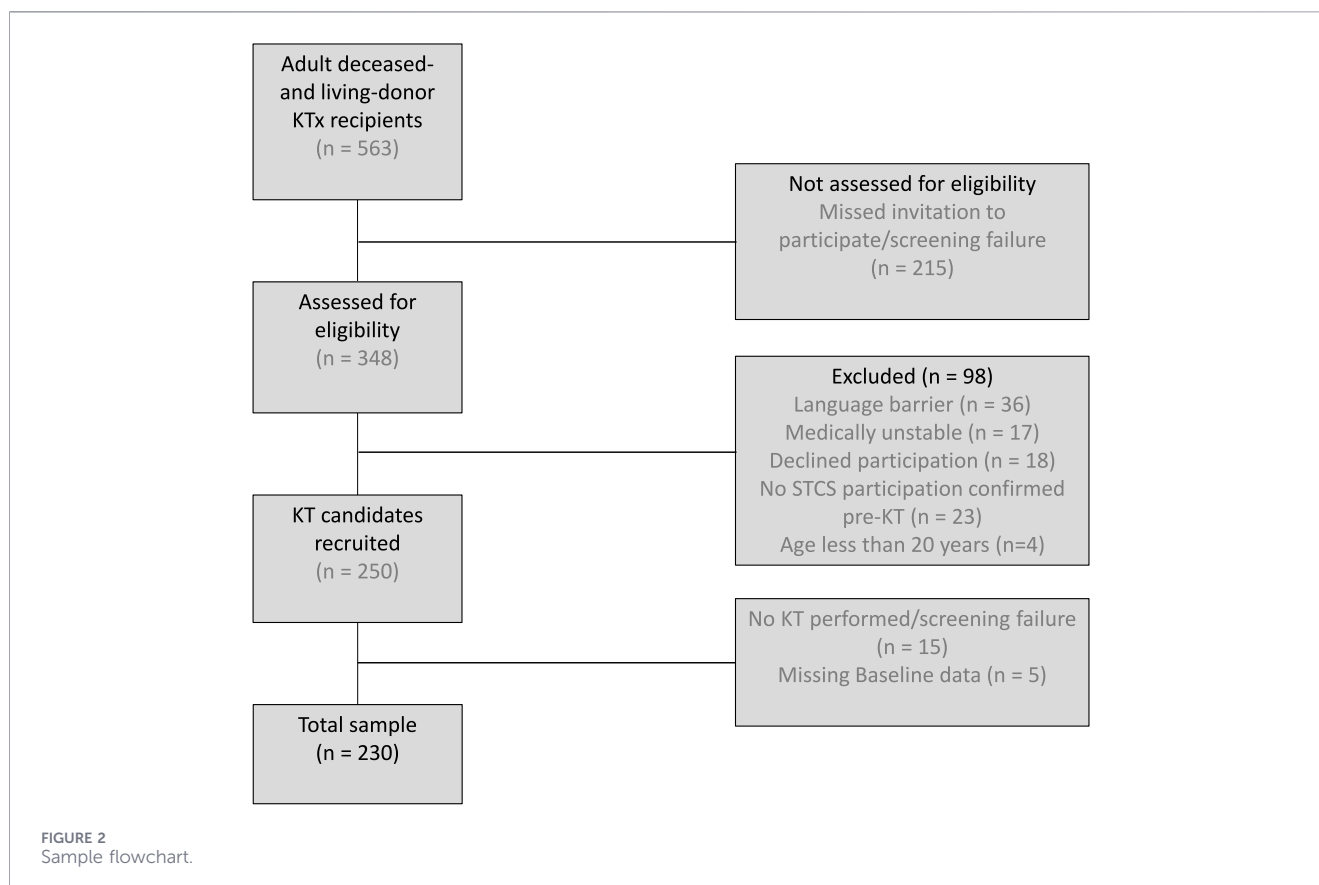
| Domain                         | Procedure                                                                                                                                                                                                                                                          | Evaluation                                                                                             |
|--------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| Weakness                       | Mean values of three tests of maximum grip strength with both hands by the hand-held Jamar dynamometer® [47] were calculated                                                                                                                                       | Weak grip strength: mean value ≤ two standard deviations of sex and age adjusted normative values [48] |
| Slowness                       | Time in seconds to complete a 5-m walk, measured, following a standardized protocol                                                                                                                                                                                | Slow walking speed: Average of three attempts ≥6 s                                                     |
| Low level of physical activity | One closed-ended question: “How often do you engage in activities that require a low or moderate level of energy, such as gardening, cleaning the car or going for a walk?”<br>Answer options: more than once a week/once a week/one to three times a month/rarely | Low level of physical activity: response of “one to three times a month” or “rarely”                   |
| Exhaustion                     | Two closed-ended questions: “In the last week, did you feel on at least 3 days, that everything you did was an effort?” and “In the last week did you feel on at least 3 days, that you could not get going?”<br>Answer options: yes/no.                           | Exhaustion: response of “yes” to at least one question                                                 |
| Appetite                       | One closed-ended question: “Have you, in the last 3 months, been eating more/unchanged/less than usual?” Answer options: less/unchanged/more                                                                                                                       | Chronic undernutrition: response of “less”                                                             |

and KT recipients from all KT centers to test face validity and estimated time investment. Following the pilot testing we made some adjustments to the wording to improve language clarity.

Variables and measurements

Physical frailty was assessed by data collectors using the adapted Fried frailty assessment for KT patients [13, 14]. The original five domains of the FFP were developed and psychometrically tested in the Cardiac Health Study [10]. Both, the original and adapted versions have shown good construct and predictive validity in

KT cohorts [28, 44–46]. The adapted version requires an approximately 10-min interview by a data collector and evaluates the five domains weakness (handgrip strength, cut-off based on age and sex), slowness (measured habitual walking speed, cut-off based on established measures), low level of physical activity (self-report item on physical activity), lower total energy expenditure (self-report item on subjective exhaustion) and chronic undernutrition (self-report item on loss of appetite) (Table 1) [10, 49]. Every domain is scored as 0 or 1, depending on its absence or presence. We calculated a summary score and grouped patients in non-frail (score 0), pre-frail (score 1–2) or frail (score 3–5) [10].



Mild cognitive impairment was assessed by data collectors using the Montreal Cognitive Assessment (MoCA) at the time of transplantation [43, 50, 51]. The MoCA is a 10-min assessment recommended for evaluating cognitive function and covers visuospatial and executive functioning, naming, memory, attention, language, abstraction, delayed recall, and orientation. The overall score is 0–30, with a score <26 indicating MCI [43].

Demographic and clinical variables: Chronological age (years), sex (male/female) and pre-KT comorbidities (cancer, endocrine-metabolic or cardiopulmonary disease) were retrieved from the medical patient record and the STCS dataset. Donor type (living donor deceased donor), LOS (days of hospitalisation starting from admission to discharge), hospital readmission rate (count of readmissions within the first 6 months after KT), renal replacement therapy received (none, peritoneal dialysis, haemodialysis), graft loss event post-KT (event, no event), mortality, transplant related complications during hospital stay (yes/no) time on dialysis (years), donor age (age) human leukocyte antigen mismatches (count in numbers) and graft rejection within the first 6 months post-KT (yes/no) were retrieved from the STCS data source and reviewed in the medical patient record.

## Statistics

All analyses were performed in R 3.5.2 for Windows and SAS 9.4. Descriptive statistics of central tendency and dispersion and graphical methods for data visualization were used to report the

prevalence of physical frailty and MCI immediately before KT and describe their post-KT evolution. As a sensitivity analysis to account for missing values, we compared the numbers of observations that were not followed up till 24 months post-KT for both, physical frailty and MCI. Regression analysis were performed applying generalized estimating equations to account for the repeated measures within patients [52, 53].

We first considered frailty as the dependent variable explained by the MoCA score and second, by treating MoCA as the dependent variable, with frailty as the explanatory variable. For each of these analyses, we adjusted for time-varying and -invariant confounding using a propensity score, calculated based on modelling the respective independent variables by a set of baseline covariates (i.e., age, gender, donor type, educational level, number of comorbidities, first vs. re-transplantation and dialysis type) as well as prior values of both MoCA and frailty scores (i.e., until two prior visits). Additionally, time-lagged models were added to predict both outcomes at subsequent follow-up visits [52, 53]. The significance level was set *a priori* to 0.05.

## Results

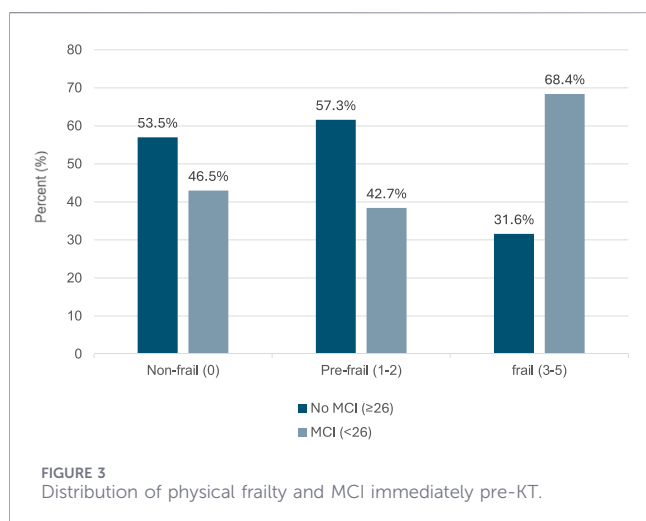
During the recruitment phase, 563 adults underwent kidney transplantation across the five participating Swiss transplant centers. Of these, 348 were assessed for eligibility and 230 fulfilled the inclusion criteria and were enrolled in the study. The discrepancy between the total number of transplant recipients and those



TABLE 2 Demographic and clinical variables.

| Sample characteristic (n = 230) | Value       |
|---------------------------------|-------------|
| <b>Recipient factors</b>        |             |
| Age in years, mean (SD)         | 53.0 ± 14.4 |
| Age ≥65 years, %                | 25.2        |
| Female sex, %                   | 31.7        |
| Education <13 years, %          | 24.3        |
| <b>Comorbidities</b>            |             |
| No comorbidities, %             | 3.9         |
| 1–2 comorbidities, %            | 39.1        |
| ≥3 comorbidities, %             | 57.0        |
| <b>LOS</b>                      |             |
| Days, mean                      | 13.2        |
| Days, median                    | 12          |
| <b>Readmission rate</b>         |             |
| No readmission, %               | 56.5        |
| Readmission, %                  | 43.5        |
| <b>Donor type</b>               |             |
| Living, %                       | 60.0        |
| Deceased, %                     | 40.0        |
| <b>Dialysis type</b>            |             |
| HD, %                           | 60.4        |
| PD, %                           | 14.35       |
| None, %                         | 24.45       |
| <b>Type of KTx</b>              |             |
| First KTx, %                    | 85.6        |
| Second KTx, %                   | 2.2         |
| Third or subsequent KTx, %      | 12.2        |

SD, Standard deviation; HD, Haemodialysis; PD, Peritonealdialysis; LOS, Length of stay.



screened was primarily attributable to logistical constraints during transplantation procedures, particularly when multiple transplantations occurred simultaneously. If several transplantations were planned simultaneously, recruitment of all

recipients was not always possible. 230 KT recipients across the five participating Swiss transplant centers fulfilled the inclusion criteria and were included in the study (Figure 2). As center-specific differences were not part of the study objectives, participants from all centers were analysed as a single multicenter cohort and results are reported for the pooled sample. Table 2 provides an overview of the sample characteristics. The study participant's mean age was 53.0 years (SD ± 14.4, range 20–75), 25.2% were aged ≥65 years and 31.7% were female.

## Prevalence of physical frailty and MCI immediately prior to KT

Immediately pre-KT, the prevalence of physical frailty was 8.3%, and 54.3% of the study participants were assessed as pre-frail (Table 3; Figure 3). Physical frailty was determined to be slightly less prevalent in female participants (6.9%) than in male participants (8.9%). Within the age group 65–75 years, the prevalence of physical frailty was lower than in the younger age group 20–64 years (6.9% versus 8.8%). Overall, the prevalence of MCI was 42.6% (Table 3). The older age group had a higher prevalence than the younger group (67.2% versus 39.0%).

## Evolution of physical frailty and MCI status up to two years post-KT

The prevalence of physical frailty declined over the 2 years follow up period and ranged between 1 and 2% between the 6, 12 and 24 months follow up time points. Physical pre frailty declined initially to 43.4% at 6 months follow up and remained stable at 12 months post KT (45.6%). At the last follow up time point a decrease to 27.6% was detected (Figure 4).

The prevalence of MCI declined to 35.0% at 6 months, 29.8% at 12 months and 31.3% at 24 months post-KT. Figure 5 presents the evolution of MCI as well as the missing data at each time point.

When calculating the number of observations that did not reach the 24-month post-KT time point, we found that 74 observations (32.2%) for physical frailty and 67 observations (29.1%) for MCI were missing (Supplementary Table).

## The relationship between frailty and cognitive function

The regression analyses revealed a significant association between cognitive function and frailty. The MoCA score was a significant predictor of frailty (OR = 0.92, 95% CI: 0.88–0.96,  $p = 0.0004$ ) indicating that higher cognitive function was associated with lower frailty levels. Interaction effects between MoCA and time were not statistically significant, suggesting that this relationship remained stable across follow-up visits, thus were omitted from the models (Table 4).

In the time-lagged models, higher MoCA scores at an earlier time point predicted lower frailty at later visits (OR = 0.88, 95% CI: 0.82–0.94;  $p = 0.0003$ ). The same was not true for frailty being predictor of cognitive function in the future (OR = 0.84, 95% CI: 0.59–1.21;  $p = 0.35$ ) suggesting that cognitive function is able to predict frailty progression, in an unidirectional way (Table 4).

TABLE 3 Participants and prevalence of physical frailty and MCI immediately prior to KT.

| Participant characteristics | Frailty (n = 230) |               |           |                      | MCI (n = 230) |         |                    |
|-----------------------------|-------------------|---------------|-----------|----------------------|---------------|---------|--------------------|
|                             | Non-frail (n)     | Pre-frail (n) | Frail (n) | Prevalence frail (%) | No MCI (n)    | MCI (n) | Prevalence MCI (%) |
| All participants            | 86                | 125           | 19        | 8.3%                 | 132           | 98      | 42.6%              |
| Age, years                  |                   |               |           |                      |               |         |                    |
| 20–64                       | 63                | 93            | 15        | 8.8%                 | 110           | 61      | 35.7%              |
| 65–75                       | 23                | 31            | 4         | 6.9%                 | 21            | 37      | 63.8%              |
| Sex                         |                   |               |           |                      |               |         |                    |
| Female                      | 29                | 39            | 5         | 6.8%                 | 45            | 28      | 38.4%              |
| Male                        | 57                | 86            | 14        | 8.9%                 | 87            | 70      | 44.6%              |
| Education level             |                   |               |           |                      |               |         |                    |
| Education <13 years         | 18                | 37            | 1         | 1.8%                 | 36            | 20      | 35.7%              |
| Education ≥13 years         | 68                | 88            | 18        | 10.3%                | 96            | 78      | 44.8%              |
| LOS                         |                   |               |           |                      |               |         |                    |
| <13 days                    | 61                | 67            | 7         | 5.2%                 | 86            | 49      | 36.3%              |
| ≥13 days                    | 25                | 58            | 12        | 12.6%                | 46            | 49      | 51.6%              |
| Dialysis type               |                   |               |           |                      |               |         |                    |
| HD                          | 56                | 71            | 12        | 8.6%                 | 75            | 64      | 46.0%              |
| PD                          | 15                | 16            | 3         | 8.8%                 | 22            | 11      | 33.3%              |
| None                        | 14                | 38            | 4         | 7.1%                 | 34            | 22      | 39.3%              |

TABLE 4 Results of the ordinal logistic regression models.

| Model                                   | Odds ratio (95% confidence intervals) | P-value |
|-----------------------------------------|---------------------------------------|---------|
| Frailty scores predicted by MoCA scores |                                       |         |
| Concurrent                              | 0.92 (0.88–0.96)                      | 0.0004  |
| Forward lag (one assessment)            | 0.88 (0.82–0.94)                      | 0.0003  |
| Forward lag (two assessments)           | 0.97 (0.87–1.08)                      | 0.53    |
| MoCA scores predicted by frailty scores |                                       |         |
| Concurrent                              | 0.77 (0.61–0.96)                      | 0.02    |
| Forward lag (one assessment)            | 0.84 (0.59–1.21)                      | 0.35    |

Models are adjusted for time, the propensity score and the propensity score squared.

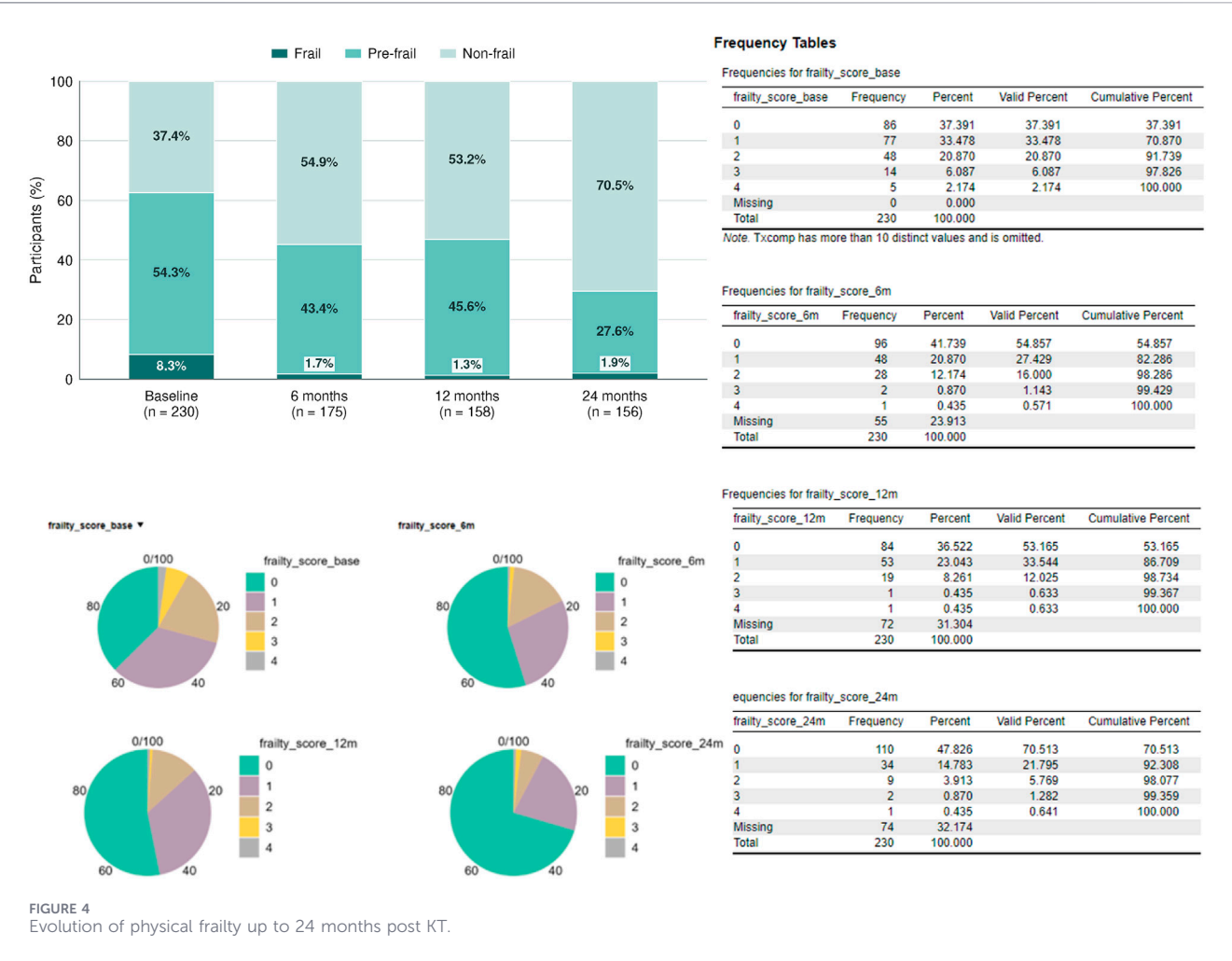
To assess potential attrition bias, baseline characteristics were compared between participants with and without available 24-month follow-up assessments. Participants without a 24-month MoCA assessment had lower baseline MoCA scores ( $p = 0.019$ ) and a higher comorbidity burden ( $p = 0.021$ ), whereas participants without a 24-month frailty assessment had higher baseline frailty scores ( $p = 0.032$ ).

## Discussion

The objective of this study was to evaluate the prevalence of physical frailty and MCI among adult KT recipients at the time of transplantation and to describe their evolution up to 2 years post-

KT. We found that in our study population, the prevalence of physical frailty amounted to 8.3% and that of MCI to 42.6%.

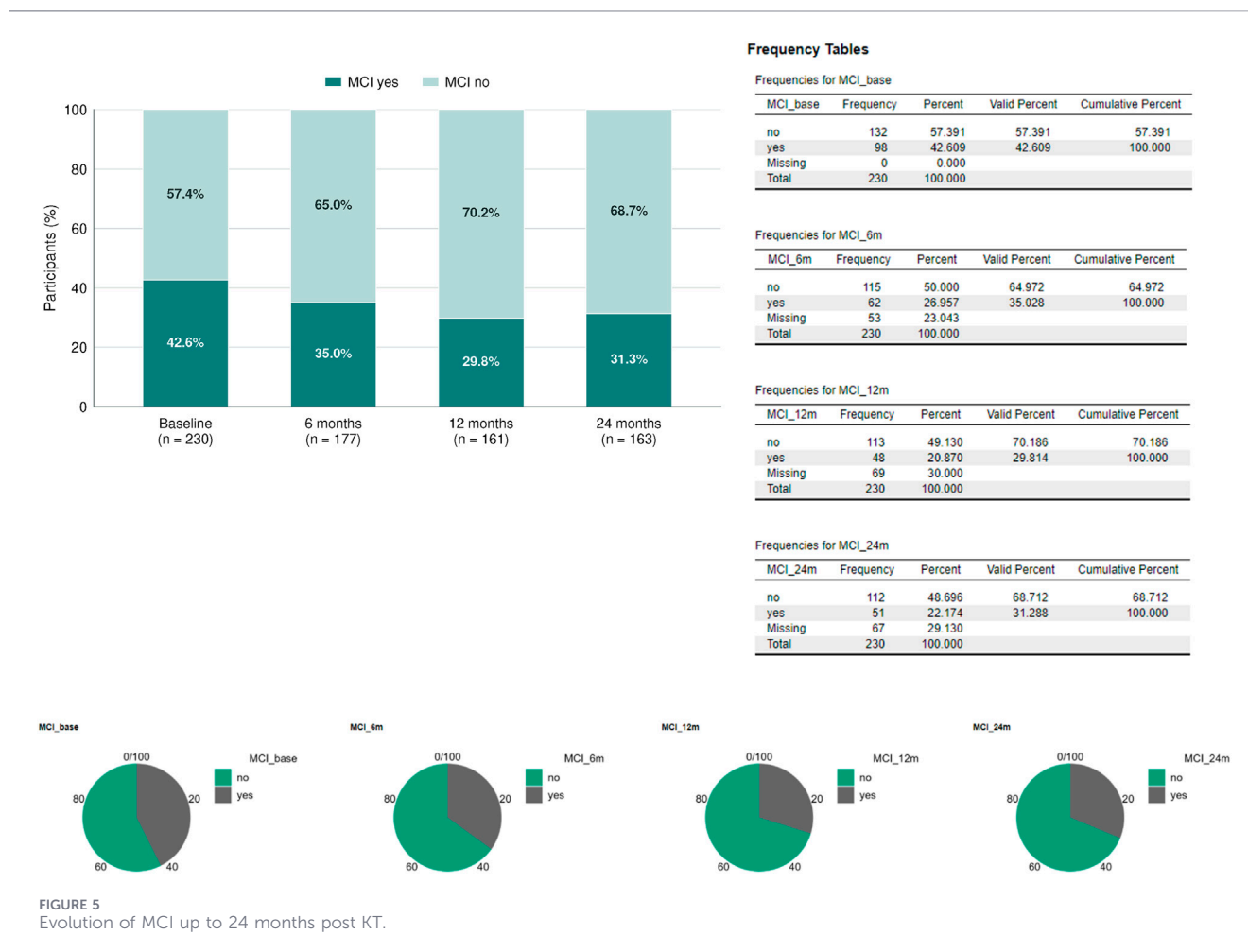
The prevalence of physical frailty in KT patients in our study was lower than that reported in the literature (8% compared to up to 20%) [6, 8, 9, 18, 44, 54]. In contrast, the percentage of patients with physical pre-frail status was higher than values previously reported in an international study (54% compared to 33%) [44, 45]. This finding might result from the fact that healthcare systems differ among countries, making direct comparisons difficult. Nevertheless, the average time on the waitlist for KT in Switzerland was 2.6 years in 2018, which is low compared to other European countries and the US (Swisstransplant, ERA-EDTA, UNOS). A shorter waiting time has been shown to be beneficial in various studies, whereas a prolonged time on the waitlist has been associated with negative



outcomes after KT [3, 55]. The relatively short waiting time may be one factor explaining why many patients were only in the physical pre-frail stage and not yet further advanced. Interestingly, pre-frailty was more prevalent among pre-emptive transplant recipients than among patients receiving haemodialysis. This difference was not explained by age in our cohort and may instead reflect other patient characteristics or selection factors that were not investigated in the present study. A further notable finding was the lower prevalence of physical frailty among recipients aged 65–75 years compared with those aged 20–64 years. Although this observation appears counterintuitive, it is likely explained by the selection process for kidney transplantation. Older transplant recipients represent a highly selected population who have undergone extensive medical and functional evaluation and are therefore more likely to have preserved physiological reserve than older individuals with kidney failure in the general population [6]. In contrast, younger recipients may accumulate a substantial burden of chronic disease, dialysis-related complications, and multimorbidity that contribute to frailty despite their younger chronological age [56]. This observation further supports the concept that chronological age alone is an insufficient surrogate for biological age in kidney transplantation and reinforces the importance of incorporating frailty assessment into transplant evaluation irrespective of age

[3, 6, 12, 54]. Nevertheless, given the relatively small number of frail participants in our cohort, this finding should be interpreted with caution and warrants confirmation in larger prospective studies. Similarly, although physical frailty appeared to be more prevalent among participants with a higher educational background, this finding should be interpreted cautiously, as participants with higher educational attainment were proportionally overrepresented in our cohort and this imbalance may have influenced the observed distribution.

This study revealed a high prevalence of MCI among kidney transplant recipients immediately prior to transplantation, consistent with previous reports [20, 36, 57, 58]. Although pre-transplant anxiety related to the anticipated transplantation procedure may have influenced cognitive performance in some individuals, baseline assessments were conducted under standardized and calm clinical conditions as part of routine pre-transplant evaluations. The observed reduction in MCI prevalence following transplantation aligns with longitudinal studies demonstrating improvements in cognitive function after kidney transplantation, particularly during the first postoperative year, although residual cognitive impairment remains common [20, 36]. Therefore, the observed trajectory is more likely to reflect genuine post-transplant cognitive recovery than a transient effect



of perioperative stress. Evidence regarding longer-term cognitive trajectories is less consistent, with some studies reporting persistent impairment or late cognitive decline, whereas others demonstrate sustained improvement or stabilization [20, 57]. Consistent with recent longitudinal evidence, we observed a decline in MCI prevalence from 42.6% before transplantation to 29.8% at 12 months, followed by stable prevalence at 24 months. These findings suggest that cognitive impairment after KT is not necessarily progressive but rather reflects an early phase of cognitive recovery followed by relative stability over the first two years after transplantation. Despite this favourable trajectory, persistent cognitive impairment remained common, highlighting the importance of integrating cognitive screening and tailored support into routine post-transplant care to optimize self-management, medication adherence, and long-term clinical outcomes [20]. Although frailty improved and cognitive function remained relatively stable over time, these findings should be interpreted in light of the observed attrition. Participants with poorer baseline cognitive function and greater physical frailty were more likely to have missing 24-month follow-up assessments, which may have resulted in a modest overestimation of the observed longitudinal improvements.

Post-KT, the study observed a marked decline in the prevalence of physical frailty, decreasing to between 1% and 2% at the six,

twelve, and twenty-four-month follow-ups. Similarly, pre-frailty levels decreased significantly, from 43.4% at six months to 27.6% at twenty-four months. This trend aligns with existing literature suggesting that rehabilitation and supportive care post-transplant can lead to improved physical outcomes. Studies have demonstrated that structured rehabilitation programs can improve physical functioning in kidney transplant recipients. Recent studies further indicate that structured exercise-based prehabilitation can improve exercise capacity, muscle strength, physical performance, and muscle morphology in kidney transplant candidates, including those with frailty. These findings support the integration of individualized exercise interventions into multidisciplinary care across the kidney transplantation pathway [59, 60]. In terms of cognitive health, MCI prevalence also decreased over time, dropping from 35.0% at six months to 31.3% at twenty-four months. This reduction, although less pronounced than that observed in physical frailty, indicates that cognitive function can improve post-transplant, possibly due to enhanced physical health and psychosocial factors.

Regression analyses further revealed a significant association between cognitive function and frailty, suggesting that cognitive decline precedes subsequent physical resilience. This is important in identifying patients at risk. Moreover, if this would constitute a causal pathway, then physical frailty may respond to interventions

targeting cognitive function, thus early cognitive assessments in kidney transplant patients may help mitigate frailty progression over time.

The strength of this study lies in its prospective, longitudinal design, which encompassed a 24-month follow-up period post-KT and employed a multicentric approach. However, two significant limitations of this study are the reliance on a convenience sample, which led to a considerable number of missed patients, and the presence of missing data at several follow-up time points particularly in later visits, reduced the statistical power to detect weaker associations and may have introduced bias, which could not be addressed by the use of less missingness-sensitive random-effects models (i.e., because of convergence problems). The follow-up duration may also have been insufficient to capture the long-term impact of frailty on cognitive decline. Further, the potential attrition bias should be considered when interpreting the longitudinal findings, although the mixed-effects models included all available observations and were therefore able to accommodate intermittent missing follow-up data. Future studies should aim to extend the follow-up period and incorporate more detailed assessments of both cognitive and physical function, such as domain-specific neuropsychological tests and further objective frailty measures. Moreover, interventional studies investigating whether improving cognitive function can mitigate frailty progression in kidney transplant recipients would be valuable.

In conclusion, this study underscores the dynamic nature of physical frailty in kidney transplant recipients, demonstrating significant improvements over time, while MCI remained more stable. These findings highlight the critical importance of ongoing monitoring of both physical frailty and MCI in clinical settings. This study further provides evidence that cognitive function plays a significant role in frailty progression among kidney transplant patients, whereas frailty does not appear to substantially impact cognitive decline within the observed timeframe. These findings highlight the importance of early cognitive screening and intervention strategies to improve long-term outcomes in this patient population. Healthcare providers should implement routine assessments of frailty and cognitive function to identify at-risk patients early to adapt how healthcare services are being provided. Additionally, developing targeted interventions aimed at reducing frailty and improving cognitive health is essential to enhance patient outcomes and overall quality of life post-KT. By prioritizing these aspects of patient care, clinicians can better support the long-term wellbeing of kidney transplant recipients.

## Data availability statement

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

## Ethics statement

The studies involving humans were approved by Ethical approval for this study was obtained from the Ethics Committee of Northwest and Central Switzerland (Ethikkommission Nordwest- und Zentralschweiz, EKNZ), Basel, Switzerland. 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

NB and OM conducted the study, NB and OM wrote the manuscript, and SD contributed as senior advisor. 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.2026.16175/full#supplementary-material>



## References

- Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS, et al. Global prevalence of chronic kidney disease - a systematic review and meta-analysis. *PloS One* (2016) 11(7):e0158765. doi: 10.1371/journal.pone.0158765
- Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: global dimension and perspectives. *Lancet (London, England)* (2013) 382(9888):260–72. doi: 10.1016/s0140-6736(13)60687-x
- Chadban SJ, Ahn C, Axelrod DA, Foster BJ, Kasiske BL, Kher V, et al. Summary of the kidney disease: improving global outcomes (KDIGO) clinical practice guideline on the evaluation and management of candidates for kidney transplantation. *Transplantation* (2020) 104(4):708–14. doi: 10.1097/tp.0000000000003137
- 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 Journal Transplantation* (2011) 11(10):2093–109. doi: 10.1111/j.1600-6143.2011.03686.x
- Laupacis A, Keown P, Pus N, Krueger H, Ferguson B, Wong C, et al. A study of the quality of life and cost-utility of renal transplantation. *Kidney International* (1996) 50(1):235–42. doi: 10.1038/ki.1996.307
- Abecassis M, Bridges ND, Clancy CJ, Dew MA, Eldadah B, Englesbe MJ, et al. Solid-organ transplantation in older adults: current status and future research. *Am Journal Transplantation* (2012) 12(10):2608–22. doi: 10.1111/j.1600-6143.2012.04245.x
- Singh P, Ng YH, Unruh M. Kidney transplantation among the elderly: challenges and opportunities to improve outcomes. *Adv Chronic Kidney Disease* (2016) 23(1):44–50. doi: 10.1053/j.ackd.2015.11.002
- Chen HJ, Wen J, Qi R, Zhong J, Schoepf UJ, Varga-Szemes A, et al. Re-Establishing brain networks in patients with ESRD after successful kidney transplantation. *Clin Journal Am Soc Nephrol : CJASN* (2018) 13(1):109–17. doi: 10.2215/cjn.00420117
- McAdams-DeMarco MA, King EA, Luo X, Haugen C, DiBrito S, Shaffer A, et al. Frailty, length of stay, and mortality in kidney transplant recipients: a national registry and prospective cohort study. *Ann Surgery* (2017) 266(6):1084–90. doi: 10.1097/sla.0000000000002025
- Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. *The Journals Gerontology Ser A, Biol Sciences Medical Sciences* (2001) 56(3):M146–56. doi: 10.1093/gerona/56.3.m146
- Fried LP, Ferrucci L, Darer J, Williamson JD, Anderson G. Untangling the concepts of disability, frailty, and comorbidity: implications for improved targeting and care. *The Journals Gerontology Ser A, Biol Sciences Medical Sciences* (2004) 59(3):255–63. doi: 10.1093/gerona/59.3.m255
- Kobashigawa J, Dadhania D, Bhorade S, Adey D, Berger J, Bhat G, et al. Report from the American society of transplantation on frailty in solid organ transplantation. *Am Journal Transplantation* (2018) 19:984–94. doi: 10.1111/ajt.15198
- Bande-Roche K, Xue QL, Ferrucci L, Walston J, Guralnik JM, Chaves P, et al. Phenotype of frailty: characterization in the women's health and aging studies. *The Journals Gerontology Ser A, Biol Sciences Medical Sciences* (2006) 61(3):262–6. doi: 10.1093/gerona/61.3.262
- Jha SR, Hannu MK, Chang S, Montgomery E, Harkess M, Wilhelm K, et al. The prevalence and prognostic significance of frailty in patients with advanced heart failure referred for heart transplantation. *Transplantation* (2016) 100(2):429–36. doi: 10.1097/tp.0000000000000991
- Romero-Ortuno R, Walsh CD, Lawlor BA, Kenny RA. A frailty instrument for primary care: findings from the survey of health, ageing and retirement in Europe (SHARE). *BMC Geriatrics* (2010) 10:57. doi: 10.1186/1471-2318-10-57
- Jha SR, Hannu MK, Gore K, Chang S, Newton P, Wilhelm K, et al. Cognitive impairment improves the predictive validity of physical frailty for mortality in patients with advanced heart failure referred for heart transplantation. *The J Heart Lung Transplantation* (2016) 35(9):1092–100. doi: 10.1016/j.healun.2016.04.008
- Silverwood RJ, Richards M, Pierce M, Hardy R, Sattar N, Ferro C, et al. Cognitive and kidney function: results from a British birth cohort reaching retirement age. *PloS One* (2014) 9(1):e86743. doi: 10.1371/journal.pone.0086743
- Quint EE, Zogaj D, Banning LBD, Benjamins S, Annema C, Bakker SJL, et al. Frailty and kidney transplantation: a systematic review and meta-analysis. *Transplant Direct* (2021) 7(6):e701. doi: 10.1097/txd.0000000000001156
- Chu NM, Ruck J, Chen X, Xue QL, Norman SP, Segev DL, et al. Long-term trajectories of frailty and its components after kidney transplantation. *The Journals Gerontology Ser A, Biol Sciences Medical Sciences* (2022) 77(12):2474–81. doi: 10.1093/gerona/glac051
- Gupta A, Mahnken JD, Bernal J, Sharma P, Lepping RJ, Montgomery RN, et al. Changes in cognitive function after kidney transplantation: a longitudinal cohort study. *Am Journal Kidney Diseases* (2024) 84(1):28–37. doi: 10.1053/j.ajkd.2023.12.022
- Azzouz S, Coté L, Doell D, Paparella AN, Cantarovich M, Gaudio K, et al. Cognitive impairment assessments in kidney transplantation: a review. *Transplant Reviews (Orlando, Fla)* (2025) 39(3):100940. doi: 10.1016/j.trre.2025.100940
- McAdams-DeMarco MA, Law A, Salter ML, Boyarsky B, Gimenez L, Jaar BG, et al. Frailty as a novel predictor of mortality and hospitalization in individuals of all ages undergoing hemodialysis. *J Am Geriatr Soc* (2013) 61(6):896–901. doi: 10.1111/jgs.12266
- Johansen KL, Dalrymple LS, Delgado C, Kaysen GA, Kornak J, Grimes B, et al. Association between body composition and frailty among prevalent hemodialysis patients: a US renal data system special study. *J Am Soc Nephrol : JASN* (2014) 25(2):381–9. doi: 10.1681/asn.2013040431
- McAdams-DeMarco MA, Law A, Salter ML, Chow E, Grams M, Walston J, et al. Frailty and early hospital readmission after kidney transplantation. *Am Journal Transplantation* (2013) 13(8):2091–5. doi: 10.1111/ajt.12300
- McAdams-DeMarco MA, Tan J, Salter ML, Gross A, Meoni LA, Jaar BG, et al. Frailty and cognitive function in incident hemodialysis patients. *Clin Journal Am Soc Nephrol : CJASN* (2015) 10(12):2181–9. doi: 10.2215/cjn.01960215
- Avila-Funes JA, Amieva H, Barberger-Gateau P, Le Goff M, Raoux N, Ritchie K, et al. Cognitive impairment improves the predictive validity of the phenotype of frailty for adverse health outcomes: the three-city study. *J Am Geriatr Soc* (2009) 57(3):453–61. doi: 10.1111/j.1532-5415.2008.02136.x
- Murray AM, Knopman DS. Cognitive impairment in CKD: no longer an occult burden. *Am Journal Kidney Diseases* (2010) 56(4):615–8. doi: 10.1053/j.ajkd.2010.08.003
- Garonzik-Wang JM, Govindan P, Grinnan JW, Liu M, Ali HM, Chakraborty A, et al. Frailty and delayed graft function in kidney transplant recipients. *Arch Surgery (Chicago, Ill : 1960)* (2012) 147(2):190–3. doi: 10.1001/archsurg.2011.1229
- Hidaka S, Nishimura A, Tanabe K, Kobayashi S. The connection between cognitive and cardiac function following kidney transplant: the VINTAGE study. *Kidney Med* (2025) 7(11):101096. doi: 10.1016/j.xkme.2025.101096
- Nair D, Liu CK, Raslan R, McAdams-DeMarco M, Hall RK. Frailty in kidney disease: a comprehensive review to advance its clinical and research applications. *Am Journal Kidney Diseases* (2025) 85(1):89–103. doi: 10.1053/j.ajkd.2024.04.018
- Mara A, McAdams-DeMarco P, Kyra Isaacs BS, Salter ML, Natasha Gupta B, Elizabeth A, et al. Changes in frailty after kidney transplantation. *J Am Geriatr Soc* (2015) 63(10):2152–7. doi: 10.1111/jgs.13657
- Cano C, Samper-Ternent R, Al Snih S, Markides K, Ottenbacher KJ. Frailty and cognitive impairment as predictors of mortality in older Mexican Americans. *The Journal Nutrition, Health and Aging* (2012) 16(2):142–7. doi: 10.1007/s12603-011-0104-7
- Houles M, Canevelli M, van Kan GA, Ousset PJ, Cesari M, Vellas B. Frailty and cognition. *The J Frailty and Aging* (2012) 1(2):56–63. doi: 10.14283/jfa.2012.11
- Robertson DA, Savva GM, Kenny RA. Frailty and cognitive impairment—a review of the evidence and causal mechanisms. *Ageing Research Reviews* (2013) 12(4):840–51. doi: 10.1016/j.arr.2013.06.004
- McAdams-DeMarco MA, Olorundare IO, Ying H, Warsame F, Haugen CE, Hall R, et al. Frailty and postkidney transplant health-related quality of life. *Transplantation* (2018) 102(2):291–9. doi: 10.1097/tp.0000000000001943
- Jurgensen A, Qannus AA, Gupta A. Cognitive function in kidney transplantation. *Curr Transplantation Reports* (2020) 7:145–53. doi: 10.1007/s40472-020-00284-0
- Sargent L, Nalls M, Amella EJ, Slattum PW, Mueller M, Bandinelli S, et al. Shared mechanisms for cognitive impairment and physical frailty: a model for complex systems. *Alzheimer's and Dementia (New York, N Y)* (2020) 6(1):e12027. doi: 10.1002/trc2.12027
- Cobo G, Lindholm B, Stenvinkel P. Chronic inflammation in end-stage renal disease and dialysis. *Nephrol Dialysis, Transplantation* (2018) 33(3):iii33–iii40. doi: 10.1093/ndt/gfy175
- Fitzpatrick J, Sozio SM, Jaar BG, Estrella MM, Segev DL, Parekh RS, et al. Frailty, body composition and the risk of mortality in incident hemodialysis patients: the predictors of arrhythmic and cardiovascular risk in end stage renal disease study. *Nephrol Dialysis, Transplantation* (2019) 34:346–54. doi: 10.1093/ndt/gfy124
- Mauthner O, Claes V, Walston J, Engberg S, Binet I, Dickenmann M, et al. Exploring frailty and mild cognitive impairment in kidney transplantation to predict biomedical, psychosocial and health cost outcomes (GERAS): protocol of a nationwide prospective cohort study. *J Advanced Nursing* (2017) 73(3):716–34. doi: 10.1111/jan.13179
- Koller MT, van Delden C, Muller NJ, Baumann P, Lovis C, Marti HP, et al. Design and methodology of the swiss transplant cohort study (STCS): a comprehensive prospective nationwide long-term follow-up cohort. *Eur Journal Epidemiology* (2013) 28(4):347–55. doi: 10.1007/s10654-012-9754-y
- De Geest S, Burkhalter H, Berben L, Bogert LJ, Denhaerynck K, Glass TR, et al. The Swiss transplant cohort Study's framework for assessing lifelong psychosocial factors in solid-organ transplants. *Prog Transplantation (Aliso Viejo, Calif)* (2013) 23(3):235–46. doi: 10.7172/ptit2013250
- Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal cognitive assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc* (2005) 53(4):695–9. doi: 10.1111/j.1532-5415.2005.53221.x
- McAdams-DeMarco MA, Law A, King E, Orandi B, Salter M, Gupta N, et al. Frailty and mortality in kidney transplant recipients. *Am Journal Transplantation* (2015) 15(1):149–54. doi: 10.1111/ajt.12992
- McAdams-DeMarco MA, Isaacs K, Darko L, Salter ML, Gupta N, King EA, et al. Changes in frailty after kidney transplantation. *J Am Geriatr Soc* (2015) 63(10):2152–7. doi: 10.1111/jgs.13657

46. Lai X, Chen G, Qiu J, Wang C, Chen L. Recipient-related risk factors for graft failure and death in elderly kidney transplant recipients. *PLoS One* (2014) 9(11):e112938. doi: 10.1371/journal.pone.0112938
47. Roberts HC, Denison HJ, Martin HJ, Patel HP, Syddall H, Cooper C, et al. A review of the measurement of grip strength in clinical and epidemiological studies: towards a standardised approach. *Age and Ageing* (2011) 40(4):423–9. doi: 10.1093/ageing/afr051
48. Mathiowetz V, Kashman N, Volland G, Weber K, Dowe M, Rogers S. Grip and pinch strength: normative data for adults. *Arch Phys Med Rehabil* (1985) 66(2):69–74.
49. Chen X, Mao G, Leng SX. Frailty syndrome: an overview. *Clin Interventions Aging* (2014) 9:433–41. doi: 10.2147/cia.s45300
50. Tiffin-Richards FE, Costa AS, Holschbach B, Frank RD, Vassiliadou A, Krüger T, et al. The Montreal cognitive assessment (MoCA) - a sensitive screening instrument for detecting cognitive impairment in chronic hemodialysis patients. *PLoS One* (2014) 9(10):e106700. doi: 10.1371/journal.pone.0106700
51. Julayanont P, Tangwongchai S, Hemrungron S, Tunvirachaisakul C, Phanthumchinda K, Hongsawat J, et al. The Montreal cognitive assessment-basic: a screening tool for mild cognitive impairment in illiterate and low-educated elderly adults. *J Am Geriatr Soc* (2015) 63(12):2550–4. doi: 10.1111/jgs.13820
52. Ertefaie A, Stephens DA. Comparing approaches to causal inference for longitudinal data: inverse probability weighting versus propensity scores. *Int J Biostat* (2010) 6(2):14. doi: 10.2202/1557-4679.1198
53. Moodie EE, Stephens DA. Estimation of dose-response functions for longitudinal data using the generalised propensity score. *Stat Methods Med Res* (2012) 21(2):149–66. doi: 10.1177/0962280209340213
54. Sandes-Freitas TV, Domingues-da-Silva RO, Sanders-Pinheiro H. Frailty in the context of kidney transplantation. *J Bras Nefrol* (2024) 46(4):e20240048. doi: 10.1590/2175-8239-JBN-2024-0048en
55. Apostolo J, Holland C, O'Connell MD, Feeney J, Tabares-Seisdedos R, Tadros G, et al. Mild cognitive decline. A position statement of the cognitive decline group of the European innovation partnership for active and healthy ageing (EIPAH). *Maturitas* (2016) 83:83–93. doi: 10.1016/j.maturitas.2015.10.008
56. Marroquin KA, Frazier K, McCulloch CE, Grimes B, Ku E. Association between age at kidney transplantation and risk of early graft loss. *Pediatr Transplant* (2026) 30(6):e70373. doi: 10.1111/ptr.70373
57. Malyszko J, Golenia A, Farisco M, Re VL, Klimkowicz-Mrowiec A, Capasso G, et al. Cognitive impairment in kidney transplanted patients. *Nephrol Dialysis, Transplantation* (2025) 40(Suppl. ment\_2):ii46–ii53. doi: 10.1093/ndt/gfae250
58. Griva K, Thompson D, Jayasena D, Davenport A, Harrison M, Newman SP. Cognitive functioning pre-to post-kidney transplantation--a prospective study. *Nephrol Dialysis, Transplantation* (2006) 21(11):3275–82. doi: 10.1093/ndt/gfl385
59. Bartlett ST, Santi I, Hachaj G, Wilund KR, Tzvetanov I, Spaggiari M, et al. Resistance-based muscle therapy, frailty, and muscle biopsy findings in kidney transplant candidates: a clinical trial. *Kidney Med* (2025) 7(4):100978. doi: 10.1016/j.xkme.2025.100978
60. Pérez-Sáez MJ, Muñoz-Redondo E, Morgado-Pérez A, Delcros-Forestier L, Bach A, Faura A, et al. Exercise-based prehabilitation for kidney transplant candidates: the FRAILMar randomized controlled trial. *Am Journal Kidney Diseases* (2025) 86(5):634–45. doi: 10.1053/j.ajkd.2025.07.003



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# Post-transplant acute pancreatitis after pancreas transplantation: application of the International Study Group for Pancreatic Surgery definition and clinical impact in a multicenter cohort study

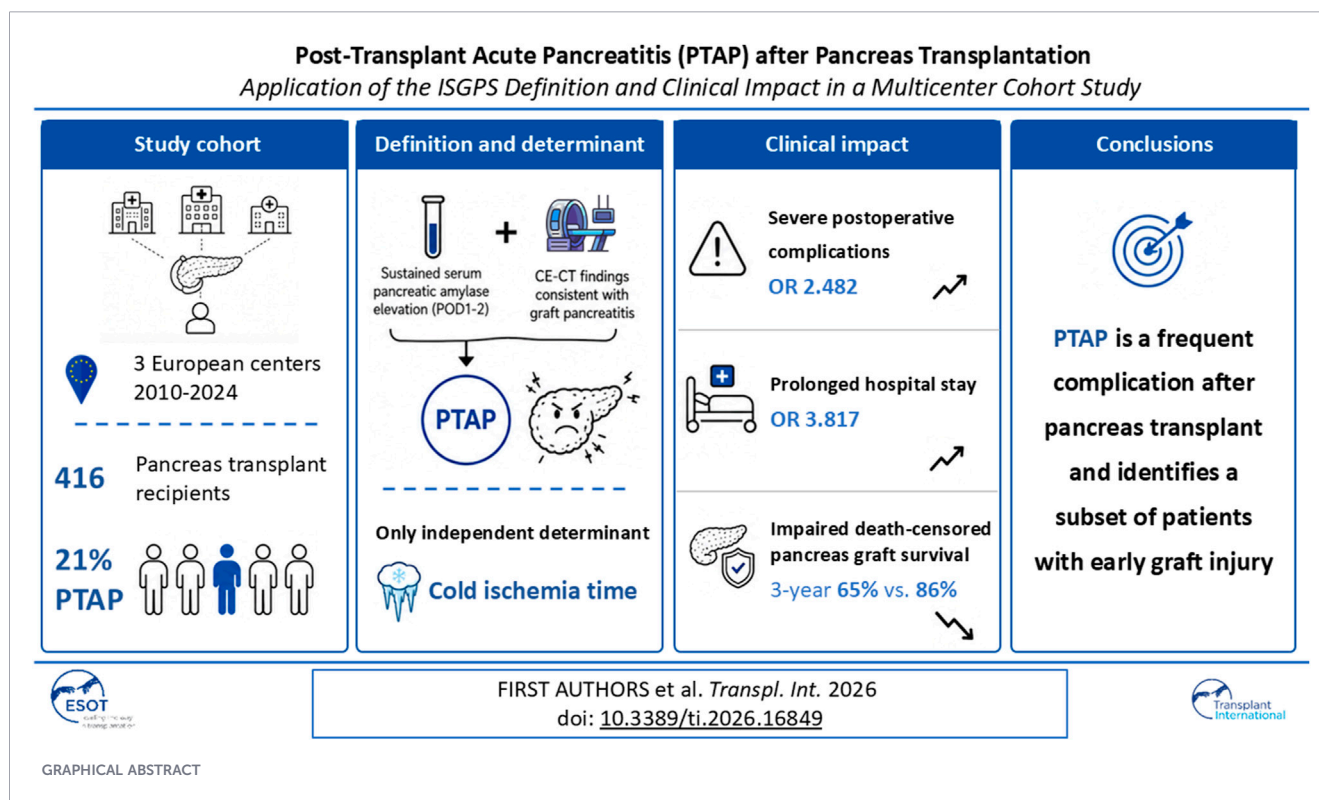
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Graft pancreatitis is a clinically relevant but poorly standardized complication after pancreas transplantation (PT). In contrast, post-pancreatectomy acute pancreatitis has been formally defined and validated by the International Study Group for Pancreatic Surgery (ISGPS). This study assessed the clinical applicability of an adapted ISGPS-based framework to define post-transplant acute pancreatitis (PTAP) after PT and its association with clinically relevant outcomes. Consecutive patients undergoing PT at three European centers between 2010 and 2024 were included. PTAP was defined by sustained postoperative hyperamylasemia (POH) for  $\geq 48$  h combined with CT findings consistent with pancreatitis within 30 days after transplantation. Imaging was performed in 89% of patients based on clinical indication and center-specific protocols. Among 432 patients, the adapted definition was applicable in 416. Sustained POH occurred in 196 patients (47%), and PTAP was diagnosed in 86 (21%). PTAP was the only independent predictor of severe postoperative complications (OR, 2.482;  $P = 0.001$ ), was independently associated with prolonged hospital stay (OR, 3.817;  $P < 0.001$ ), and with worse death-censored graft survival ( $P < 0.001$ ). Cold ischemia time was the only independent determinant of PTAP ( $P < 0.001$ ). PTAP was a frequent and clinically meaningful complication after PT, occurring in approximately one in five patients and associated with worse short- and long-term outcomes.

### KEYWORDS

amylase, graft pancreatitis, graft survival, hyperamylasemia, pancreas transplantation, post-transplant acute pancreatitis, postoperative complications



## Introduction

Graft pancreatitis (GP) is a clinically relevant complication after pancreas transplantation (PT), and has been associated with increased postoperative morbidity, including early relaparotomy and graft loss [1, 2]. However, GP remains a poorly standardized clinical entity that encompasses a broad spectrum of pancreatic graft injury. Multiple mechanisms have been implicated in its development, including ischemia-reperfusion injury and technical or preservation-related factors, such as the use of histidine tryptophan ketoglutarate (HTK) solution, graft overflushing, and exocrine bladder drainage [3, 4]. Donor-related factors may also contribute. As a result, both clinical management and outcome reporting remain inconsistent across transplant programs.

To address this gap, a recent scoping review systematically examined the definitions of GP reported in the literature [5]. Twenty studies were identified, each using a different definition based on variable combinations of clinical, biochemical, radiological, and pathological criteria [5]. This lack of standardization is reflected in the wide variation in reported GP rates, ranging from 0% to 87%, which limits meaningful comparisons of postoperative outcomes across institutions [5]. Importantly, the definitions proposed in the transplant setting remain circumstantial and lack formal validation.

The established definition of native acute pancreatitis [6] cannot be directly extrapolated to the surgical and immunological context of PT because of the unique contribution of perioperative ischemia-reperfusion injury, graft denervation, and immunosuppression-related factors. In contrast, the International Study Group for Pancreatic Surgery (ISGPS) has recently proposed and validated a

standardized definition of post-pancreatectomy acute pancreatitis (PPAP), based on persistent elevation of serum pancreatic amylase above the institutional upper limit of normal (ULN) for at least 48 h postoperatively and radiological evidence of pancreatitis [7, 8]. Although the mechanisms of pancreatic injury differ substantially between PT and pancreatic resection, both settings are characterized by early perioperative pancreatic injury that may range from transient biochemical abnormalities to clinically relevant inflammatory damage with prognostic implications. Because the ISGPS framework relies on objective biochemical and radiological criteria rather than on a specific etiology [7, 8], it may be applicable to the perioperative setting of PT and could provide a standardized basis for defining post-transplant acute pancreatitis (PTAP).

The aim of this study was to assess the clinical applicability of an adapted ISGPS-based framework in a multicenter cohort of PT recipients to define PTAP and to evaluate its association with clinically relevant short- and long-term outcomes, including postoperative morbidity and pancreas graft survival.

## Materials and methods

### Study design and participants

This multicenter retrospective observational study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [9]. Consecutive patients undergoing PT, including simultaneous pancreas-kidney (SPK), pancreas-after-kidney (PAK), and pancreas transplant alone (PTA), at three European pancreas



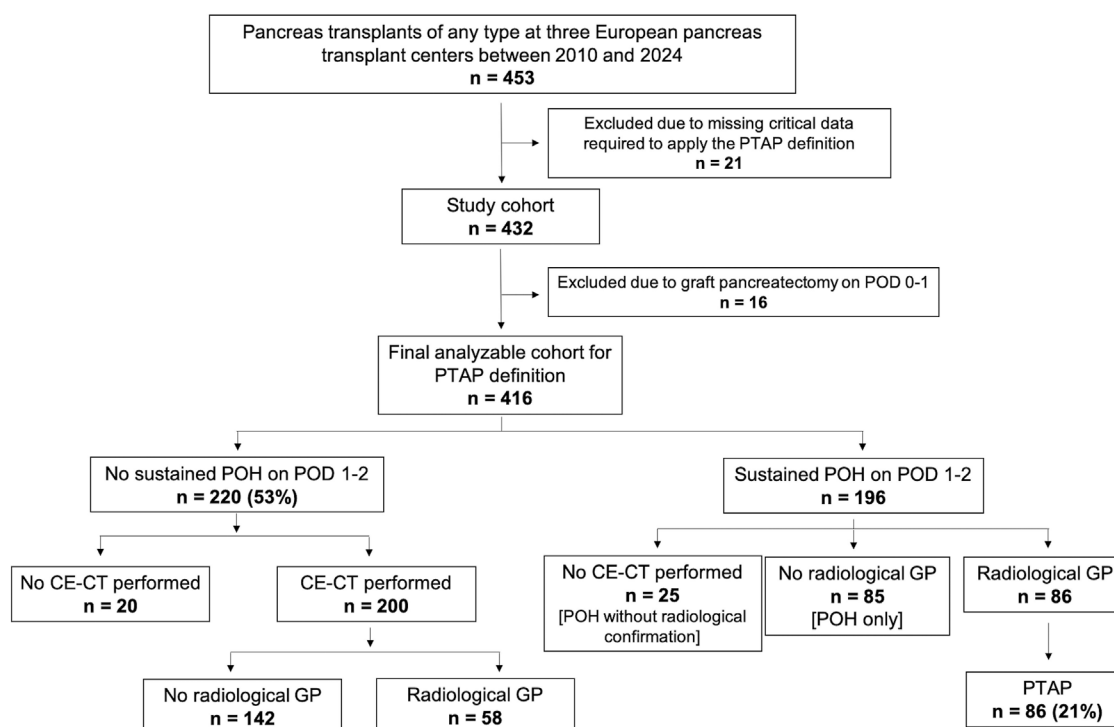


FIGURE 1

Study flow diagram illustrating patient screening, exclusions, and classification according to sustained postoperative hyperamylasemia and radiological graft pancreatitis. Patients undergoing graft pancreatectomy on postoperative day (POD) 0–1 were excluded because sustained postoperative hyperamylasemia over 48 h could not be assessed, and the adapted ISGPS-based definition of post-transplant acute pancreatitis (PTAP) could therefore not be applied. CE-CT, contrast-enhanced computed tomography; GP, graft pancreatitis; POD, postoperative day; POH, postoperative hyperamylasemia; PTAP, post-transplant acute pancreatitis.

transplant centers between 2010 and 2024 were considered for inclusion. Patients with missing data required to apply the PTAP definition, including postoperative serum amylase measurements, were excluded. Patients undergoing graft explantation on postoperative day (POD) 0 or 1 were also excluded because sustained postoperative hyperamylasemia could not be assessed and the adapted ISGPS-based definition could therefore not be applied. The patient selection process is summarized in Figure 1.

The study was conducted in accordance with the Declaration of Helsinki. Given the retrospective observational design and the use of anonymized routinely collected clinical data, approval by the local ethics committees was waived at all participating centers.

## PTAP definition

PTAP was defined using an adapted ISGPS framework for PPAP [7, 8]. Specifically, PTAP was defined by the presence of both of the following objective criteria:

1. Sustained postoperative hyperamylasemia (POH): persistent elevation of serum pancreatic amylase above the institutional upper limit of normal (Supplementary Table 1) for at least 48 h postoperatively, defined by elevated values on both POD1 and POD2;
2. Radiological evidence of GP: findings consistent with pancreatitis on contrast-enhanced computed tomography

(CE-CT) performed within 30 days after transplantation. Radiological GP was defined by the presence of at least 1 of the following features: interstitial parenchymal edema, inflammatory changes in the peripancreatic graft fat, intra- and/or peripancreatic graft fluid collections, or parenchymal and/or peripancreatic graft necrosis.

Postoperative CE-CT scans were interpreted locally by radiologists at each participating center as part of routine clinical care. No central radiological review was performed. Because imaging was obtained and interpreted in the clinical setting, radiologists were not systematically blinded to serum amylase values or clinical information. For study purposes, PTAP status was assigned retrospectively according to prespecified biochemical and radiological criteria.

Serum pancreatic amylase was routinely measured on POD1 and POD2 in all patients. CE-CT was not performed systematically in all patients with sustained POH but was obtained according to clinical indication or center-specific protocols. CE-CT was the standard postoperative cross-sectional imaging modality across the three participating centers and was performed according to center-specific imaging strategies. In one center, CE-CT was performed according to clinical indication, whereas the other two centers adopted protocol-driven CE-CT for all PT recipients, implemented from 2013 onward in one center and routinely performed throughout the study period in



the other. Patients with sustained POH who did not undergo postoperative cross-sectional imaging were classified as having POH without radiological confirmation, because the radiological criterion for PTAP could not be assessed. These patients were therefore considered not to fulfill the PTAP definition, but were not regarded as radiologically negative. This pragmatic imaging strategy, which reflects real-world clinical practice, may have led to underdiagnosis of mild or subclinical forms of PTAP but was expected to enhance specificity for clinically meaningful PTAP, which was the primary focus of the present study.

The clinical criterion included in the original ISGPS definition, namely clinically relevant changes in postoperative management, was intentionally not applied. This choice was made to avoid incorporation bias, because postoperative management decisions are intrinsically linked to short-term clinical outcomes, which constituted primary study endpoints. By relying exclusively on objective biochemical and radiological criteria, the analysis aimed to preserve independence between exposure (PTAP) and outcome assessment.

## Definition of clinical outcomes

The clinical impact of PTAP was evaluated using two primary outcomes: (i) severe postoperative complications and (ii) length of index hospital stay (LOS). Postoperative complications occurring within 30 days after transplantation or during the index hospitalization were graded according to the Clavien-Dindo classification [10] and were considered severe if grade IIIA or higher. This threshold was selected to capture complications of clear clinical relevance requiring invasive management. Both systemic and pancreas-related complications were included, whereas complications exclusively related to the kidney graft were excluded. LOS was calculated from the day of transplantation (POD0) to hospital discharge and dichotomized using the third quartile of the cohort distribution; prolonged LOS was defined as more than 23 days.

Secondary outcomes included death-censored pancreas graft survival (GS) and patient survival. Death-censored pancreas GS was defined as the time from transplantation to pancreas graft loss from any cause, excluding death with a functioning graft. Pancreas graft loss was defined as graft explantation or persistent return to insulin dependence during follow-up. Temporary insulin therapy during the early postoperative period was not considered graft loss if graft endocrine function subsequently recovered. Patient survival was defined as the time from transplantation to death from any cause. Patients without events were censored at last available follow-up.

## Data collection

Clinical data were retrospectively collected from prospectively maintained institutional databases using standardized data collection procedures. Collected variables included recipient- and donor-related intraoperative and postoperative characteristics.

Recipient-related variables included sex, age at transplantation, body mass index (BMI), duration and type of diabetes, comorbidities, preoperative dialysis status and modality, and history of previous transplants. Donor-related variables included

donor type (deceased donor after brain death [DBD] or after circulatory death [DCD]), sex, age and BMI.

Intraoperative variables collected included transplant type (SPK, PAK, PTA), cold ischemia time (CIT), anastomotic time, duration of surgery, and intraoperative blood transfusions. CIT was defined as the interval between initiation of cold perfusion in the donor and removal of the graft from ice for implantation. Anastomotic time was defined as the interval between the end of cold preservation and restoration of graft blood flow in the recipient.

Postoperative variables included early and late complications, relaparotomy, hemorrhage, thrombosis (occurring from POD2 onwards), graft explantation, duodenal leakage, and graft rejection before discharge. Clinically relevant graft exocrine leakage was captured as duodenal leakage and recorded as a distinct graft-related complication. Duodenal leakage was defined on the basis of clinical findings, drain characteristics including suspicious drain output and increased drain amylase levels, operative findings, and radiological findings when CT imaging was available. Complications occurring within 30 days after surgery were classified as early, whereas those occurring between 30 days and 3 months were classified as late. Long-term outcomes included rejection episodes, graft loss (with or without graft pancreatectomy), and patient survival.

## Immunosuppression and anticoagulation

Induction immunosuppression consisted of rabbit anti-thymocyte globulin (rATG) and corticosteroids in all patients. Maintenance immunosuppression consisted of a triple-drug regimen including tacrolimus, mycophenolate mofetil (MMF), and corticosteroids.

Perioperative anticoagulation protocols differed across centers and over time, and included prophylactic-dose low molecular weight heparin (LMWH), therapeutic-dose LMWH, or continuous intravenous unfractionated heparin (UFH), with escalation to therapeutic-dose anticoagulation in selected patients with thrombotic defects detected on postoperative CE-CT, as summarized in [Supplementary Table 2](#). Antimicrobial prophylaxis did not differ across centers and followed standard institutional transplant protocols.

## Statistical analysis

Categorical variables are reported as absolute numbers and percentages and were compared using the  $\chi^2$  test or Fisher's exact test, as appropriate. Continuous variables are reported as median with interquartile range (IQR); normality was assessed using the Kolmogorov-Smirnov test. Group comparisons were performed using Student's *t* test or Mann-Whitney *U* test, as appropriate.

Univariable and multivariable logistic regression analyses were performed to identify determinants of severe postoperative complications, prolonged LOS, and PTAP. LOS was primarily analyzed as a binary outcome using the third quartile as the threshold for prolonged hospitalization. As a sensitivity analysis, LOS was also analyzed as a continuous outcome using multivariable linear regression after logarithmic transformation to account for its right-skewed distribution. Logistic regression was selected for early postoperative outcomes occurring within a fixed 30-day window.

Results are reported as odds ratios (OR) with 95% confidence intervals (CIs). Multivariable models were constructed by entering variables significantly associated with the outcome on univariable analysis, followed by backward elimination for covariate selection. Center-specific effects were included in multivariable models to account for between-center heterogeneity. The number of covariates included in each model was limited according to the number of outcome events, aiming for up to 10 outcome events per covariate, to reduce the risk of overfitting. Missing data were not imputed; multivariable analyses were performed as complete-case analyses for the variables included in each model. Variables with low event counts were interpreted cautiously because of the potential for unstable estimates. To address the potential impact of non-systematic imaging, a sensitivity analysis restricted to patients who underwent postoperative CE-CT was performed for severe postoperative complications and prolonged LOS.

Death-censored pancreas GS and patient survival were estimated using the Kaplan-Meier method and compared using the log-rank test. Predictors of death-censored graft loss were assessed using a Fine-Gray competing-risk regression [11], with death with a functioning graft treated as a competing event. Results are reported as subdistribution hazard ratios (sHRs) with 95% CIs.

Receiver operating characteristic (ROC) curve analysis was used to assess the discriminatory performance of serum amylase levels for predicting radiological GP. Optimal cut-off values were determined using the Youden index.

All tests were 2-sided, and  $P < 0.05$  was considered statistically significant. Analyses were performed using SPSS software version 29.0.2 for Mac (IBM Corp., Armonk, NY, USA), GraphPad Prism version 10.2.2 for Mac (GraphPad Software, Boston, MA, USA) and R version 4.4.2 (R Foundation for Statistical Computing, Vienna, Austria) through RStudio version 2024.12.1 + 563 (RStudio, Boston, MA, USA), using the “survival” and “cmprsk” packages.

## Results

### Study population

A total of 453 consecutive patients undergoing PT were screened across the three participating centers. Twenty-one patients were excluded because of missing critical data required to apply the PTAP definition, namely unavailable serum pancreatic amylase measurements on POD1/POD2. The study cohort therefore included 432 PT recipients. Among these, 16 patients underwent graft pancreatectomy on POD0-1 because of early graft thrombosis and were excluded from the PTAP analysis because sustained POH over 48 h could not be assessed. The final analyzable cohort included 416 patients. All analyses assessing PTAP and its association with postoperative and long-term outcomes were performed in this cohort. Sustained postoperative hyperamylasemia (POH) occurred in 196 patients (47%), and PTAP was diagnosed in 86 (21%). Overall, postoperative CE-CT was performed in 371/416 patients (89%) in the analyzable cohort. Center-level CE-CT rates were 97/128 (76%) in the center performing CE-CT according to clinical indication, 138/152 (91%) in the center where protocol-driven CE-CT was implemented from 2013 onward, and 136/136

(100%) in the center performing routine protocol-driven CE-CT throughout the study period. Center-level PTAP rates were 38/128 (30%), 32/152 (21%), and 16/136 (12%), respectively ( $P = 0.002$ ). PTAP rates were stable across transplant eras: 15/84 patients (18%) in 2010–2014, 29/141 patients (21%) in 2015–2019, and 42/191 patients (22%) in 2020–2024 ( $P = 0.738$ ).

Among the remaining patients with sustained POH ( $n = 110$ ), 85 had no radiological evidence of GP, whereas 25 did not undergo CE-CT. The latter patients were classified as having POH without radiological confirmation. Among these patients, 9 experienced a major postoperative complication, including 7 hemorrhagic and 2 thrombotic events. Among patients without sustained POH ( $n = 220$ ), CE-CT was performed postoperatively in 200 (91%), and radiological findings consistent with GP were identified in 58 (29%). The study flow diagram is shown in [Figure 1](#).

### Comparison of clinical characteristics according to PTAP status

Recipient and donor baseline characteristics were largely comparable between patients with and without PTAP ([Table 1](#)). In contrast, operative and preservation-related variables differed between groups. Median CIT was significantly longer in patients with PTAP than in those without PTAP [538 min (IQR, 458–630) vs. 489 min (IQR, 410–560);  $P < 0.001$ ]. Median anastomotic time was also longer in the PTAP group [35 min (IQR, 30–40) vs. 30 min (IQR, 27–36),  $P = 0.036$ ], as was overall duration of surgery ( $P = 0.022$ ).

With respect to early postoperative outcomes, graft explantation between POD2 and POD30 was more frequent in patients with PTAP than in those without PTAP [13/86 (15%) vs. 13/330 (4%),  $P < 0.001$ ]. Duodenal leakage was also more common in the PTAP group [7/86 (8%) vs. 6/330 (2%),  $P = 0.007$ ], whereas rates of early hemorrhage and thrombosis did not differ significantly between groups. Among the 13 patients with duodenal leakage, 11 had sustained POH and 7 fulfilled the adapted ISGPS-based PTAP definition.

### Primary outcomes

Severe postoperative complications (Clavien-Dindo grade  $\geq$  IIIA) occurred in 112 patients (27%) and were significantly more frequent in patients with PTAP than in those without PTAP [33/86 (38%) vs. 79/330 (24%);  $P = 0.008$ ]. Univariable and multivariable logistic regression analyses assessing determinants of severe postoperative complications are reported in [Table 2](#). After adjustment for center-specific effects and clinically relevant covariates, PTAP was the only independent predictor of severe postoperative complications (OR, 2.482; 95% CI, 1.432–4.305;  $P = 0.001$ ).

Median LOS was significantly longer in patients with PTAP than in the remainder of the cohort (20 vs. 15 days,  $P < 0.001$ ). On multivariable analysis ([Table 3](#)), PTAP (OR, 3.817; 95% CI, 2.146–6.792,  $P < 0.001$ ) and graft rejection before discharge (OR, 16.662; 95% CI, 3.063–90.621;  $P = 0.001$ ) were independently associated with prolonged LOS ( $>23$  days;  $n = 98$ ). PTAP remained independently associated with longer hospital stay when LOS was

TABLE 1 Comparison of recipient and donor characteristics, surgical details and postoperative outcomes between patients with and without post-transplant acute pancreatitis (PTAP).

| Variable                                        | Overall <i>n</i> = 416 (%) | No PTAP <i>n</i> = 330 (%) | PTAP <i>n</i> = 86 (%) | <i>p</i>         |
|-------------------------------------------------|----------------------------|----------------------------|------------------------|------------------|
| <b>Recipient features</b>                       |                            |                            |                        |                  |
| Sex, male                                       | 231 (56)                   | 178 (54)                   | 53 (62)                | 0.201            |
| Age at transplant, years <sup>a</sup>           | 41 (35–47)                 | 41 (34–47)                 | 43 (38–48)             | 0.059            |
| BMI, Kg/m <sup>2a</sup>                         | 22.8 (20.9–25.5)           | 23 (21–25.5)               | 22.6 (20.6–25.7)       | 0.592            |
| Dialysis                                        | 292 (71)                   | 233 (71)                   | 59 (70)                | 0.947            |
| Diabetes type                                   |                            |                            |                        | 1.000            |
| Type I                                          | 406 (98)                   | 322 (98)                   | 84 (98)                |                  |
| Other                                           | 10 (2)                     | 8 (2)                      | 2 (2)                  |                  |
| Previous pancreas transplant                    | 13 (3)                     | 12 (4)                     | 1 (1)                  | 0.240            |
| <b>Donor features</b>                           |                            |                            |                        |                  |
| Donor type, DBD                                 | 407 (98)                   | 321 (97)                   | 86 (100)               | 0.214            |
| Sex, male                                       | 252 (62)                   | 204 (63)                   | 48 (57)                | 0.328            |
| Age, years <sup>a</sup>                         | 30 (22–38)                 | 30 (22–38)                 | 30 (22–40)             | 0.584            |
| BMI, Kg/m <sup>2a</sup>                         | 23.4 (21–25.4)             | 23.5 (21–25.4)             | 23.4 (21.2–25.7)       | 0.573            |
| Cold ischemia time, minutes <sup>a,b</sup>      | 496 (420–578)              | 489 (410–560)              | 538 (458–630)          | <b>&lt;0.001</b> |
| Anastomotic time, minutes <sup>a,b</sup>        | 30 (28–40)                 | 30 (27–36)                 | 35 (30–40)             | <b>0.036</b>     |
| <b>Intraoperative details</b>                   |                            |                            |                        |                  |
| Transplant type                                 |                            |                            |                        | 0.135            |
| SPK                                             | 362 (87)                   | 283 (86)                   | 79 (92)                |                  |
| PAK                                             | 13 (3)                     | 13 (4)                     | 0 (0)                  |                  |
| PTA                                             | 41 (10)                    | 34 (10)                    | 7 (8)                  |                  |
| Duration of surgery, minutes <sup>a</sup>       | 290 (240–330)              | 285 (240–322)              | 304 (257–341)          | <b>0.022</b>     |
| Intraoperative transfusions                     | 78 (19)                    | 60 (19)                    | 18 (21)                | 0.579            |
| <b>Postoperative outcomes</b>                   |                            |                            |                        |                  |
| Postoperative complications, CD ≥ IIIA [10]     | 112 (27)                   | 79 (24)                    | 33 (38)                | <b>0.008</b>     |
| Length of hospital stay, days <sup>a</sup>      | 16 (13–23)                 | 15 (13–21)                 | 20 (14–33)             | <b>&lt;0.001</b> |
| Early relaparotomy <sup>c</sup>                 | 101 (24)                   | 71 (22)                    | 30 (35)                | <b>0.010</b>     |
| Early hemorrhage <sup>c</sup>                   | 88 (21)                    | 65 (20)                    | 23 (27)                | 0.154            |
| Early thrombosis <sup>c</sup>                   | 83 (20)                    | 60 (18)                    | 23 (27)                | 0.077            |
| Early graft explantation (POD 2–30)             | 26 (6)                     | 13 (4)                     | 13 (15)                | <b>&lt;0.001</b> |
| Duodenal leakage                                | 13 (3)                     | 6 (2)                      | 7 (8)                  | <b>0.007</b>     |
| Graft rejection (before discharge) <sup>b</sup> | 10 (3)                     | 8 (3)                      | 2 (2)                  | 1.000            |

BMI, body mass index; CD, Clavien-Dindo; DBD, deceased after brain death; PAK, pancreas after kidney; POD, postoperative day; PTA, pancreas transplant alone; SPK, simultaneous pancreas and kidney. Bold values indicate statistical significance.

<sup>a</sup>Expressed as median (IQR).

<sup>b</sup>Missing data: CIT *n* = 12, anastomotic time *n* = 169, graft rejection before discharge *n* = 10.

<sup>c</sup>Complications defined as early when occurring within 30 days after surgery.

analyzed as a continuous log-transformed outcome (adjusted exp [β], 1.301; 95% CI, 1.166–1.451; *P* < 0.001 – [Supplementary Table 3](#)).

In a sensitivity analysis restricted to the 371 patients who underwent postoperative CE-CT, severe postoperative complications occurred in 33/86 patients with PTAP (38%) and

in 67/285 patients without PTAP (23%; *P* = 0.008). Median hospital stay was longer in patients with PTAP than in those without PTAP (20 days [IQR, 14–33] vs. 15 days [IQR, 13–22]; *P* < 0.001). On multivariable analysis, PTAP remained independently associated with severe postoperative complications (OR, 2.362; 95% CI, 1.369–

TABLE 2 Univariable and multivariable analyses assessing the determinants of severe postoperative complications (n = 404).

| Variable                               | Univariable |             |              | Multivariable <sup>a</sup> |             |              |
|----------------------------------------|-------------|-------------|--------------|----------------------------|-------------|--------------|
|                                        | OR          | 95% CI      | p            | OR                         | 95% CI      | p            |
| Recipient sex, female                  | 0.865       | 0.558–1.340 | 0.515        |                            |             |              |
| Recipient age, years                   | 0.983       | 0.957–1.009 | 0.188        |                            |             |              |
| Recipient BMI, kg/m <sup>2</sup>       | 0.996       | 0.939–1.056 | 0.885        |                            |             |              |
| Recipient diabetes type, type I        | 0.859       | 0.218–3.382 | 0.828        |                            |             |              |
| Recipient on dialysis                  | 1.094       | 0.675–1.772 | 0.716        |                            |             |              |
| Donor sex, female                      | 0.904       | 0.576–1.418 | 0.660        |                            |             |              |
| Donor age, years                       | 1.002       | 0.981–1.024 | 0.863        |                            |             |              |
| Donor BMI, kg/m <sup>2</sup>           | 1.044       | 0.970–1.123 | 0.252        |                            |             |              |
| Donor type, DCD                        | 1.362       | 0.335–5.542 | 0.666        |                            |             |              |
| Cold ischemia time, minutes            | 0.998       | 0.996–1.000 | <b>0.049</b> | –                          | –           | –            |
| Anastomotic time, minutes <sup>b</sup> | 1.014       | 0.992–1.036 | 0.221        |                            |             |              |
| Transplant type, PAK/PTA               | 1.327       | 0.712–2.473 | 0.373        |                            |             |              |
| Previous PT                            | 0.899       | 0.239–3.383 | 0.875        |                            |             |              |
| Intraoperative transfusions            | 0.541       | 0.290–1.011 | 0.054        |                            |             |              |
| PTAP                                   | 1.970       | 1.192–3.258 | <b>0.008</b> | 2.482                      | 1.432–4.305 | <b>0.001</b> |

BMI, body mass index; CI, confidence interval; DCD, deceased after circulatory death; OR, odds ratio; PAK, pancreas after kidney; PT, pancreas transplant; PTA, pancreas transplant alone; PTAP, post-transplant acute pancreatitis. Bold values indicate statistical significance.

<sup>a</sup>Multivariable analysis adjusted for center.

<sup>b</sup>Available for n = 247 patients.

4.077;  $P = 0.002$ ) and prolonged hospital stay (OR, 3.767; 95% CI, 2.140–6.632;  $P < 0.001$ ).

## Outcomes according to biochemical and radiological PTAP components

To further characterize the relative contribution of sustained POH and radiological GP to postoperative outcomes, a four-group comparison was performed among patients who underwent postoperative CE-CT. Overall, 142 (38%) patients had neither sustained POH nor radiological GP, 85 (23%) had sustained POH without radiological GP, 58 (16%) had radiological GP without sustained POH, and 86 (23%) fulfilled the PTAP definition. CE-CT timing was comparable across groups ( $P = 0.442$ ). Later serum pancreatic amylase trajectories (POD3, POD5, and POD10), normalized to center-specific ULN, differed significantly across groups ( $P < 0.001$ ), with the highest median values observed in patients with sustained POH, either with or without radiological GP. Patients with radiological GP only did not show a delayed median enzyme rise above the ULN.

Patients with radiological GP without sustained POH showed intermediate outcomes, with rates of severe postoperative complications (26%) and hospital stay (median 18 days; IQR, 13–28) closer to those observed in the no POH/no radiological GP and POH-only groups than to those observed in patients fulfilling the combined PTAP definition (severe complications, 38%; median LOS, 20 days). The full four-group comparison is reported in [Supplementary Table 4](#).

## Long-term outcomes

After a median follow-up of 51 months (IQR, 24–87), graft loss occurred in 85 patients (20%), and 30 patients (7%) died. Death-censored pancreas GS was significantly worse in patients with PTAP than in those without PTAP ( $P < 0.001$ ). Three-year death-censored pancreas graft survival was 86% in patients without PTAP and 65% in patients with PTAP ([Figure 2A](#)). Overall pancreas graft rejection during follow-up occurred in 58/416 patients (14%).

Results of univariable and multivariable competing-risk regression analyses for predictors of death-censored pancreas graft loss are reported in [Table 4](#). On multivariable analysis, independent predictors of pancreas graft loss included donor type (DCD; sHR, 6.586; 95% CI, 1.409–30.774;  $P = 0.017$ ), CIT (sHR, 1.004 per minute; 95% CI, 1.001–1.006;  $P = 0.003$ ), transplant type (PAK/PTA; sHR, 2.722; 95% CI, 1.314–5.641;  $P = 0.007$ ), PTAP (sHR, 3.412; 95% CI, 2.005–5.808;  $P < 0.001$ ), early thrombosis (sHR, 2.821; 95% CI, 1.626–4.895;  $P < 0.001$ ), and graft rejection (sHR, 3.975; 95% CI, 2.338–6.616;  $P < 0.001$ ).

To further characterize death-censored pancreas graft loss, timing, associated graft-related events, and main causes of graft loss were assessed according to PTAP status ([Supplementary Table 5](#)). Among patients experiencing graft loss ( $n = 85$ ), median time to graft loss was shorter in patients with PTAP than in those without PTAP (1.0 months [IQR, 0–7.5] vs. 9.3 months [IQR, 1–41];  $P = 0.015$ ). Early graft explantation between POD2 and POD30 was more frequent among patients with PTAP (13/31, 42%) than among those without PTAP (13/54, 24%), although this

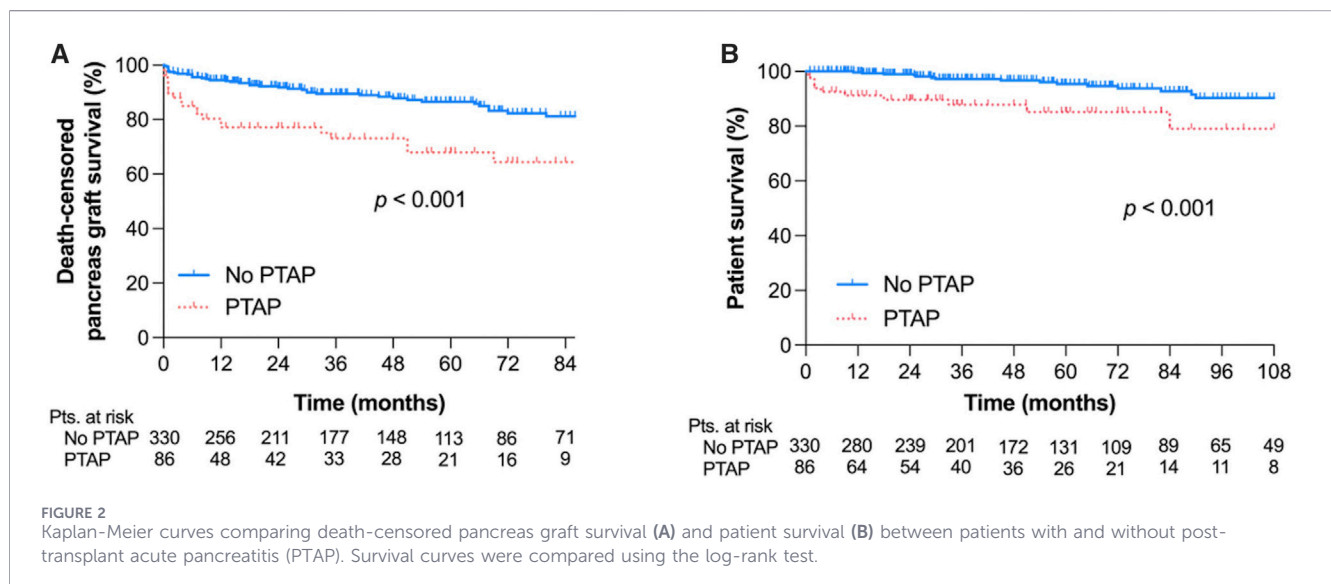
TABLE 3 Univariable and multivariable analyses assessing the determinants of prolonged length of hospital stay (&gt;23 days) (n = 395).

| Variable                               | Univariable |              |                  | Multivariable <sup>a</sup> |              |                  |
|----------------------------------------|-------------|--------------|------------------|----------------------------|--------------|------------------|
|                                        | OR          | 95% CI       | p                | OR                         | 95% CI       | p                |
| Recipient sex, female                  | 0.813       | 0.514–1.288  | 0.378            |                            |              |                  |
| Recipient age, years                   | 0.981       | 0.954–1.008  | 0.167            |                            |              |                  |
| Recipient BMI, kg/m <sup>2</sup>       | 0.974       | 0.915–1.036  | 0.399            |                            |              |                  |
| Recipient diabetes type, type I        | 0.717       | 0.182–2.828  | 0.635            |                            |              |                  |
| Recipient on dialysis                  | 1.281       | 0.766–2.143  | 0.344            |                            |              |                  |
| Donor sex, female                      | 1.294       | 0.815–2.055  | 0.274            |                            |              |                  |
| Donor age, years                       | 1.019       | 0.996–1.042  | 0.104            |                            |              |                  |
| Donor BMI, kg/m <sup>2</sup>           | 1.014       | 0.940–1.094  | 0.714            |                            |              |                  |
| Donor type, DCD                        | 2.647       | 0.697–10.057 | 0.153            |                            |              |                  |
| Cold ischemia time, minutes            | 0.998       | 0.996–1.000  | <b>0.044</b>     | –                          | –            | –                |
| Anastomotic time, minutes <sup>b</sup> | 1.005       | 0.989–1.021  | 0.560            |                            |              |                  |
| Transplant type, PAK/PTA               | 0.522       | 0.237–1.147  | 0.106            |                            |              |                  |
| Previous PT                            | 0.966       | 0.261–3.582  | 0.959            |                            |              |                  |
| Intraoperative transfusions            | 0.549       | 0.283–1.067  | 0.549            |                            |              |                  |
| PTAP                                   | 2.887       | 1.733–4.809  | <b>&lt;0.001</b> | 3.817                      | 2.146–6.792  | <b>&lt;0.001</b> |
| Graft rejection (before discharge)     | 13.511      | 2.819–64.764 | <b>0.001</b>     | 16.662                     | 3.063–90.621 | <b>0.001</b>     |

BMI, body mass index; CI, confidence interval; DCD, deceased after circulatory death; OR, odds ratio; PAK, pancreas after kidney; PT, pancreas transplant; PTA, pancreas transplant alone; PTAP, post-transplant acute pancreatitis. Bold values indicate statistical significance.

<sup>a</sup>Multivariable analysis adjusted for center.

<sup>b</sup>Available for n = 247 patients.



difference did not reach statistical significance ( $P = 0.085$ ). Pancreas graft rejection before discharge occurred in 10/416 patients (2%), while overall pancreas graft rejection during follow-up occurred in 58/416 patients (14%). The distribution of graft-loss causes differed according to PTAP status ( $P = 0.036$ ), with early postoperative complication-related graft loss accounting for a larger proportion of

events among patients with PTAP, whereas rejection-related graft loss was more common among patients without PTAP.

To assess whether the association between PTAP and graft loss was driven by very early technical failures, a sensitivity analysis was performed after excluding patients who underwent early graft explantation between POD2 and POD30. In Kaplan-Meier



TABLE 4 Univariable and multivariable Fine-Gray competing risks regression analyses assessing the determinants of death-censored pancreas graft survival after pancreas transplantation (n = 395).

| Variable                               | Univariable |              |                  | Multivariable <sup>a</sup> |              |                  |
|----------------------------------------|-------------|--------------|------------------|----------------------------|--------------|------------------|
|                                        | sHR         | 95% CI       | p                | sHR                        | 95% CI       | p                |
| Recipient sex, female                  | 1.042       | 0.683–1.591  | 0.848            |                            |              |                  |
| Recipient age, years                   | 1.006       | 0.984–1.030  | 0.581            | –                          | –            | –                |
| Recipient BMI, kg/m <sup>2</sup>       | 0.990       | 0.936–1.049  | 0.743            |                            |              |                  |
| Recipient diabetes type, type I        | 2.240       | 0.294–17.055 | 0.436            |                            |              |                  |
| Recipient on dialysis <sup>b</sup>     | 0.583       | 0.394–0.863  | <b>0.007</b>     |                            |              |                  |
| Donor type, DCD                        | 1.759       | 0.441–7.023  | 0.424            | 6.586                      | 1.409–30.774 | <b>0.017</b>     |
| Donor sex, female                      | 0.993       | 0.638–1.544  | 0.974            |                            |              |                  |
| Donor age, years                       | 0.989       | 0.966–1.012  | 0.343            | –                          | –            | –                |
| Donor BMI, kg/m <sup>2</sup>           | 0.949       | 0.876–1.029  | 0.207            |                            |              |                  |
| Cold ischemia time, minutes            | 1.002       | 1.001–1.004  | <b>0.004</b>     | 1.004                      | 1.001–1.006  | <b>0.003</b>     |
| Anastomotic time, minutes <sup>c</sup> | 1.011       | 0.996–1.025  | 0.153            |                            |              |                  |
| Intraoperative transfusions            | 0.992       | 0.593–1.659  | 0.976            |                            |              |                  |
| Transplant type, PAK/PTA               | 2.791       | 1.780–4.378  | <b>&lt;0.001</b> | 2.722                      | 1.314–5.641  | <b>0.007</b>     |
| Previous PT                            | 0.951       | 0.280–3.232  | 0.936            |                            |              |                  |
| PTAP                                   | 2.580       | 1.654–4.025  | <b>&lt;0.001</b> | 3.412                      | 2.005–5.808  | <b>&lt;0.001</b> |
| Early thrombosis <sup>d</sup>          | 2.904       | 1.850–4.558  | <b>&lt;0.001</b> | 2.821                      | 1.626–4.895  | <b>&lt;0.001</b> |
| Early hemorrhage <sup>d</sup>          | 1.021       | 0.597–1.746  | 0.939            |                            |              |                  |
| Rejection                              | 3.552       | 2.305–5.473  | <b>&lt;0.001</b> | 3.975                      | 2.338–6.616  | <b>&lt;0.001</b> |

BMI, body mass index; CI, confidence interval; DCD, deceased after circulatory death; sHR, subdistribution hazard ratio; PAK, pancreas after kidney; PT, pancreas transplant; PTA, pancreas transplant alone; PTAP, post-transplant acute pancreatitis. Bold values indicate statistical significance.

<sup>a</sup>Multivariable analysis adjusted for center.

<sup>b</sup>Not included in the multivariable model due to collinearity with transplant type.

<sup>c</sup>Available for n = 247 patients.

<sup>d</sup>Complications defined as early when occurring within 30 days after surgery.

analysis, death-censored pancreas graft survival remained significantly worse in patients with PTAP ( $P = 0.003$ ). In a center-adjusted competing-risk sensitivity analysis, PTAP remained associated with a higher cumulative incidence of pancreas graft loss (sHR, 1.964; 95% CI, 1.087–3.550;  $P = 0.025$ ).

Patient survival was also significantly worse among patients with PTAP ( $P < 0.001$ , Figure 2B).

## Radiological graft pancreatitis and predictors of PTAP

Overall, 371 patients (89%) underwent at least one CE-CT scan during the postoperative period, at a median of POD8 (IQR, 4–10). Among these patients, 171 (45%) had sustained POH. Radiological signs consistent with GP were identified in 144 patients (35%), of whom 86 met the biochemical criterion for sustained POH and were therefore classified as having PTAP.

Serum amylase levels on POD1 and POD2, expressed as multiples of the upper limit of normal (ULN), were significantly higher in patients with radiological GP than in the remainder of the cohort ( $P < 0.001$ , Figure 3). The discriminatory performance of

POD1 and POD2 amylase levels for predicting radiological GP was modest, with an AUC of 0.630 (95% CI, 0.570–0.690;  $P < 0.001$ ) and 0.648 (95% CI, 0.589–0.708;  $P < 0.001$ ), respectively (Supplementary Figure 1). Optimal cut-off values were 3.25×ULN on POD1 and 2.25×ULN on POD2. Radiological GP was present in 74% of patients (37/50) who exceeded both these thresholds, compared with 33% of those below at least one threshold ( $P < 0.001$ ).

Determinants of PTAP were further explored using logistic regression analyses (Supplementary Table 6). On multivariable analysis, CIT was the only independent determinant of PTAP (OR, 1.004 per minute; 95% CI 1.002–1.006;  $P < 0.001$ ).

## Discussion

In this multicenter study, the ISGPS definition of PPAP [7] was adapted to the setting of pancreas transplantation to define post-transplant acute pancreatitis (PTAP) and assess its clinical relevance in a large cohort of PT recipients. PTAP was identified in approximately 1 in 5 patients and was consistently associated with increased postoperative morbidity, prolonged

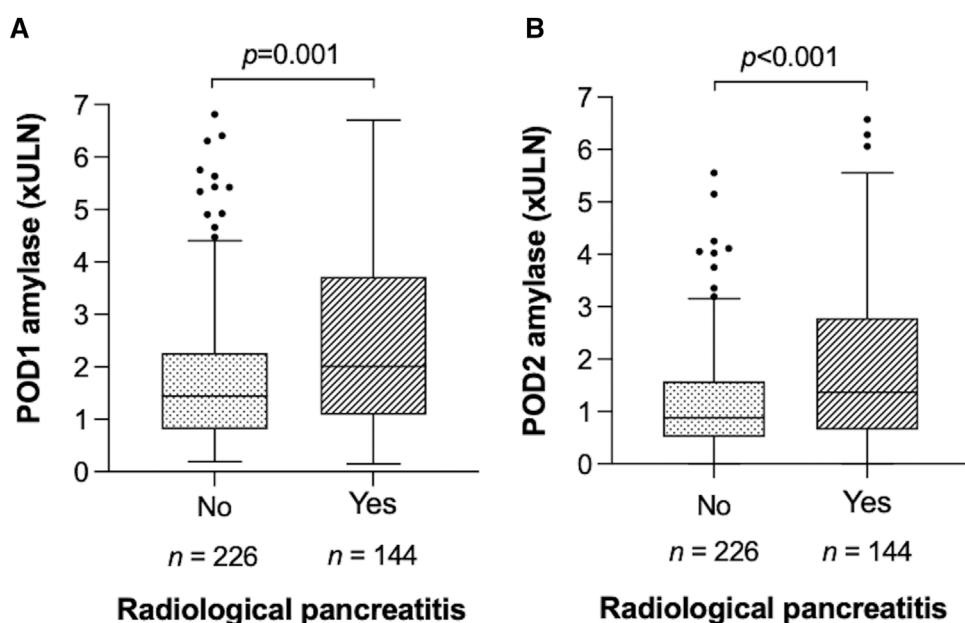


**FIGURE 3**  
Comparison of serum amylase levels on postoperative day (POD) 1 (A) and POD 2 (B), expressed as multiples of the laboratory upper limit of normal (ULN), between patients with and without radiological evidence of graft pancreatitis. Box plots represent median and interquartile range, with whiskers according to Tukey (1.5×IQR). Black dots indicate outliers.

hospitalization, and worse long-term graft survival. These findings suggest that PTAP represents a clinically meaningful manifestation of early graft injury rather than a benign biochemical epiphenomenon. Importantly, applying the ISGPS framework to the transplant setting does not imply shared pathophysiological mechanisms between PTAP and PPAP, but rather the use of a standardized, outcome-oriented approach to identify clinically relevant perioperative pancreatic graft injury.

Although the present study does not support specific changes in perioperative management, the findings may have important implications for clinical decision-making after PT. Identification of PTAP using objective biochemical and radiological criteria may enable early risk stratification of patients at increased risk for major postoperative complications and graft loss. In this context, PTAP may be viewed as a warning signal prompting closer clinical surveillance and timely imaging, while potentially informing individualized postoperative drain management rather than serving as a trigger for predefined therapeutic interventions.

GP after PT has long been recognized as a relevant complication, but its reported incidence and clinical significance have varied markedly across studies. This variability largely reflects the absence of a standardized and validated definition [5, 12], with previous reports relying on heterogeneous combinations of clinical symptoms, enzyme elevations, imaging findings, and pathological features [4, 13–16]. As highlighted by a recent scoping review [5], reported rates of GP range from 0% to 87%, reflecting both true inter-center variability (i.e. differences in donor characteristics, CIT, operative complexity, and perioperative factors) and the lack of a universally accepted definition. This heterogeneity has limited comparability across centers and hindered both clinical decision-making and research in the field. Established definitions of native

acute pancreatitis are not directly applicable to the transplant setting because of the unique perioperative and immunological milieu of PT. Early graft injury is strongly influenced by ischemia-reperfusion damage [17, 18], microvascular dysfunction, denervation, and immunosuppression-related factors, all of which may lead to pancreatic enzyme release without overt inflammatory sequelae. Accordingly, POH is common after PT and has often been regarded as a benign biochemical finding [17, 19]. However, increasing evidence suggests that persistent enzyme elevation may identify a subset of patients at increased risk of clinically relevant complications [15, 20–22].

In pancreatic surgery, the ISGPS has recently proposed and validated a standardized definition of PPAP [7, 8] based on sustained POH and radiological evidence of pancreatitis. This framework was developed to distinguish clinically relevant pancreatitis from isolated biochemical abnormalities and has shown prognostic significance in surgical cohorts. Although PPAP arises in a different clinical context, its conceptual structure, based on objective biochemical and imaging criteria, appears well suited to the perioperative phase of PT. In the present study, this framework was adapted to the transplant setting by applying these two objective components [7] while intentionally excluding the clinical management criterion. This choice was made to avoid incorporation bias, because changes in postoperative management are intrinsically linked to short-term outcomes, which were primary study endpoints. By relying exclusively on biochemical and radiological criteria, we sought to preserve the independence between exposure and outcome assessment.

Using this adapted framework, PTAP was diagnosed in 21% of patients. PTAP was the only independent predictor of severe postoperative complications and was strongly associated with

prolonged length of stay. These findings indicate that PTAP identifies a subgroup of patients with clinically significant early graft injury and tangible consequences for postoperative recovery and resource utilization. PTAP was also independently associated with death-censored graft loss, with early separation of survival curves after transplantation. Although the association between PTAP and death-censored pancreas graft survival persisted after excluding very early graft failures requiring graft explantation between POD2 and POD30, this finding should be interpreted as prognostic rather than causal. Indeed, PTAP and other early postoperative complications, including thrombosis, rejection and graft exocrine leakage, may occur within the same postoperative trajectory, and their temporal and biological relationships cannot be fully disentangled in a retrospective study. Taken together, these observations suggest that PTAP likely captures a severe phenotype of early graft injury associated with a higher subsequent risk of graft failure, rather than a transient inflammatory process without longer-term implications.

Postoperative imaging played a central role in confirming the diagnosis of PTAP. In this cohort, CE-CT was performed in most patients on the basis of clinical indication rather than systematically. Although this approach may have led to underdiagnosis of mild or subclinical PTAP, it reflects real-world clinical practice and likely enriched the analysis for clinically meaningful cases. Interpretation of early postoperative imaging remains challenging, as inflammatory changes may overlap with expected postoperative findings or coexist with other graft-related complications, including thrombosis and rejection. Nevertheless, in the presence of sustained POH, radiological findings such as graft edema or peripancreatic fluid collections should raise suspicion for PTAP and prompt closer clinical surveillance [23].

Serum amylase levels on postoperative days 1 and 2 were significantly higher in patients with radiological GP, supporting their role as an early risk signal. However, the modest discriminatory performance observed in ROC analyses indicates that amylase levels alone should not be considered diagnostic for PTAP. Rather, persistent enzyme elevation may serve as an early warning marker to identify patients who may benefit from targeted imaging and closer monitoring during the postoperative course. It should also be acknowledged that POH may arise from causes other than GP, including duodenal leakage, although this complication was uncommon in the present cohort.

Although the etiological context of GP after PT differs from that of post-pancreatectomy acute pancreatitis, key downstream features, including parenchymal edema, inflammatory activation, and microcirculatory impairment, are conceptually translatable across settings. The four-group comparison highlighted the clinical relevance of combining biochemical and radiological criteria: patients fulfilling the PTAP definition had the highest burden of postoperative morbidity and the longest hospital stay, whereas those with either sustained POH alone or radiological GP alone showed a less pronounced clinical impact. Radiological abnormalities in the absence of sustained POD1-2 hyperamylasemia may therefore identify a different, less clinically severe phenotype. Consistently, postoperative enzyme trajectories further showed that patients with sustained POH had median serum pancreatic amylase values above the center-specific ULN up to POD10, whereas patients without sustained POH, including those with radiological GP alone, did

not show a delayed median enzyme rise above the ULN. This suggests that sustained early POH may represent an early marker of clinically relevant graft injury rather than a merely transient postoperative enzyme elevation. Overall, the adapted ISGPS-based framework may represent a candidate operational definition for clinically meaningful GP after PT.

Identification of potentially modifiable risk factors for PTAP is of particular clinical interest. In the present study, prolonged CIT emerged as the only independent determinant of PTAP among the variables uniformly available for analysis. Although the OR per minute appears numerically small, the cumulative effect over prolonged ischemic intervals is likely to be clinically meaningful. This finding is consistent with a potential role of ischemia-reperfusion injury in the pathogenesis of PTAP and with experimental and clinical evidence showing impaired graft microcirculation, inflammatory activation, and tissue edema after prolonged cold preservation [24]. However, this association should be interpreted cautiously. CIT likely reflects not only cold ischemic injury itself, but also preservation-related, procurement-related, and logistical factors. Data on additional donor characteristics, procurement distance, detailed transport logistics, and pancreas procurement time were not available with sufficient completeness across centers, and residual confounding from these factors cannot be excluded. Future prospective studies should clarify whether strategies aimed at reducing ischemia-preservation burden can decrease the risk of PTAP.

Several limitations should be acknowledged. First, the retrospective design introduces the potential for selection and information bias. Second, patients requiring graft pancreatectomy within the first postoperative day were necessarily excluded because sustained hyperamylasemia could not be assessed. As these cases may represent the most severe end of the early GP spectrum, including ischemia-reperfusion-related pancreatitis, their exclusion may have led to underestimation of the incidence of severe early GP after PT. However, retrospective pathological distinction between ischemia-reperfusion-related pancreatitis and thrombosis-related ischemic injury would be unreliable. Third, postoperative imaging was not performed systematically in all patients, which may have resulted in underdiagnosis of mild disease and differential ascertainment across centers and time periods. In particular, patients with sustained POH who did not undergo CE-CT could not be definitively classified with respect to radiological GP and may have included mild or subclinical cases. However, CE-CT was performed in most patients, and the association between PTAP and the main postoperative outcomes remained consistent in a sensitivity analysis restricted to patients who underwent postoperative CE-CT. In addition, postoperative CE-CT scans were interpreted locally as part of routine clinical care, and no central blinded radiological review was performed. Therefore, prospective validation using standardized imaging protocols is warranted. Fourth, radiological findings consistent with pancreatitis may overlap with other early graft complications, such as thrombosis, acute graft rejection, or graft exocrine leakage, and causal relationships between PTAP and associated outcomes cannot be definitively established. We also did not retrospectively classify clinically relevant management changes as attributable to PTAP. In the early postoperative course after PT, management decisions are frequently driven by overlapping complications. Because no standardized definition of GP was

available at the time of clinical management, attributing specific therapeutic decisions retrospectively to PTAP would have been prone to misclassification and circular reasoning. For this reason, the adapted definition was intentionally restricted to objective biochemical and radiological criteria. In addition, serum lipase levels, complete enzyme kinetics beyond the first 48 postoperative hours, and drain amylase measurements were not systematically available across centers. Finally, perioperative management strategies, including anticoagulation protocols, varied across centers and over time. PTAP rates were stable across transplant eras, but center-level differences were observed and may reflect differences in surgical technique, imaging strategies, postoperative management, case mix, and unmeasured recipient-related factors, including vascular complexity. Although adjustment for center-specific effects was performed to mitigate the impact of this heterogeneity, residual confounding related to center-specific management strategies cannot be excluded.

In conclusion, an adapted ISGPS-based definition identified PTAP as a frequent and clinically meaningful complication after PT. PTAP identifies a subgroup of patients with clinically relevant early graft injury and was associated with major postoperative complications, prolonged hospitalization, and impaired pancreas graft survival, supporting its role as a prognostic marker rather than establishing a direct causal relationship. Standardized diagnostic criteria may improve recognition of clinically meaningful GP, support closer clinical surveillance and early imaging, facilitate comparisons across transplant programs, and provide a foundation for prospective studies aimed at refining diagnostic thresholds and informing postoperative management. Given the retrospective design, non-systematic imaging, and potential overlap with other early graft-related complications, prospective validation using standardized imaging protocols, prespecified CT timing and central radiological adjudication is warranted.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the corresponding author upon reasonable request, in anonymized form and in accordance with applicable privacy and ethical regulations.

## Ethics statement

The requirement of ethical approval was waived by IRCCS San Raffaele Hospital, Padua University Hospital, Hôpital Universitaire Paul Brousse for the studies involving humans because of the retrospective observational design and use of anonymized routinely

collected clinical data. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board also waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because of the retrospective observational design and use of anonymized routinely collected clinical data.

## Author contributions

SP, VA, OC, RC, PR, LP, LF, and MF contributed to research conception and design. MS, OC, AB, VT, DC, AI, CDB, GP, CS, and GS contributed to data acquisition. SP, VA, OC, VT, CDB, PR, ASC, LF, and MF contributed to data interpretation. VA, LF, and MF contributed to data analysis. SP and VA drafted 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

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2026.16849/full#supplementary-material>

## References

- Manrique A, Jiménez C, López RM, Cambra F, Morales JM, Andrés A, et al. Relaparotomy after pancreas transplantation: causes and outcomes. *Transpl Proc* (2009) 41(6):2472–4. doi: 10.1016/j.transproceed.2009.06.165
- Nadalin S, Girotti P, Königsrainer A. Risk factors for and management of graft pancreatitis. *Curr Opin Organ Transpl* (2013) 18(1):89–96. doi: 10.1097/MOT.0b013e32835c6f0f
- Benz S, Pfeffer F, Adam U, Schareck W, Hopt UT. Impairment of pancreatic microcirculation in the early reperfusion period during simultaneous pancreas-kidney transplantation. *Transpl Int* (1998) 11(Suppl. 1):S433–435. doi: 10.1007/s001470050515
- Alonso D, Dunn TB, Rigley T, Skorupa JY, Schriener ME, Wrenshall LE, et al. Increased pancreatitis in allografts flushed with histidine-tryptophan-ketoglutarate

- solution: a cautionary tale. *Am J Transpl* (2008) 8(9):1942–5. doi: 10.1111/j.1600-6143.2008.02312.x
5. Partelli S, Andreasi V, Tomajer V, Tamburrino D, Caldara R, Rigotti P, et al. Exploring definitions of graft pancreatitis following pancreas transplantation: a scoping review. *Transpl Rev (Orlando)* (2024) 38(4):100861. doi: 10.1016/j.trre.2024.100861
6. Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, et al. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. *Gut* (2013) 62(1):102–11. doi: 10.1136/gutjnl-2012-302779
7. Marchegiani G, Barreto SG, Bannone E, Sarr M, Vollmer CM, Connor S, et al. Postpancreatectomy acute pancreatitis (PPAP): definition and grading from the international study group for pancreatic surgery (ISGPS). *Ann Surg* (2022) 275(4):663–72. doi: 10.1097/SLA.0000000000005226
8. Bannone E, Cattelan A, Corvino G, Marchetti A, Andreasi V, Ferri F, et al. Clinical and financial validation of the international study group for pancreatic surgery (ISGPS) definition of post-pancreatectomy acute pancreatitis (PPAP): International multicenter prospective study. *Ann Surg* (2024) 284:e12–e21. doi: 10.1097/SLA.0000000000006569
9. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al. The strengthening of reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Int J Surg* (2014) 12(12):1495–9. doi: 10.1016/j.ijsu.2014.07.013
10. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* (2004) 240(2):205–13. doi: 10.1097/01.sla.0000133083.54934.ae
11. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* (1999) 94(446):496–509. doi: 10.1080/01621459.1999.10474144
12. Shin J, Woo HY, Jo EA, Cho A, Han A, Ahn S, et al. Outcomes of pancreas transplantation over two decades: a single-center retrospective cohort study. *Ann Surg Treat Res* (2025) 108(5):271–8. doi: 10.4174/astr.2025.108.5.271
13. Kopp WH, Lam HD, Schaapherder AFM, Huurman VAL, van der Boog PJJ, de Koning EJP, et al. Pancreas transplantation with grafts from donors deceased after circulatory death: 5 years single-center experience. *Transplantation* (2018) 102(2):333–9. doi: 10.1097/TP.0000000000001940
14. Singh RP, Vrakas G, Hayek S, Hayek S, Anam S, Aqueel M, et al. Clinically significant peripancreatic fluid collections after simultaneous pancreas-kidney transplantation. *Transplantation* (2013) 95(10):1263–9. doi: 10.1097/TP.0b013e318289c978
15. Rössler F, Kümmerli C, Hügli S, Jonas JP, Hübel K, Oberkofler CE, et al. Effect of donor pancreas extraction time on pancreas transplantation—a Swiss tertiary center experience. *Clin Transpl* (2023) 37(1):e14846. doi: 10.1111/ctr.14846
16. Connor S. Defining post-operative pancreatitis as a new pancreatic specific complication following pancreatic resection. *HPB* (2016) 18(8):642–51. doi: 10.1016/j.hpb.2016.05.006
17. Perez Daga JA, Perez Rodriguez R, Santoyo J. Immediate post-operative complications (I): post-operative bleeding; vascular origin: thrombosis pancreatitis. *World J Transpl* (2020) 10(12):415–21. doi: 10.5500/wjt.v10.i12.415
18. Prudhomme T, Mulvey JF, Young LAJ, Mesnard B, Lo Faro ML, Ogbemudia AE, et al. Ischemia-reperfusion injuries assessment during pancreas preservation. *Int J Mol Sci* (2021) 22(10):5172. doi: 10.3390/ijms22105172
19. Troppmann C. Complications after pancreas transplantation. *Curr Opin Organ Transpl* (2010) 15(1):112–8. doi: 10.1097/MOT.0b013e3283355349
20. Montiel-Casado MC, Fernández-Burgos I, Pérez-Daga JA, Aranda-Narváez JM, Sánchez-Pérez B, González-Sánchez AJ, et al. Impact of blood amylase peak over vascular graft thrombosis in pancreas transplantation. *Transpl Proc* (2012) 44(9):2627–30. doi: 10.1016/j.transproceed.2012.09.103
21. Bonsdorff A, Sallinen V, Rähä J, Ekstrand A, Nordin A, Lempinen M, et al. First-day plasma amylase detects patients at risk of complications after simultaneous pancreas-kidney transplantation. *Clin Transpl* (2021) 35(4):e14233. doi: 10.1111/ctr.14233
22. Tomihara K, Hisadome Y, Noguchi H, Kaku K, Okabe Y, Nakamura M. Serum pancreatic enzymes in the early postoperative period predict complications associated with pancreatic fluid after pancreas transplantation: a retrospective, single-center, observational cohort study. *J Hepatobiliary Pancreat Sci* (2021) 28(4):365–75. doi: 10.1002/jhbp.895
23. Lieberman JM, Marks WH, Stuart FP, Abecassis MM, Florence LS, Kauffman D, et al. Co-monitoring serum anodal trypsinogen, serum amylase, and serum creatinine accurately differentiates rejection from other causes of allograft dysfunction after simultaneous pancreas-kidney transplantation. *Transpl Proc* (1997) 29(1-2):676–7. doi: 10.1016/s0041-1345(96)00397-1
24. Maglione M, Hermann M, Hengster P, Schneeberger S, Mark W, Obrist P, et al. A novel technique for heterotopic vascularized pancreas transplantation in mice to assess ischemia reperfusion injury and graft pancreatitis. *Surgery* (2007) 141(5):682–9. doi: 10.1016/j.surg.2006.07.037





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# Locus-specific HLA matching and induction therapy in simultaneous pancreas–kidney transplantation: a national UK cohort study

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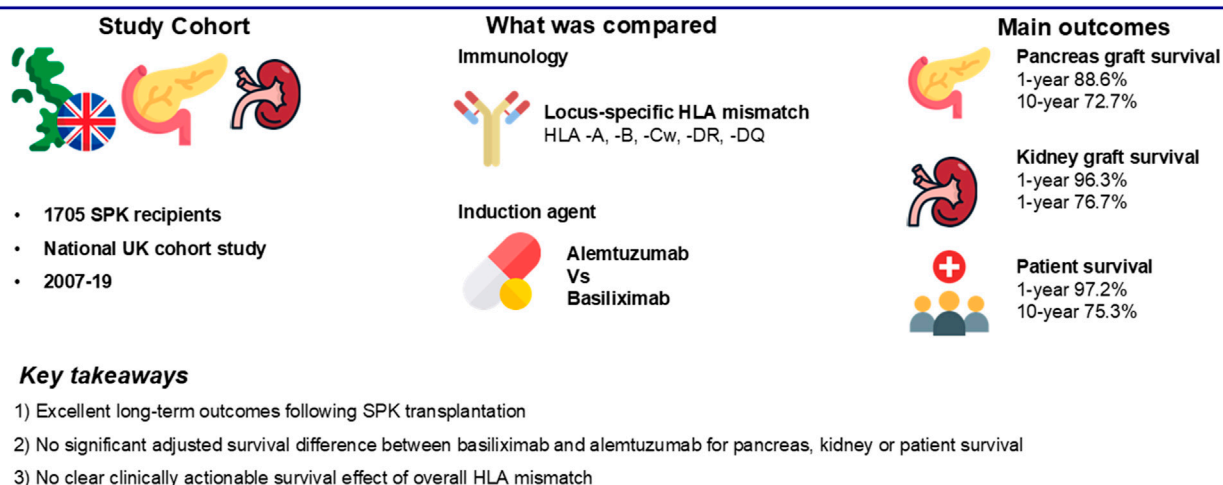
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The impact of HLA mismatch and induction therapy in simultaneous pancreas–kidney transplantation on outcomes remains incompletely defined. We conducted a retrospective cohort study of 1705 SPK recipients transplanted in the UK between 2007 and 2019. Using national transplant registry data, we analysed the impact of locus-specific HLA mismatch and induction therapy (Alemtuzumab vs. Basiliximab) on pancreas graft survival primarily. Kidney graft and patient survival were also analysed as secondary outcomes. Multivariable Cox proportional hazards models were adjusted for donor, recipient, and transplant variables. Pancreas graft survival at 1- and 10-year post-transplant was estimated to be 88.6% and 72.7%, respectively. Kidney graft and patient survival at 10 years were 76.7% and 75.3%. In adjusted analyses, donor age, cold ischaemic time, and HLA-DQ mismatch were significantly associated with pancreas graft loss. No significant survival difference was seen between Alemtuzumab and Basiliximab induction therapy. Induction therapy and HLA-mismatch status were not associated with pancreas graft outcome in this large registry study.

### KEYWORDS

graft failure, HLA mismatch, induction agent, outcomes, simultaneous pancreas–kidney transplantation

## Locus-Specific HLA Matching and Induction Therapy in Simultaneous Pancreas–Kidney Transplantation: A National UK Cohort Study



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GRAPHICAL ABSTRACT

## Introduction

Simultaneous pancreas-kidney (SPK) transplantation is the optimum treatment for selected patients with insulin-dependent diabetes mellitus and end-stage kidney disease, offering superior glycaemic control, renal function and survival compared to dialysis or kidney transplantation alone [1–5]. Recent improvements in immunosuppression, donor and recipient selection, and perioperative care have contributed to enhanced outcomes over recent decades [4, 6–8]. However, long-term pancreas graft survival remains variable, and optimising immunological matching and immunosuppression protocols is warranted to improve graft outcome.

The role of human leucocyte antigen (HLA) mismatching in pancreas transplantation is incompletely understood. HLA matching at the A-, B-, and -DR loci is routine in kidney transplantation [9–11], however the impact of mismatches at the -Cw and -DQ loci is not well studied. Although some studies have suggested that class II mismatches (particularly -DQ) may contribute to *de novo* donor-specific antibody (DSA) development and chronic rejection following deceased donor

kidney transplantation [12–14], such associations are not well-established in SPK transplantation. The clinical significance of individual locus-specific mismatches in SPK graft recipients is uncertain [15–17].

In the UK, organ allocation for SPK transplantation incorporates a HLA mismatch component to favour better-matched donor–recipient pairs. Candidates with fewer HLA mismatches receive higher allocation points, which increases their priority for available organs. The system also accounts for recipient sensitisation, quantified as calculated reaction frequency (cRF, analogous to calculated panel-reactive antibody [cPRA] in the United States), such that highly sensitised patients receive additional priority when a compatible donor becomes available. This scoring framework aims to balance immunological compatibility with equitable access for sensitised recipients.

Induction agents administered at the time of graft implantation vary between centres, with Alemtuzumab (a lymphocyte-depleting agent) and Basiliximab (a non-depleting IL-2 receptor antagonist) the commonest agents used. While both agents are widely used in SPK transplantation, direct comparisons of long-term graft and patient survival are lacking, particularly in relation to HLA-mismatch status.

This cohort study investigated the association between HLA-mismatch status and induction agent in terms of long-term outcome following SPK transplantation in the UK. The aim of this study was to inform immunological risk stratification that may potentially guide future allocation and immunosuppression strategies in SPK transplantation.

**Abbreviations:** CI, Confidence interval; CMV, Cytomegalovirus; cRF, calculated Reaction frequency; cPRA, calculated Panel-reactive antibody; DCD, Donation after circulatory death; DSA, Donor-specific antibody; HLA, Human leucocyte antigen; HR, Hazard ratio; IQR, Interquartile range; SPK, Simultaneous Pancreas-Kidney Transplantation.

## Patients and methods

### Setting

This study used data extracted from the UK Transplant Registry (maintained by NHS Blood and Transplant), following approval from the Pancreas Advisory Group. All adult ( $\geq 18$  years) patients who underwent SPK transplantation in the UK from 1st April 2007 to 31st March 2019 were included. Patients who received a normothermic-regional perfusion preserved graft, recipients who underwent re-transplantation, recipients who received an induction agent other than Alemtuzumab or Basiliximab, and recipients with missing outcome data were excluded. One centre exclusively uses anti-thymocyte globulin as their induction agent, therefore all patients from this centre were excluded as numbers were too few for any meaningful analysis.

### Immunosuppression

Induction immunosuppression was with either Alemtuzumab or Basiliximab, according to local centre protocols. Maintenance immunosuppression typically comprised tacrolimus, mycophenolate mofetil, and corticosteroids (tapered according to centre-specific protocols), but detailed maintenance regimens were not uniformly available in the dataset.

### Definitions and outcomes

Demographic and clinical variables included donor and recipient age, sex, ethnicity, blood group, donor type, cytomegalovirus (CMV) status, cause of death, body mass index, creatinine at retrieval, recipient diabetes aetiology, dialysis modality, sensitisation at transplantation, waiting time to transplant, cold ischaemic time, and transplant era (grouped into three time periods by financial year: 2007–2011, 2011–2015, and 2015–2019). HLA mismatches were recorded separately for each of the following loci: A, B, Cw, DR, and DQ. NHS Blood and Transplant defined HLA mismatch groups was also included for comparison (grouped into three categories: Level 1 & 2 due to small numbers of Level 1 transplants, Level 3 and Level 4, [Supplementary Table S1](#)).

The primary outcome was pancreas graft survival at 10-year post-transplant. Pancreas graft survival at 1-year post-transplant, and additionally kidney graft survival and patient survival at both horizons were analysed as secondary outcomes. Graft survival was defined as the time from transplant to graft failure, censoring for death with a functioning graft. Patient survival was defined as the time from transplant to patient death. Patients lost to follow-up were censored at the date of last known status.

Acute pancreas graft rejection rates at 3- and 12-month post-transplant were obtained. For follow-up which was 'Not reported', it was assumed that no rejection episodes occurred.

### Statistical analysis

Demographic and clinical factors are summarised by induction therapy group to describe the cohort. Categorical variables are presented as counts and percentage, and comparisons between the groups made using Fisher's exact test or Chi-Squared test.

Continuous variables are reported as medians and interquartile range (IQR), and comparisons made using the Student's t-test.

Kaplan-Meier survival analysis was used to estimate the unadjusted survival for each outcome (pancreas graft, kidney graft and patient mortality). Survival curves were stratified by induction therapy group, and log-rank p-values allowed for comparisons between the groups.

Observations with missing data for the outcome or exposure variables were excluded from the analysis. To retain the sample size when multivariable modelling, missing data for the covariates were addressed using simple imputation. For categorical variables, the most frequently occurring category was imputed while continuous variables were imputed using median values. A comparison of this analysis and a complete-case analysis for the primary outcome was performed.

Separate Cox proportional hazards models were built for each survival outcome (pancreas graft, kidney graft, and patient survival). Each model was stratified by transplant centre to account for centre-specific baseline hazard functions. Stepwise backward selection was used, with variables retained if  $p < 0.10$ . Continuous variables were assessed for non-linearity using natural cubic splines and included in that form where appropriate.

A final model was created for each outcome incorporating any covariate found to be significant at any time horizon. The proportional hazards assumption was assessed using cumulative hazard function plots. Model checking involved assessing covariate functional form by Martingale residuals and checking for influential observations using df betas and likelihood displacement. Individual HLA locus models and induction therapy covariates were added to the model to evaluate their significance. The final model for analysis of the primary outcome was assessed for multicollinearity using the Variance Inflation Factor.

Further exploratory analyses of these outcomes at 3 and 5 years were performed to assess consistency with the results at other time horizons; however, these results are not presented in this paper. An interaction term between mismatch at HLA-DQ and induction therapy was included in the multivariable model for the primary outcome to assess potential effect modification. A 1-month landmark survival analysis was performed for the unadjusted pancreas graft survival, excluding any patients who died or were censored within the first month of transplantation.

All analyses were performed using SAS Enterprise Guide version 7.13 (SAS Institute Inc., Cary, NC).

## Results

### Demographics, induction agent and HLA-mismatch status

During the study period, 1897 adult patients underwent first-time SPK transplantation. The final analysis cohort consisted of 1705 SPK graft recipients after excluding recipients transplanted with a graft preserved normothermic regional perfusion ( $n = 26$ ), recipients who received an induction agent other than Alemtuzumab or Basiliximab ( $n = 142$ ), and recipients with missing outcome data ( $n = 24$ ).

TABLE 1 Donor and recipient demographics for adult first time simultaneous pancreas and kidney transplants performed in the UK between 1st April 2007 and 31st March 2019.

| Factor                                  | Total (n = 1705) | Alemtuzumab (n = 1,196) | Basiliximab (n = 509) | P-value |
|-----------------------------------------|------------------|-------------------------|-----------------------|---------|
| <b>Donor</b>                            |                  |                         |                       |         |
| Transplant era (n, %)                   |                  |                         |                       | <0.0001 |
| 1 Apr 2007–31 Mar 2011                  | 564 (33.1%)      | 361 (30.2%)             | 203 (39.9%)           |         |
| 1 Apr 2011–31 Mar 2015                  | 612 (35.9%)      | 424 (35.5%)             | 188 (36.9%)           |         |
| 1 Apr 2015–31 Mar 2019                  | 529 (31.0%)      | 411 (34.4%)             | 118 (23.2%)           |         |
| Donor type (n, %)                       |                  |                         |                       | 0.025   |
| DBD                                     | 1,385 (81.2%)    | 955 (79.9%)             | 430 (84.5%)           |         |
| DCD                                     | 320 (18.8%)      | 241 (20.1%)             | 79 (15.5%)            |         |
| Donor age (years)                       |                  |                         |                       | <0.0001 |
| Median (IQR)                            | 36 (23–47)       | 38 (24–48)              | 34 (22–44)            |         |
| Donor BMI (kg/m <sup>2</sup> )          |                  |                         |                       | 0.091   |
| Median (IQR)                            | 23.5 (21.4–25.9) | 23.6 (21.5–26.0)        | 23.2 (21.2–25.5)      |         |
| Not reported (n, %)                     | 7 (0.4%)         | 4 (0.3%)                | 3 (0.6%)              |         |
| Donor CMV status (n, %)                 |                  |                         |                       | 0.591   |
| Positive                                | 966 (56.7%)      | 683 (57.1%)             | 283 (55.6%)           |         |
| Negative                                | 721 (42.3%)      | 501 (41.9%)             | 220 (43.2%)           |         |
| Not reported                            | 18 (1.1%)        | 12 (1.0%)               | 6 (1.2%)              |         |
| Cold ischaemic time (hours)             |                  |                         |                       | <0.0001 |
| Median (IQR)                            | 11.0 (9.5–13.0)  | 10.6 (9.2–12.5)         | 12.0 (10.3–14.0)      |         |
| Not reported (n, %)                     | 88 (5.2%)        | 69 (5.8%)               | 19 (3.7%)             |         |
| <b>Recipient</b>                        |                  |                         |                       |         |
| Recipient age (years)                   |                  |                         |                       | 0.005   |
| Median (IQR)                            | 43 (36–49)       | 43 (36–49)              | 42 (35–48)            |         |
| Recipient sex (n, %)                    |                  |                         |                       | 0.108   |
| Female                                  | 720 (42.2%)      | 706 (59.0%)             | 279 (54.8%)           |         |
| Male                                    | 985 (57.8%)      | 490 (41.0%)             | 230 (45.2%)           |         |
| Recipient blood group (n, %)            |                  |                         |                       | 0.002   |
| O                                       | 739 (43.3%)      | 507 (42.4%)             | 232 (45.6%)           |         |
| A                                       | 717 (42.0%)      | 524 (43.8%)             | 193 (37.9%)           |         |
| B                                       | 189 (11.1%)      | 116 (9.7%)              | 73 (14.3%)            |         |
| AB                                      | 60 (3.5%)        | 49 (4.1%)               | 11 (2.2%)             |         |
| Recipient cause of diabetes (n, %)      |                  |                         |                       | 0.597   |
| Type I                                  | 1,468 (86.1%)    | 1,014 (84.8%)           | 454 (89.2%)           |         |
| Type II                                 | 70 (4.1%)        | 46 (3.9%)               | 24 (4.7%)             |         |
| Not reported                            | 167 (9.8%)       | 136 (11.4%)             | 31 (6.1%)             |         |
| Waiting time to transplantation (years) |                  |                         |                       | 0.479   |
| Median (IQR)                            | 1.16 (0.55–1.71) | 1.17 (0.54–1.71)        | 1.16 (0.55–1.71)      |         |
| Not reported (n, %)                     | 1 (0.1%)         | 0 (0.0%)                | 1 (0.1%)              |         |
| Recipient sensitisation (n, %)          |                  |                         |                       | 0.382   |
| <85%                                    | 1,625 (95.3%)    | 1,136 (95.0%)           | 489 (96.1%)           |         |
| >85%                                    | 80 (4.7%)        | 60 (5.0%)               | 20 (3.9%)             |         |

The donor and recipient demographic factors for the whole cohort and by induction agent are presented in Table 1 (with further data in Supplementary Table S2). Median recipient age was 43 years (IQR 36–49 years), 720 recipients were female (42.2%), and

70 recipients had Type II diabetes (4.1%). At the time of transplantation, 682 recipients were pre-dialysis (40.0%), and 1,019 recipients (59.8%) were on either haemodialysis (n = 627, 36.8%) or peritoneal dialysis (n = 392, 23.0%). In 1,625 recipients

TABLE 2 HLA mismatches in the whole cohort, and by induction agent.

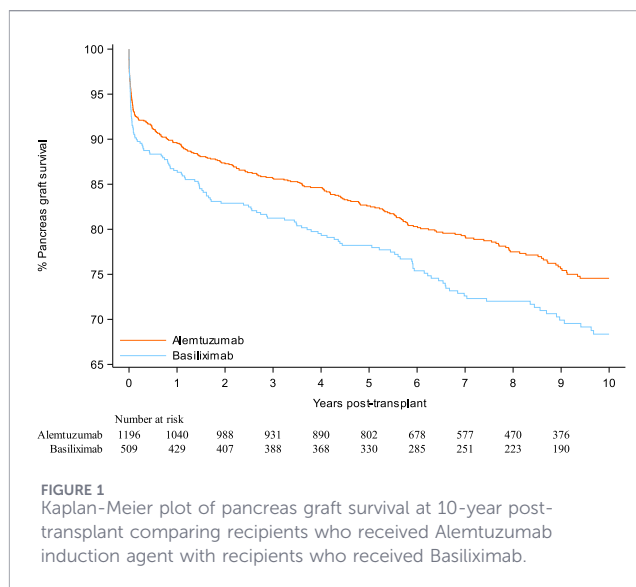
| HLA mismatches            | Total (n = 1705) | Alemtuzumab (n = 1,196) | Basiliximab (n = 509) | P-value |
|---------------------------|------------------|-------------------------|-----------------------|---------|
| HLA-A (n, %)              |                  |                         |                       | 0.311   |
| 0                         | 252 (14.8%)      | 187 (15.6%)             | 65 (12.8%)            |         |
| 1                         | 1,019 (59.8%)    | 707 (59.1%)             | 312 (61.3%)           |         |
| 2                         | 434 (25.5%)      | 302 (25.3%)             | 132 (25.9%)           |         |
| HLA-B (n, %)              |                  |                         |                       | 0.704   |
| 0                         | 77 (4.5%)        | 55 (4.6%)               | 22 (4.3%)             |         |
| 1                         | 804 (47.2%)      | 571 (47.7%)             | 233 (45.8%)           |         |
| 2                         | 824 (48.3%)      | 570 (47.7%)             | 254 (49.9%)           |         |
| HLA-Cw (n, %)             |                  |                         |                       | 0.942   |
| 0                         | 231 (13.6%)      | 163 (13.6%)             | 68 (13.4%)            |         |
| 1                         | 853 (50.0%)      | 595 (49.8%)             | 258 (50.7%)           |         |
| 2                         | 621 (36.4%)      | 438 (36.6%)             | 183 (36.0%)           |         |
| HLA-DQ (n, %)             |                  |                         |                       | 0.248   |
| 0                         | 593 (34.8%)      | 408 (34.1%)             | 185 (36.4%)           |         |
| 1                         | 1,004 (58.9%)    | 705 (59.0%)             | 299 (58.7%)           |         |
| 2                         | 108 (6.3%)       | 83 (6.9%)               | 25 (4.9%)             |         |
| HLA-DR (n, %)             |                  |                         |                       | 0.059   |
| 0                         | 153 (9.0%)       | 95 (7.9%)               | 58 (11.4%)            |         |
| 1                         | 969 (56.8%)      | 694 (58.0%)             | 275 (54.0%)           |         |
| 2                         | 583 (34.2%)      | 407 (34.0%)             | 176 (34.6%)           |         |
| HLA mismatch level (n, %) |                  |                         |                       | 0.243   |
| Level 1 and 2             | 86 (5.0%)        | 54 (4.5%)               | 32 (6.3%)             |         |
| Level 3                   | 550 (32.3%)      | 394 (32.9%)             | 156 (30.7%)           |         |
| Level 4                   | 1,069 (62.7%)    | 748 (62.5%)             | 321 (63.1%)           |         |

(95.3%), sensitisation was less than 85%. The median waiting time to transplantation was 1.16 years (IQR 0.55–1.17 years), and 1,385 recipients received a graft from a brainstem-death donor (81.2%). Alemtuzumab was used as the induction agent in 1,196 recipients (70.1%) compared to 509 recipients who received Basiliximab (29.9%). The proportion of donation after circulatory death (DCD) graft recipients was greater with Alemtuzumab compared to Basiliximab (20.2% vs. 15.5%,  $P = 0.025$ ).

HLA mismatch distribution varied across loci (Table 2). At the HLA-A locus, 14.8% of recipients were fully matched, 59.8% had one mismatch, and 25.5% had two. For HLA-B, most recipients had either one (47.2%) or two mismatches (48.3%), with complete matches in only 4.5%. HLA-Cw matching was similar: 13.6% had no mismatches, 50.0% had one, and 36.4% had two. A single HLA-DQ mismatch occurred in 58.9%, whereas 34.8% had no mismatches and 6.3% had two mismatches. At the HLA-DR locus, 9.0% were fully matched, while 56.8% and 34.2% had one or two mismatches, respectively. Mismatches were similar across induction agent groups.

## Pancreas graft, kidney graft, and patient survival

The unadjusted estimated pancreas, kidney, and patient survival were high over the study period. Pancreas graft survival was 88.6% (95% CI 87.0%–90.0%) at 1-year post-transplant and 72.7% (95% CI 70.1%–75.0%) at 10 years. Kidney graft survival was estimated to be



higher with rates of 96.3% (95% CI 95.3%–97.1%) and 76.7% (95% CI 74.2%–79.1%) at 1 and 10 years respectively. Patient survival followed a similar trend, with estimated survival rates of 97.2% (95% CI 96.2%–97.9%) at 1 year, and 75.3% (95% CI 72.6%–77.8%) at 10 years. Most of the graft losses and deaths occurred in the first year following transplantation, with relative stability thereafter.



TABLE 3 Multivariable Cox regression model estimates for predictors of pancreas graft survival at 1- and 10-year post-transplant.

| Factor                      | One-year pancreas graft failure |              | Ten-year pancreas graft failure |              |
|-----------------------------|---------------------------------|--------------|---------------------------------|--------------|
|                             | HR (95% CI)                     | P-value      | HR (95% CI)                     | P-value      |
| Era of transplant           |                                 | 0.35         |                                 | 0.038        |
| Apr 2007-Mar 2011           | Reference                       |              | Reference                       |              |
| Apr 2011-Mar 2015           | 0.81 (0.56–1.15)                |              | 0.81 (0.64–1.03)                |              |
| Apr 2015-Mar 2019           | 0.76 (0.51–1.13)                |              | 0.70 (0.52–0.93)                |              |
| Donor type                  |                                 | 0.14         |                                 | 0.36         |
| DBD                         | Reference                       |              | Reference                       |              |
| DCD                         | 1.33 (0.91–1.93)                |              | 1.14 (0.87–1.49)                |              |
| Donor age (years)           | 1.03 (1.01–1.04)                | <0.0001      | 1.02 (1.01–1.03)                | <0.0001      |
| Donor CMV status            |                                 | 0.36         |                                 | 0.03         |
| Negative                    | Reference                       |              | Reference                       |              |
| Positive                    | 1.14 (0.86–1.53)                |              | 1.25 (1.02–1.53)                |              |
| Recipient age (years)       | 0.98 (0.96–1.00)                | 0.031        | 0.97 (0.96–0.98)                | <0.0001      |
| Recipient blood group       |                                 | Global 0.37  |                                 | Global 0.059 |
| O                           | Reference                       |              | Reference                       |              |
| A                           | 1.19 (0.88–1.62)                |              | 1.16 (0.94–1.43)                |              |
| B                           | 0.84 (0.49–1.42)                |              | 0.75 (0.52–1.08)                |              |
| AB                          | 0.68 (0.25–1.88)                |              | 0.70 (0.37–1.33)                |              |
| Sensitisation group         |                                 | 0.4          |                                 | 0.22         |
| <85%                        | Reference                       |              | Reference                       |              |
| ≥85%                        | 0.71 (0.33–1.56)                |              | 0.72 (0.42–1.22)                |              |
| Cold ischaemic time (hours) | 1.07 (1.02–1.13)                | 0.005        | 1.05 (1.01–1.08)                | 0.015        |
| HLA-A mismatches            |                                 | Global 0.5   |                                 | Global 0.65  |
| 0                           | Reference                       |              | Reference                       |              |
| 1                           | 1.30 (0.82–2.06)                |              | 1.11 (0.81–1.51)                |              |
| 2                           | 1.16 (0.69–1.95)                |              | 1.18 (0.84–1.65)                |              |
| HLA-B mismatches            |                                 | Global 0.26  |                                 | Global 0.008 |
| 0                           | Reference                       |              | Reference                       |              |
| 1                           | 0.87 (0.44–1.75)                |              | 1.25 (0.74–2.11)                |              |
| 2                           | 0.68 (0.33–1.41)                |              | 0.89 (0.52–1.53)                |              |
| HLA-Cw mismatches           |                                 | Global 0.081 |                                 | Global 0.14  |
| 0                           | Reference                       |              | Reference                       |              |
| 1                           | 1.28 (0.77–2.12)                |              | 1.22 (0.88–1.71)                |              |
| 2                           | 1.71 (1.00–2.91)                |              | 1.42 (0.99–2.03)                |              |
| HLA-DQ mismatches           |                                 | Global 0.33  |                                 | Global 0.033 |
| 0                           | Reference                       |              | Reference                       |              |
| 1                           | 0.79 (0.57–1.09)                |              | 0.75 (0.59–0.94)                |              |
| 2                           | 0.73 (0.36–1.48)                |              | 0.94 (0.60–1.47)                |              |
| HLA-DR mismatches           |                                 | Global 0.76  |                                 | Global 0.52  |
| 0                           | Reference                       |              | Reference                       |              |
| 1                           | 0.97 (0.59–1.61)                |              | 1.10 (0.76–1.59)                |              |

(Continued)

TABLE 3 Continued

| Factor          | One-year pancreas graft failure |         | Ten-year pancreas graft failure |         |
|-----------------|---------------------------------|---------|---------------------------------|---------|
|                 | HR (95% CI)                     | P-value | HR (95% CI)                     | P-value |
| 2               | 0.86 (0.48–1.52)                |         | 0.97 (0.64–1.46)                |         |
| Induction agent |                                 | 0.93    |                                 | 0.91    |
| Alemtuzumab     | Reference                       |         | Reference                       |         |
| Basiliximab     | 0.97 (0.55–1.72)                |         | 0.98 (0.66–1.45)                |         |

Abbreviations: DBD, donor after brainstem death; DCD, donor after circulatory death; CMV, cytomegalovirus; HLA, human leucocyte antigen; HR, hazard ratio; CI, confidence interval.

## Predictors of pancreas graft outcome and acute pancreas graft rejection

Unadjusted pancreas graft survival, based on induction agent, is presented in [Figure 1](#), with some evidence to suggest significantly better 10-year survival ( $p = 0.012$ ) in the Alemtuzumab group (74.6%, 95% CI 71.5%–77.3%) compared to Basiliximab (68.4%, 95% CI 63.6%–72.6%). However, after excluding graft failures that occurred in the first 30 days post-transplant, there was no evidence of a difference in long term pancreas graft survival between the groups, as shown in [Supplementary Table S3](#).

Multivariable modelling of predictors of pancreas graft survival at 1- and 10-year post-transplant are presented in [Table 3](#). Several factors were associated with pancreas graft survival at 10-year. Increasing donor age and longer cold ischaemic time were significantly associated with a higher risk of graft failure ( $p < 0.0001$  and  $p = 0.015$  respectively), and older recipient age was associated with a reduced risk of graft failure ( $p < 0.0001$ ). Donor CMV positivity was associated with increased risk of graft loss when compared to CMV negative donors (HR 1.25, 95% CI 1.02–1.53,  $p = 0.030$ ). A sensitivity analysis for handling missing data is presented in [Supplementary Table S4](#), comparing the 10-year pancreas graft survival model estimates for the simple imputation cohort and a complete-case cohort, but the pattern of results was similar between the cohorts.

Regarding immunological factors, HLA mismatches at most loci were not significantly associated with pancreas graft outcomes. However, one HLA-DQ mismatch was associated with improved long-term pancreas graft survival at 10 years (HR 0.75, 95% CI 0.59–0.94,  $p = 0.033$ ), compared to zero mismatches. This finding was not observed for two DQ mismatches, however the sample size in this category is small (6.3%) presenting high uncertainty in the true effect. HLA-B mismatch status was identified as a predictor of graft loss at 10-year (global  $p = 0.008$ ), however the confidence intervals for one and two mismatches crossed 1.0, therefore the true effect remains uncertain. No significant associations were identified for mismatches at HLA-A, -Cw, or -DR. Induction therapy type (Alemtuzumab vs. Basiliximab) was not significantly associated with pancreas graft survival at 10 years when adjusted for other factors.

An interaction term between mismatch at HLA-DQ and induction therapy was included in the multivariable model for 10-year pancreas graft survival to explore effect modification. There was no evidence of effect modification by induction therapy ( $p = 0.71$ ) ([Supplementary Table S5](#)).

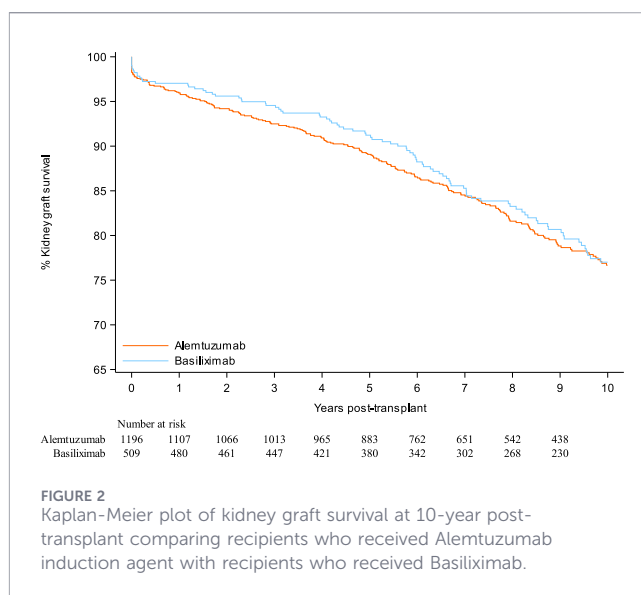


FIGURE 2  
Kaplan-Meier plot of kidney graft survival at 10-year post-transplant comparing recipients who received Alemtuzumab induction agent with recipients who received Basiliximab.

After adjusting for confounders, there were no significant associations between HLA mismatches at any locus and pancreas graft survival at 1 year and induction therapy was also not significantly associated with this outcome, as all estimates were consistent with no effect. At 3-month post-transplantation, there was a non-significant difference in the incidence of at least one acute pancreas graft rejection episode when comparing Alemtuzumab with Basiliximab (6.1% vs. 4.0%,  $p = 0.06$ ). There was no difference when comparing HLA-B mismatch status ( $p = 0.34$ ) or -DQ status ( $p = 0.99$ ). Follow-up data for acute rejection rates at 12 months post-transplant was available for 1,544 (91%) patients, and was no difference when comparing Alemtuzumab with Basiliximab (6.8% vs. 7.5%,  $p = 0.60$ ), HLA-B mismatch status ( $p = 0.17$ ) or -DQ status ( $p = 0.30$ ).

## Predictors of kidney-graft outcome

Unadjusted kidney graft survival is presented in [Figure 2](#), with no evidence to suggest a difference in 10-year graft survival ( $p = 0.601$ ) when comparing Alemtuzumab (76.7%, 95% CI 73.6%–79.4%) with Basiliximab (77.0%, 95% CI 72.3%–81.0%). Predictors of kidney graft survival at 1- and 10-year post-transplant are presented in [Table 4](#). Kidney graft survival improved significantly, with the most recent era (2015–2019) associated with the lowest risk of graft failure at 10 years (HR

TABLE 4 Multivariable Cox regression model estimates for predictors of kidney graft survival at 1- and 10-year post-transplant.

| Factor                                              | One-year kidney graft failure  |               | Ten-year kidney graft failure  |              |
|-----------------------------------------------------|--------------------------------|---------------|--------------------------------|--------------|
|                                                     | HR (95% CI)                    | P-value       | HR (95% CI)                    | P-value      |
| Era of transplant                                   |                                | Global 0.0001 |                                | Global 0.008 |
| Apr 2007-Mar 2011                                   | Reference                      |               | Reference                      |              |
| Apr 2011-Mar 2015                                   | 0.25 (0.12–0.51)               |               | 0.71 (0.54–0.93)               |              |
| Apr 2015-Mar 2019                                   | 0.36 (0.18–0.70)               |               | 0.62 (0.44–0.87)               |              |
| Donor type                                          |                                | 0.4           |                                | 0.39         |
| DBD                                                 | Reference                      |               | Reference                      |              |
| DCD                                                 | 1.35 (0.67–2.71)               |               | 1.15 (0.84–1.57)               |              |
| Donor age (years)                                   | Fitted as natural cubic spline | 0.042         | Fitted as natural cubic spline | <0.0001      |
| Donor creatinine at retrieval ( $\mu\text{mol/L}$ ) | 1.31 (0.85–2.00)               | 0.22          | 1.29 (1.07–1.56)               | 0.008        |
| Donor ethnicity                                     |                                | 0.92          |                                | 0.031        |
| White                                               | Reference                      |               | Reference                      |              |
| Other ethnic minority                               | 0.95 (0.34–2.69)               |               | 1.52 (1.04–2.22)               |              |
| Recipient sex                                       |                                | 0.87          |                                | 0.002        |
| Male                                                | Reference                      |               | Reference                      |              |
| Female                                              | 0.96 (0.56–1.63)               |               | 1.46 (1.16–1.85)               |              |
| Recipient age (years)                               | Fitted as natural cubic spline | 0.13          | Fitted as natural cubic spline | <0.0001      |
| Recipient dialysis status                           |                                | Global 0.15   |                                | Global 0.005 |
| Not on dialysis                                     | Reference                      |               | Reference                      |              |
| Haemodialysis                                       | 1.81 (0.99–3.27)               |               | 1.41 (1.09–1.83)               |              |
| Peritoneal dialysis                                 | 1.48 (0.73–2.99)               |               | 0.89 (0.64–1.23)               |              |
| HLA-A mismatches                                    |                                | Global 0.5    |                                | Global 0.75  |
| 0                                                   | Reference                      |               | Reference                      |              |
| 1                                                   | 1.60 (0.62–4.15)               |               | 1.10 (0.77–1.56)               |              |
| 2                                                   | 1.83 (0.67–5.04)               |               | 1.16 (0.79–1.72)               |              |
| HLA-B mismatches                                    |                                | Global 0.99   |                                | Global 0.66  |
| 0                                                   | Reference                      |               | Reference                      |              |
| 1                                                   | 0.92 (0.25–3.40)               |               | 0.78 (0.46–1.33)               |              |
| 2                                                   | 0.95 (0.25–3.60)               |               | 0.80 (0.46–1.39)               |              |
| HLA-Cw mismatches                                   |                                | Global 0.63   |                                | Global 0.54  |
| 0                                                   | Reference                      |               | Reference                      |              |
| 1                                                   | 0.89 (0.38–2.08)               |               | 0.95 (0.66–1.37)               |              |
| 2                                                   | 1.16 (0.48–2.83)               |               | 1.10 (0.74–1.62)               |              |
| HLA-DQ mismatches                                   |                                | Global 0.96   |                                | Global 0.76  |
| 0                                                   | Reference                      |               | Reference                      |              |
| 1                                                   | 0.97 (0.53–1.78)               |               | 0.91 (0.69–1.20)               |              |
| 2                                                   | 0.87 (0.31–2.46)               |               | 1.00 (0.61–1.63)               |              |
| HLA-DR mismatches                                   |                                | Global 0.34   |                                | Global 0.057 |
| 0                                                   | Reference                      |               | Reference                      |              |
| 1                                                   | 1.17 (0.42–3.32)               |               | 0.92 (0.59–1.41)               |              |
| 2                                                   | 1.76 (0.58–5.34)               |               | 1.26 (0.79–2.02)               |              |

(Continued)

TABLE 4 Continued

| Factor          | One-year kidney graft failure |         | Ten-year kidney graft failure |         |
|-----------------|-------------------------------|---------|-------------------------------|---------|
|                 | HR (95% CI)                   | P-value | HR (95% CI)                   | P-value |
| Induction agent |                               | 0.8     |                               | 0.74    |
| Alemtuzumab     | Reference                     |         | Reference                     |         |
| Basiliximab     | 0.89 (0.36–2.17)              |         | 0.92 (0.58–1.48)              |         |

Abbreviations: DBD, donor after brainstem death; DCD, donor after circulatory death; HLA, human leucocyte antigen; HR, hazard ratio; CI, confidence interval.

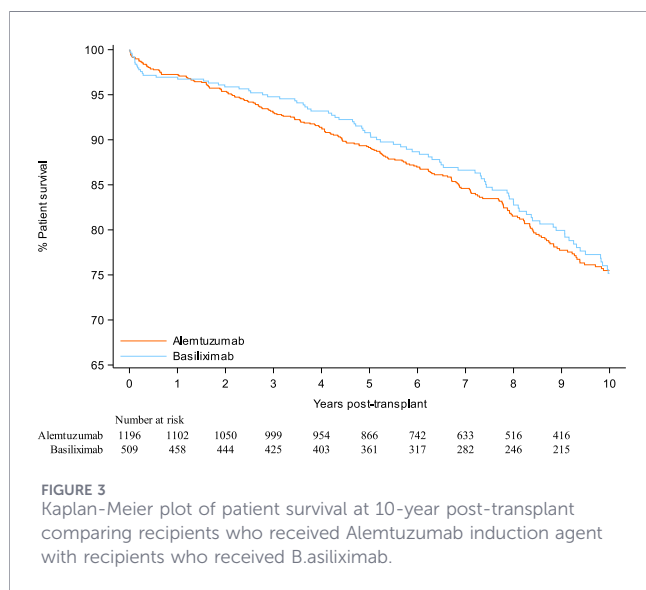


FIGURE 3  
Kaplan-Meier plot of patient survival at 10-year post-transplant comparing recipients who received Alemtuzumab induction agent with recipients who received Basiliximab.

0.62, 95% CI 0.44–0.87,  $p = 0.008$ ). Donor age showed a significant non-linear association with graft loss at 10 years ( $p < 0.0001$ ). Higher donor creatinine at retrieval was associated with increased graft failure risk at 10 years (HR 1.29, 95% CI 1.07–1.56,  $p = 0.008$ ). Non-white donor ethnicity and female recipient sex were significantly associated with increased risk of graft failure at 10 years (HR 1.52, 95% CI 1.04–2.22,  $p = 0.031$ ; and HR 1.46, 95% CI 1.16–1.85,  $p = 0.002$ , respectively).

Recipient dialysis modality also impacted outcomes, with haemodialysis (vs. no dialysis) significantly associated with worse kidney graft survival at 10 years (HR 1.41, 95% CI 1.09–1.83;  $p = 0.005$ ). In contrast, peritoneal dialysis was not associated with excess risk when compared to no dialysis at 10 years (HR 0.89, 95% CI 0.64–1.23).

HLA mismatches at all loci were not significantly associated with kidney graft loss at 10 years, when adjusted for other factors. Similarly, induction therapy had no significant impact on kidney graft survival in adjusted models. The same conclusion was observed for 1-year kidney graft survival, as neither the association with HLA mismatch nor induction therapy was significant after adjusting for other factors.

## Predictors of patient mortality

Unadjusted patient mortality is presented in Figure 3, with no evidence to suggest a difference in 10-year patient survival ( $p = 0.699$ ) when comparing Alemtuzumab (75.5%, 95% CI 72.3%–

78.4%) with Basiliximab (75.2%, 95% CI 70.1%–79.5%). Predictors of patient survival at 1- and 10-year post-transplant are presented in Table 5. Increasing recipient age was a predictor of higher mortality risk with significant association at 10 years (HR 1.04, 95% CI 1.02–1.05,  $p < 0.0001$ ). Cold ischaemic time was significantly associated with increased 1-year mortality (HR 1.13, 95% CI 1.02–1.24,  $p = 0.020$ ), but not at 10-year. Longer waiting time to transplant also emerged as a significant non-linear predictor of mortality at 1- and 10-year post-transplant ( $p \leq 0.033$ .) Neither transplant era, donor age, donor CMV status, nor dialysis modality were significant predictors of early mortality.

HLA mismatches at all loci and induction therapy regimen were not significantly associated with patient mortality at either timepoint, when adjusted for other factors.

## Discussion

In this large, contemporary national cohort of SPK transplant recipients, we demonstrate excellent long-term outcomes, with 10-year pancreas graft, kidney graft, and patient survival rates exceeding 70%. Our analysis identifies donor age, cold ischaemic time, and donor CMV status as key predictors of pancreas graft failure and further highlights the adverse impact of haemodialysis on long-term kidney graft survival. Crucially, we identified an unexpected association between a single HLA-DQ mismatch and improved long-term pancreas graft survival, a finding not observed for kidney or patient outcomes. In contrast, induction therapy choice between Alemtuzumab and Basiliximab was not significantly associated with differences in graft or patient survival.

The 3C study, comparing Alemtuzumab with Basiliximab induction therapy in renal transplantation, demonstrated that alemtuzumab significantly reduced the risk of biopsy-proven rejection without increasing serious infections [18]. However, in our study we observed no difference in SPK graft, kidney graft or patient survival on adjusted analyses. This may reflect the distinct immunological challenges of SPK recipients, who may have an alloimmune burden distinct to kidney-only recipients. This also underscores the need for caution in extrapolating induction strategies validated in kidney transplantation to SPK recipients.

The observation that a single HLA-DQ mismatch was associated with improved long-term pancreas graft survival is unexpected and, to our knowledge, has not been previously reported. Although two mismatches were not significantly associated with graft outcome, the overlapping confidence intervals suggests that there is no difference in the effect of two mismatches compared with the effect of a single

TABLE 5 Multivariable Cox regression model estimates for predictors of patient survival at 1- and 10-year post-transplant.

| Factor                      | One-year patient mortality     |             | Ten-year patient mortality     |              |
|-----------------------------|--------------------------------|-------------|--------------------------------|--------------|
|                             | HR (95% CI)                    | P-value     | HR (95% CI)                    | P-value      |
| Era of transplant           |                                | Global 0.23 |                                | Global 0.5   |
| Apr 2007-Mar 2011           | Reference                      |             | Reference                      |              |
| Apr 2011-Mar 2015           | 0.75 (0.37–1.52)               |             | 0.96 (0.73–1.28)               |              |
| Apr 2015-Mar 2019           | 0.45 (0.18–1.12)               |             | 0.81 (0.56–1.17)               |              |
| Donor age (years)           | 1.01 (0.99–1.03)               | 0.34        | 1.01 (1.00–1.02)               | 0.053        |
| Donor CMV                   |                                | 0.17        |                                | 0.071        |
| Negative                    | Reference                      |             | Reference                      |              |
| Positive                    | 0.65 (0.35–1.20)               |             | 1.24 (0.98–1.56)               |              |
| Recipient age (years)       | 1.02 (0.99–1.06)               | 0.25        | 1.04 (1.02–1.05)               | <0.0001      |
| Recipient dialysis status   |                                | Global 0.77 |                                | Global 0.033 |
| Not on dialysis             | Reference                      |             | Reference                      |              |
| Haemodialysis               | 1.27 (0.64–2.53)               |             | 1.19 (0.92–1.54)               |              |
| Peritoneal dialysis         | 1.23 (0.56–2.69)               |             | 0.76 (0.55–1.07)               |              |
| Cold ischaemic time (hours) | 1.13 (1.02–1.24)               | 0.02        | 1.01 (0.97–1.05)               | 0.61         |
| Waiting time (years)        | Fitted as natural cubic spline | 0.033       | Fitted as natural cubic spline | 0.019        |
| HLA-A mismatches            |                                | Global 0.49 |                                | Global 0.98  |
| 0                           | Reference                      |             | Reference                      |              |
| 1                           | 0.67 (0.31–1.45)               |             | 0.99 (0.71–1.38)               |              |
| 2                           | 0.59 (0.23–1.47)               |             | 1.01 (0.70–1.47)               |              |
| HLA-B mismatches            |                                | Global 0.73 |                                | Global 0.95  |
| 0                           | Reference                      |             | Reference                      |              |
| 1                           | 0.64 (0.14–3.02)               |             | 0.96 (0.52–1.77)               |              |
| 2                           | 0.79 (0.17–3.78)               |             | 1.00 (0.54–1.87)               |              |
| HLA-Cw mismatches           |                                | Global 0.5  |                                | Global 0.32  |
| 0                           | Reference                      |             | Reference                      |              |
| 1                           | 1.94 (0.63–5.95)               |             | 1.33 (0.90–1.97)               |              |
| 2                           | 1.92 (0.59–6.31)               |             | 1.35 (0.89–2.06)               |              |
| HLA-DQ mismatches           |                                | Global 0.7  |                                | Global 0.79  |
| 0                           | Reference                      |             | Reference                      |              |
| 1                           | 1.37 (0.65–2.91)               |             | 1.06 (0.80–1.39)               |              |
| 2                           | 1.46 (0.40–5.31)               |             | 0.91 (0.52–1.58)               |              |
| HLA-DR mismatches           |                                | Global 0.94 |                                | Global 0.15  |
| 0                           | Reference                      |             | Reference                      |              |
| 1                           | 0.92 (0.28–3.03)               |             | 0.68 (0.45–1.02)               |              |
| 2                           | 1.03 (0.28–3.78)               |             | 0.75 (0.47–1.17)               |              |
| Induction agent             |                                | 0.92        |                                | 0.7          |
| Alemtuzumab                 | Reference                      |             | Reference                      |              |
| Basiliximab                 | 1.06 (0.34–3.26)               |             | 1.09 (0.70–1.69)               |              |

Abbreviations: CMV, cytomegalovirus; HLA, human leucocyte antigen; HR, hazard ratio; CI, confidence interval.



-DQ mismatch. While class II mismatches, particularly at the DQ locus, have been linked to adverse outcomes in kidney transplantation [12, 19], including increased risk of donor-specific antibody formation and chronic rejection, such associations have not been well established in SPK recipients. This observation could be specific to the model fitted, and not represent true causality warranting cautious interpretation and is, at best, hypothesis-generating. Additionally, the DQ and B mismatches were not associated with acute rejection, suggesting that any observations associated with outcomes may be due to chance rather than a true effect. Further mechanistic investigation using high-resolution HLA typing, epitope analysis, immune profiling, and validation in other populations may provide further insight into the effects of HLA matching on pancreas graft outcomes.

In a retrospective analysis of 1219 pancreas graft recipients (SPK  $n = 355$ ), HLA mismatch status was not associated with pancreas graft or patient survival following transplantation [17], similar to our results. However, the study reported an increased risk of acute rejection with HLA-B and -DR mismatches with -DQ mismatches having no impact, although this observation was not apparent when SPK graft recipients were examined as a subgroup. These results suggest that the immunological relevance of specific HLA loci may differ by transplant type. These findings suggest that while overall HLA mismatch may not influence long-term outcomes, locus-specific mismatching may contribute to pancreas graft loss through immune mechanisms that are not fully mitigated by standard immunosuppression. This highlights the potential importance of considering HLA matching when evaluating immunological risk, particularly in the context of non-depleting induction strategies, warranting further investigation.

The absence of a clear association between HLA mismatch and kidney graft survival in our cohort may, at least in part, reflect the relatively short cold ischaemia times observed in the UK for SPK grafts. Both HLA mismatch and prolonged cold ischaemia are well-established determinants of kidney graft outcomes, and previous large registry studies have demonstrated that the adverse effect of HLA mismatch is attenuated when cold ischaemia times are short [20, 21]. In our study, the median cold ischaemia time was substantially lower than in comparable international series, likely diminishing the incremental impact of HLA mismatching on kidney graft survival.

Induction therapy did not significantly impact pancreas graft, kidney graft or patient survival on the adjusted analyses. Although unadjusted analyses suggested slightly improved pancreas graft survival with Alemtuzumab, this effect was not observed in multivariable models. The rates of early acute rejection episodes were also comparable between induction agents. Our findings are in line with a retrospective study [22], reporting similar pancreas and kidney graft outcomes between Alemtuzumab and Basiliximab in a large single-centre SPK cohort, though with a higher incidence of CMV infection in Alemtuzumab-treated recipients. More recently, Aziz et al. compared T cell-depleting agents with IL-2 receptor blockade in 417 pancreas transplant recipients and similarly found no difference in graft survival on multivariable analysis [23]. Importantly, both studies highlighted differences in infection profiles rather than survival, with Aziz et al. also noting increased CMV and bacterial infections in the T cell-depleting group. Taken together with our findings, these data suggest that

while induction agent choice does not appear to affect long-term graft or patient survival, the decision should be individualised based on recipient risk profile, infection risk, and centre-specific protocols.

Our findings have several potential implications for clinical practice and transplant policy. The absence of a survival benefit from any specific induction agent supports a more individualised approach to induction therapy. Rather than a uniform preference, the choice between T cell-depleting agents and interleukin-2 receptor antagonists may be better guided by recipient comorbidity, CMV serostatus, and institutional experience. Furthermore, we did not observe a significant reduction in graft or patient survival with locus-specific mismatching warranting specific investigation into the organ-specific effects in larger external cohorts.

This study has several limitations inherent to its retrospective design and use of national registry data. While the dataset is robust and population-based, it lacks granularity in certain key areas, including high-resolution HLA typing, DSA status, maintenance immunosuppression regimens, and biopsy-proven rejection. As such, we were unable to explore mechanistic explanations for the observed DQ mismatch effect or evaluate potential interactions between induction, maintenance therapy, and immunological risk. Additionally, selection bias may influence the choice of induction agent, although our multivariable analyses aimed to adjust for relevant confounders. Finally, while the finding of a protective association with a single DQ mismatch is statistically robust and biologically plausible, it is hypothesis-generating and requires validation in other cohorts with complementary mechanistic data.

This national study demonstrates excellent long-term outcomes following simultaneous pancreas-kidney transplantation and provides new insights into the impact of immunological matching and induction therapy. The absence of significant differences in outcomes by induction agent supports a personalised approach to immunosuppression. Although the associations described are observational, and causality cannot be determined in this registry study, these findings highlight the complex interplay between immunogenetic matching and induction therapy in SPK transplantation. Our results emphasise the importance of tailoring immunological risk assessment beyond aggregate mismatch scores.

## Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The data is held by NHS Blood and Transplant, and may be available upon reasonable request. Requests to access these datasets should be directed to [statistical.enquiries@nhsbt.nhs.uk](mailto:statistical.enquiries@nhsbt.nhs.uk).

## Ethics statement

The requirement of ethical approval was waived by Pancreas Advisory Group, NHS Blood and Transplant for the studies involving humans because Retrospective review of anonymised registry data complying with local legislation. The studies were

conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board also waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because Retrospective review of anonymised registry data complying with local legislation.

## Author contributions

AM, JB, LS, and SW were responsible for the design of the study. AM, JB, LS, and CC performed the analysis. All authors contributed to the interpretation of the results. AM, JB, LS, ST and SW wrote the first draft. All authors contributed to the article and approved the submitted version.

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## References

- Montagud-Marrahi E, Molina-Andujar A, Pane A, Ruiz S, Amor AJ, Esmatjes E, et al. Impact of simultaneous pancreas-kidney transplantation on cardiovascular risk in patients with diabetes. *Transplantation* (2022) 106(1):158–66. doi: 10.1097/TP.0000000000003710
- Sollinger HW, Odorico JS, Becker YT, D'Alessandro AM, Pirsch JD. One thousand simultaneous pancreas-kidney transplants at a single center with 22-year follow-up. *Ann Surg* (2009) 250(4):618–30. doi: 10.1097/SLA.0b013e3181b76d2b
- Venkatanarasimhamoorthy VS, Barlow AD. Simultaneous pancreas-kidney transplantation versus living donor kidney transplantation alone: an outcome-driven choice? *Curr Diab Rep* (2018) 18(9):67. doi: 10.1007/s11892-018-1039-8
- White SA, Shaw JA, Sutherland DE. Pancreas transplantation. *Lancet* (2009) 373(9677):1808–17. doi: 10.1016/S0140-6736(09)60609-7
- van Dellen D, Worthington J, Mitu-Pretorian OM, Ghazanfar A, Forgacs B, Pararajasingam R, et al. Mortality in diabetes: pancreas transplantation is associated with significant survival benefit. *Nephrol Dial Transpl* (2013) 28(5):1315–22. doi: 10.1093/ndt/gfs613
- Al-Naseem AO, Attia A, Gonnah AR, Al-Naseem AOAS, Spiers HVM, Gruessner A, et al. Pancreas transplantation today: quo vadis? *Eur J Endocrinol* (2023) 188(4):R73–R87. doi: 10.1093/ajendo/lvad032
- Callaghan CJ, Ibrahim M, Counter C, Casey J, Friend PJ, Watson CJE, et al. Outcomes after simultaneous pancreas-kidney transplantation from donation after circulatory death donors: a UK registry analysis. *Am J Transpl* (2021) 21(11):3673–83. doi: 10.1111/ajt.16604
- 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 J Transpl* (2024) 24(7):1247–56. doi: 10.1016/j.ajt.2024.02.008
- Milner J, Melcher ML, Lee B, Veale J, Ronin M, D'Alessandro T, et al. HLA matching trumps donor age: donor-recipient pairing characteristics that impact long-term success in living donor kidney transplantation in the era of paired kidney exchange. *Transplant Direct* (2016) 2(7):e85. doi: 10.1097/TXD.0000000000000597
- Tambur AR, Audry B, Glotz D, Jacquelinet C. Improving equity in kidney transplant allocation policies through a novel genetic metric: the matched donor potential. *Am J Transpl* (2023) 23(1):45–54. doi: 10.1016/j.ajt.2022.08.001
- Foster BJ, Dahhou M, Zhang X, Platt RW, Smith JM, Hanley JA. Impact of HLA mismatch at first kidney transplant on lifetime with graft function in young recipients. *Am J Transpl* (2014) 14(4):876–85. doi: 10.1111/ajt.12643
- DeVos JM, Gaber AO, Knight RJ, Land GA, Suki WN, Gaber LW, et al. Donor-specific HLA-DQ antibodies may contribute to poor graft outcome after renal transplantation. *Kidney Int* (2012) 82(5):598–604. doi: 10.1038/ki.2012.190
- Tambur AR, Kosmoliaptis V, Claas FJH, Mannon RB, Nickerson P, Naesens M. Significance of HLA-DQ in kidney transplantation: time to reevaluate human leukocyte antigen-matching priorities to improve transplant outcomes? An expert review and recommendations. *Kidney Int* (2021) 100(5):1012–22. doi: 10.1016/j.kint.2021.06.026
- Wiebe C, Kosmoliaptis V, Pochinco D, Gibson IW, Ho J, Birk PE, et al. HLA-DR/DQ molecular mismatch: a prognostic biomarker for primary alloimmunity. *Am J Transpl* (2019) 19(6):1708–19. doi: 10.1111/ajt.15177
- Berney T, Malaise J, Morel P, Toso C, Demuylder-Mischler S, Majno P, et al. Impact of HLA matching on the outcome of simultaneous pancreas-kidney transplantation. *Nephrol Dial Transpl* (2005) 20(Suppl. 2):ii48–53, ii62. doi: 10.1093/ndt/gfh1082
- Malaise J, Berney T, Morel P, Toso C, Demuylder-Mischler S, Majno P, et al. Effect of HLA matching in simultaneous pancreas-kidney transplantation. *Transpl Proc* (2005) 37(6):2846–7. doi: 10.1016/j.transproceed.2005.05.023
- Rudolph EN, Dunn TB, Mauer D, Noreen H, Sutherland DER, Kandaswamy R, et al. HLA-A, -B, -C, -DR, and -DQ matching in pancreas transplantation: effect on graft rejection and survival. *Am J Transpl* (2016) 16(8):2401–12. doi: 10.1111/ajt.13734
- Group TCSC, Haynes R, Harden P, Judge P, Blackwell L, Emberson J, et al. Alemtuzumab-based induction treatment versus basiliximab-based induction

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## Conflict of interest

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## Supplementary material

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treatment in kidney transplantation (the 3C study): a randomised trial. *The Lancet* (2014) 384(9955):1684–90. doi: 10.1016/S0140-6736(14)61095-3

19. Isaacson D, Schold JD, Gmeiner MW, Copley HC, Kosmoliaptsis V, Tambur AR. HLA-DQ mismatches lead to more unacceptable antigens, greater sensitization, and increased disparities in repeat transplant candidates. *J Am Soc Nephrol* (2022) 33(12):2293–305. doi: 10.1681/ASN.2022030296

20. Echterdiek F, Latus J, Döhler B, Schwenger V, Süsal C. Influence of cold ischemia time on the outcome of kidney transplants from donors aged 70 years and Above-A collaborative transplant study report. *Transplantation* (2021) 105(11):2461–9. doi: 10.1097/TP.0000000000003629

21. Vinson AJ, Kiberd BA, Tennankore KK. Prolonged cold ischemia time offsets the benefit of human leukocyte antigen matching in deceased donor kidney transplant. *Transplant Proc* (2020) 52(3):807–14. doi: 10.1016/j.transproceed.2019.12.049

22. Magliocca JF, Odorico JS, Pirsch JD, Becker YT, Knechtle SJ, Levenson GE, et al. A comparison of alemtuzumab with basiliximab induction in simultaneous pancreas-kidney transplantation. *Am J Transplant* (2008) 8(8):1702–10. doi: 10.1111/j.1600-6143.2008.02299.x

23. Aziz F, Parajuli S, Kaufman D, Odorico J, Mandelbrot D. Induction in pancreas transplantation: T-cell depletion versus IL-2 receptor blockade. *Transplant Direct* (2022) 8(12):e1402. doi: 10.1097/TXD.0000000000001402



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# Multi-site cytokine levels are predictive of primary graft dysfunction following lung transplantation

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There is an urgent need to better understand the pathophysiology of primary graft dysfunction (PGD) to develop point-of-care methods predicting those at risk. We utilized a multiplex multivariable approach to define cytokines, chemokines, and growth factors in patient-matched biospecimens to identify factors predictive of PGD. Biospecimens were collected from patients undergoing bilateral lung transplantation (LTx) from three sites: donor lung perfusate, post-transplant bronchoalveolar lavage (BAL) fluid (2h), and plasma (2h, 24h, 72h, and 1 and 2 wks). A 71-multiplex panel was performed on each. Cross-validated logistic regression (LR) and random forest (RF) models determined whether analytes from each site, alone or combined with clinical data, discriminated PGD grade 0 (n = 9) vs. 3 (n = 8). BAL fluid at 2h was most predictive of PGD (LR, 0.825; RF, 0.919), followed by multi-timepoint plasma (LR, 0.841; RF, 0.653), then perfusate (LR, 0.565; RF, 0.448). Combined clinical, BAL, and plasma data yielded the strongest performance (LR, 1.000; RF, 1.000). BAL collected 2h post-transplant showed the strongest discriminatory signal for severe PGD in this exploratory cohort. This integrative approach identified IL-1RA, BCA-1, and Fractalkine as hypothesis-generating candidate biomarkers that warrant validation in larger independent studies.

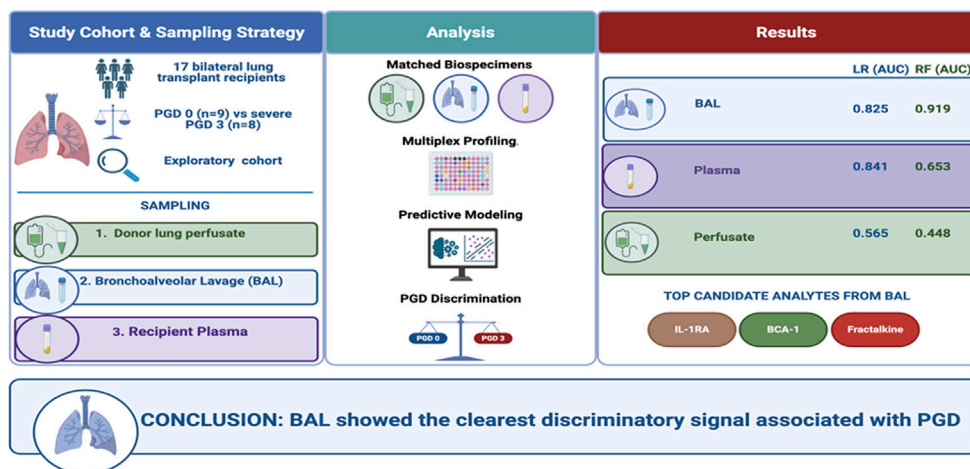
### KEYWORDS

cytokines, lung transplantation, machine learning, predictive models, primary graft dysfunction

## Introduction

Lung transplantation (LTx) is an acceptable therapy for end-stage lung disease. However, successful post-transplantation outcomes are significantly impacted by the development of severe primary graft dysfunction (PGD), which is associated with early mortality and a more rapid onset of chronic lung allograft dysfunction (CLAD) [1–4]. As such, there is an urgent need to better understand PGD pathobiology and identify those at enhanced risk. The pathophysiology of PGD is complex and involves a multifactorial inflammatory process that likely starts in the donor lung and is exacerbated by ischemia

# Multi-Site Cytokine Levels are Predictive of Primary Graft Dysfunction following Lung Transplantation



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GRAPHICAL ABSTRACT

reperfusion injury (IRI). Given the contribution of inflammation in PGD, several studies have leveraged the presence of pro-inflammatory cytokines as prognostic biomarkers. These studies have largely measured the presence of soluble factors in recipient plasma, with elevated levels of plasma cytokines such as IL-6, IL-8, IP-10, MCP-1, and endothelial/epithelial injury markers shown to be associated with severe PGD [5–13].

Though plasma provides a valuable window into the biological/inflammatory processes detected on a systemic level, a handful of studies have analyzed bronchoalveolar lavage (BAL) fluid as a more representative sample of the transplanted lung-specific microenvironment. Elevated BAL levels of IL-6, IL-8, and Eotaxin were shown to be associated with PGD [14–16]. Further, *ex vivo* lung perfusion (EVLVP) studies have shown that elevated IL-8, IL-6, and GRO- $\alpha$  levels in perfusion solution are associated with poorer graft outcomes [17, 18].

Together, these studies show the potential value of measuring inflammatory and injury markers as predictors of PGD. Most factors identified to date have been selected for investigation by association, in that they have been previously implicated in other acute lung injuries and then subsequently investigated in LTx. While these studies have guided our understanding of PGD, their hypothesis-driven focus may limit the discovery of other mediators or predictors of PGD. Thus, here we utilized a 71-analyte multiplex approach composed of a broad array of cytokine, chemokines, and growth factors in concert with matched donor perfusate, recipient plasma, and BAL to identify novel biomarkers and/or mechanistic factors associated with PGD. By examining multiple analytes across diverse samples, our study aimed to mitigate selection bias and uncover new correlates to PGD. We further hypothesized that a machine learning approach may serve as a valuable tool that

complements classical statistics for biomarker discovery. Here we demonstrate that cytokine expression in BAL collected 2h post-LTx best predicts PGD development. Further, using a model reduction process, we identified the three strongest BAL predictors of PGD development were BCA-1 (CXCL13), Fractalkine, and IL-1Ra, factors that have not been extensively studied in the context of PGD. Together, our results provide a methodological foundation for further development of predictive modeling that could result in the discovery of analytes that can be used in a clinically relevant point-of-care test and provide novel targets for PGD mechanistic studies.

## Methods and materials

### Inclusion/exclusion criteria

Samples were acquired from the University of Florida Transplant Tissue Bank from patients undergoing a first-time bilateral lung transplant between May 2021 and May 2022. Nineteen patients who had a complete sample set and had the same PGD grade at all time points, either 0 ( $n = 10$ ) or 3 ( $n = 9$ ), were selected. PGD was graded as described [19]. To maximize phenotypic contrast within a limited cohort, the primary analyses were restricted to recipients with persistently absent PGD (grade 0 at all assessed time points) or persistently severe PGD (grade 3 at all assessed time points). This design was selected to reduce ambiguity introduced by transient, intermediate, or fluctuating PGD grades and improve the likelihood of detecting biologically meaningful signals in an exploratory setting. For accuracy and consistency, transplant recipients without severe graft dysfunction are referred to throughout as PGD0 or PGD– recipients, whereas patients with



TABLE 1 Baseline donor and recipient characteristics.

| Donor Characteristics             |  | PGD– (n = 9)      | PGD+ (n = 8)      | p-value |
|-----------------------------------|--|-------------------|-------------------|---------|
| Median donor age                  |  | 37 (16, 60)       | 37 (18, 60)       | .902    |
| Donor sex                         |  |                   |                   |         |
| Male                              |  | 89% (8)           | 25% (2)           | .020    |
| Female                            |  | 11% (1)           | 75% (6)           |         |
| Donor race/Ethnicity              |  |                   |                   |         |
| White                             |  | 44% (4)           | 50% (4)           | –       |
| Hispanic/Latino                   |  | 33% (3)           | 38% (3)           |         |
| Black or african american         |  | 22% (2)           | –                 |         |
| American indian                   |  | –                 | 13% (1)           |         |
| Static storage                    |  |                   |                   | .033    |
| Standard 4 °C                     |  | 100% (9)          | 50% (4)           |         |
| 10 °C                             |  | –                 | 50% (4)           |         |
| Smoking history                   |  |                   |                   |         |
| Yes                               |  | 56% (5)           | 38% (3)           | .637    |
| No                                |  | 44% (4)           | 63% (5)           |         |
| Heavy alcohol use history         |  |                   |                   |         |
| Yes                               |  | 22% (2)           | 13% (1)           | .999    |
| No                                |  | 78% (7)           | 88% (7)           |         |
| Recipient characteristics         |  | PGD– (n = 9)      | PGD+ (n = 8)      | p-value |
| Median recipient age              |  | 61 (48, 74)       | 59 (35, 73)       | .418    |
| Mean recipient BMI at listing     |  | 24.9              | 26.9              | .256    |
| Recipient sex                     |  |                   |                   | .015    |
| Male                              |  | 78% (7)           | 13% (1)           |         |
| Female                            |  | 22% (2)           | 88% (7)           |         |
| UNOS lung disease diagnosis       |  |                   |                   |         |
| Group                             |  |                   |                   |         |
| Group A: Obstructive lung disease |  | 3                 | 0                 | –       |
| Group B: Pulmonary vascular       |  | 1                 | 0                 |         |
| Disease                           |  |                   |                   |         |
| Group C: Cystic fibrosis          |  | 0                 | 0                 |         |
| Group D: Restrictive lung disease |  | 5                 | 9                 |         |
| Mean LAS Score                    |  | 47.9 (33.1, 92.4) | 57.1 (36.5, 93.3) | .462    |
| % ECMO pre-Tx                     |  | 10% (1)           | 22% (2)           | .577    |
| % ECMO post-Tx                    |  | 10% (1)           | 67% (5)           | .013    |
| % Sensitization protocol          |  | – (0)             | 50% (4)           | .023    |

Donor and Recipient characteristics by primary graft dysfunction status. Differences between groups in categorical variables were analyzed by Fisher's Exact Comparison and differences in numerical variables were analyzed by unpaired Student's t-test, with unadjusted p-values  $p < 0.05$ . Abbreviations. PGD–: Primary Graft Dysfunction grade 0 at all time points; PGD+: Primary Graft Dysfunction grade 3 at all time points; BMI: Body Mass Index; UNOS: United Network for Organ Sharing. LAS: Lung Allocation Score, ECMO: Extracorporeal Membrane Oxygenation.

persistently severe PGD, will be termed PGD+. Donor and recipient demographics for each group are detailed in [Table 1](#). This study was approved by the University of Florida Institutional Review Board IRB#202200536.

## Sample collection

We analyzed three distinct sample types: (1) plasma, (2) bronchoalveolar lavage fluid (BAL), and (3) donor lung perfusate solution. Plasma was processed from whole blood collected 12h prior to transplant (pre) and at 2h, 24h, 72h, 1wk, and 2wk post-LTx. BAL fluid was obtained following reperfusion, at a time point matched to the 2h blood sample, “T0”. BAL was collected following the ISHLT recommendations [20]. Donor lung Perfadex® (Xvivo, Sweden) perfusate solution was obtained by collecting the initial volume of 2–3 mL of preservation solution passed out of the lung vasculature at the time of reperfusion. All processed samples were stored at –80 °C until time of analysis.

## Multiplex assays

We utilized Eve Technologies’ Cytokine, Chemokine, Growth Factor 71-Plex Clinical RUO Test (HD71-CLIN). Samples were run on Luminex 100/200™ instruments (Luminex/DiaSorin, Saluggia, Italy), with Bio-Plex Manager™ (BPM) software (BioRad, Hercules, CA) by Eve Technologies (Calgary, Canada), a Clinical Laboratory Improvement Amendments (CLIA)-approved facility. The full analyte list, with analyte abbreviations and assay detection limits, is listed in [Supplementary Tables 1A, B](#). Since a relative absence (or low levels) of any particular analyte could be meaningful with respect to patterns of cytokine expression, any sample with a fluorescence value below the range of its standard curve was included in the analysis by assigning a concentration value corresponding to the minimum sample value for the same analyte. We recognize that this approach may introduce bias. Therefore, we performed prespecified robustness analyses using alternative preprocessing strategies: first, repeating analyses without concentration imputation, and second, analyzing fluorescence intensity values directly, for which missingness was minimal (see robustness checks section).

## Statistical, computational analysis software, and preprocessing

Computations were performed in R [21], using Tidyverse and Tidymodels workflows [22, 23] and additional packages for specific analyses [24–35]. Smoking and alcohol history were collapsed to logicals. We offset log-transformed concentrations before analysis. As this study was designed as an exploratory biomarker discovery analysis, our objective was to identify candidate inflammatory mediators and specimen types associated with severe PGD rather than to test a small number of prespecified hypotheses. Accordingly, p-values from univariable screening analyses were not adjusted for multiple comparisons and should be interpreted as descriptive signals intended to prioritize candidates for further validation rather than as definitive evidence of association. All findings, particularly those emerging from high-dimensional multiplex and

modeling analyses, should therefore be considered hypothesis-generating.

## Protein concentrations and pairwise associations

We visualized protein co-concentration patterns within each medium using dendrogram-annotated heatmaps [27] and correlation plots [28]. We used Kruskal–Wallis (KW) tests to identify proteins whose plasma concentrations changed over time. We included these in a principal components analysis (PCA) and generated a biplot with an average trajectory represented by segments between consecutive centroids.

## Discrimination of PGD

KW (perfusate, BAL fluid) and multivariate Cramér [36] (plasma at all time points) tests were used to identify proteins that discriminated between the PGD– and PGD+ subgroups. Log-concentrations between the subgroups were visualized using jitter and line plots. Biplots of plasma concentrations were also generated with PGD-stratified average trajectories and are reported in the Methodological Supplement (M1).

## Prediction of PGD

Cross-validated logistic regression (LR), random forest (RF), and nearest neighbors (NN) models were used to determine whether several sets of predictors could discriminate between PGD– and PGD+. To better reflect practice conditions, no missing values were imputed for predictive analysis; analytes with several missing values were excluded. The pre-processing steps are detailed in the Methodological Supplement. Model families were optimized over hyperparameter grids. For each model specification, the area under the receiver operating characteristic curve (AUROC) was calculated from stratified leave-one-out cross-validation, in which each test set comprised one PGD– and one PGD+ case. We repeated this procedure 12 times to ensure diversity in the test sets.

The predictive value of data from each site, namely perfusate, BAL fluid, plasma (pre-Tx, T0, and 24h), and clinic (donor, recipient, and perioperative), was assessed. We ranked the model specifications by mean AUROC. Results were summarized using line–range plots and tabulated optimal and near-optimal performance by model family using each combination of sites. Clinical variables were incorporated as a separate predictor set consisting of available donor, recipient, and perioperative characteristics contained within the de-identified tissue bank dataset.

We next fitted optimized model specifications to complete data from each combination of sites and used absolute coefficient estimates (LR) and mean decrease in accuracy (RF) to rank predictors. To assess the potential feasibility of a point-of-care test, we included the top predictors from both families in a LR LASSO, using data from the site that yielded the best predictions.

## Robustness checks

We tested the robustness of our results by re-running the full analysis after each of two alternative pre-processing choices: (1) for

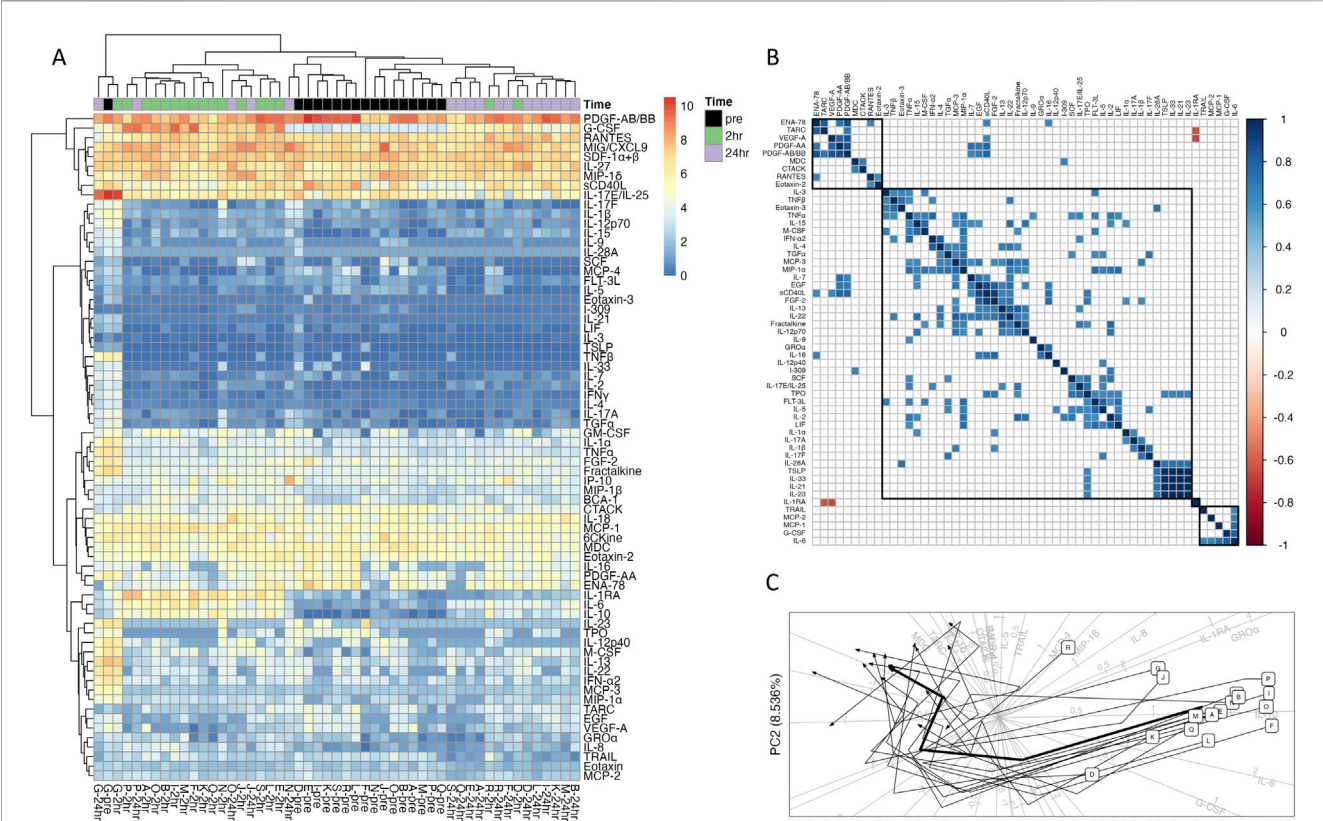


**FIGURE 1** Log-concentrations of 71 proteins in plasma at near-transplantation time points. Protein levels and their changes during the peri-operative period were similar across all but one subject. **(A)** Clustered heatmap for all subjects at pre-Tx, T0, and 24h time points, using complete-linkage clustering on cases and proteins. Blue (red) indicates lower (higher) levels. **(B)** Correlation matrix of soluble factors measured in plasma from all subjects at T0. Only correlations with unadjusted  $p < 0.01$  in Spearman tests are filled. Proteins are ordered by complete-linkage clustering in order to juxtapose more correlated proteins. Note that complete-linkage clustering is heuristic, so the order may differ from that of **(A)**. **(C)** Principle component analysis (PCA) biplot of time-discriminating proteins at all subject–time points. Values at pre-Tx are labeled by unique keys. Segments connect adjacent time points for each subject. Segments are grouped by primary graft dysfunction (PGD) status. Thickened segments connect time point centroids, stratified by PGD status.

exploratory analysis, not imputing out-of-range values and, for predictive analysis, imputing; and (2) using fluorescence intensities (FIs), for which very few values were missing, rather than concentrations.

Results

Sample characteristics and group differences

Seventeen patients with complete data were included in the analysis (PGD–,  $n = 9$ ; PGD+,  $n = 8$ ). Consistent with published risk factors, PGD+ patients were predominantly female and pre-sensitized (Table 1).

Plasma protein concentrations and pairwise comparisons

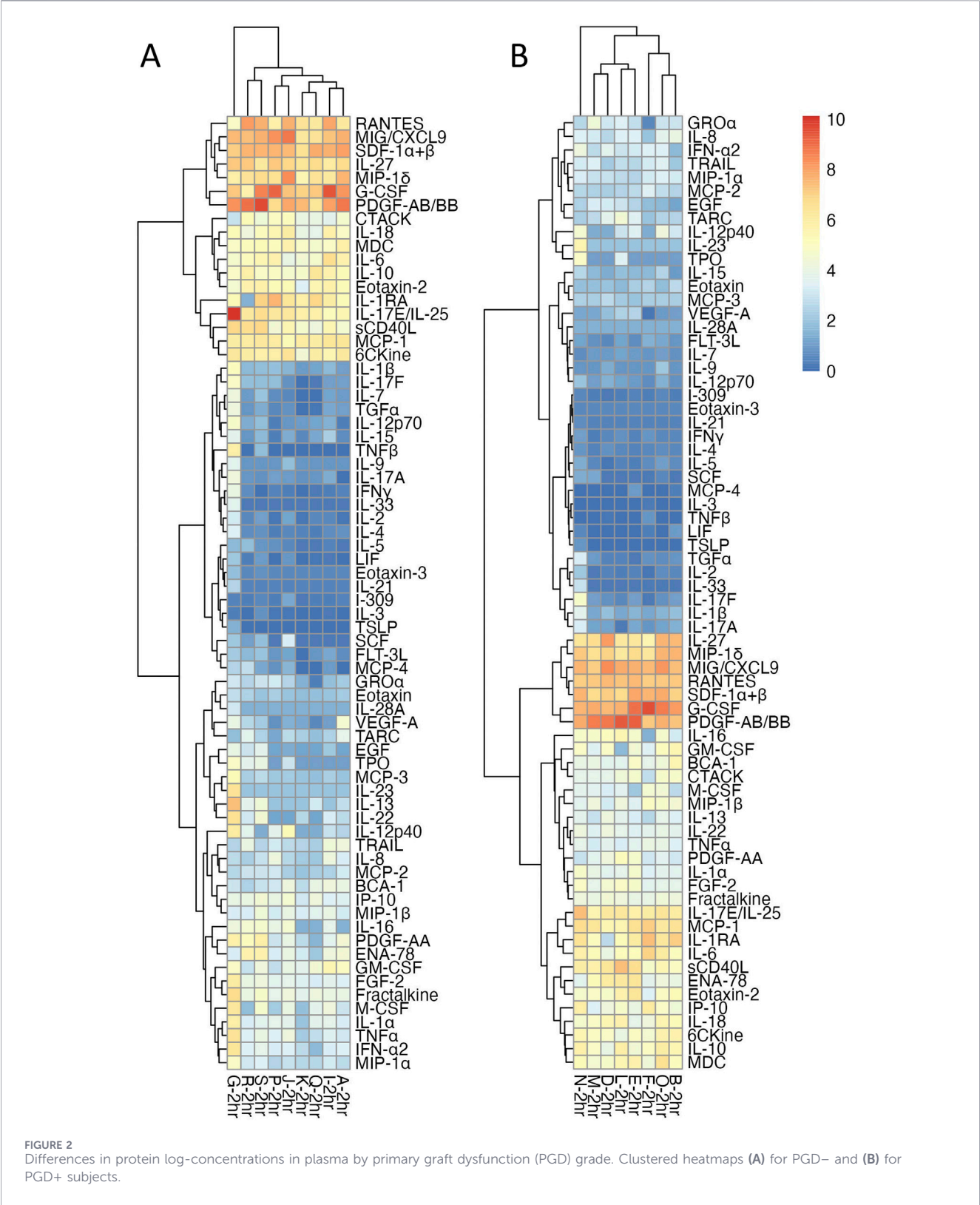
We observed consistent protein clustering based on concentrations of analytes in plasma over the first three time points (pre-Tx, T0, and 24h) (Figure 1A). One outlier case (G) had higher levels in low-concentration clusters. With six exceptions, panels clustered by time point. Next, we selected a distance threshold, based on visual inspection of dendrograms to

identify clusters at T0 (Figure 1B). There was one singleton, IL-1RA, the only analyte to be detectably negatively correlated with others (TARC and VEGF-A); a small cluster containing its negative correlates with ENA-78, PDGF-AA, PDGF-AB/BB, MDC, CTACK, RANTES, and Eotaxin-2; another small cluster comprising TRAIL, MCP-2, MCP-1, G-CSF, and IL-6; and the remaining proteins in one large cluster.

The PCA distinguished two dimensions of variation in plasma concentrations over time (Figure 1C). IL-1RA, GRO $\alpha$ , IL-10, IL-6, and G-CSF loaded strongly onto PC1, all positively. From pre-Tx to 24h, PC1 decreased, then remained low through 2wk. MDC, TARC, IL-3, MIP-1 $\delta$ , CTACK, Eotaxin, IL-2, IL-5, and TRAIL loaded strongly and negatively onto PC2, MDC, and TARC. PC2 decreased from pre-Tx to T0 then increased gradually through 2wk. MCP-1, MIP-1 $\beta$ , and IL-8 correlated more with each other than with either PC. The average trajectory reflected these patterns.

Plasma protein discrimination of PGD

We next analyzed whether concentration changes in plasma differed by PGD status. Analysis identified two-to-four clusters at T0, which broadly agree between the PGD– and PGD+ subgroups (Figure 2). Mean trajectories did not differ systematically between the



PGD- and PGD+ groups (Supplementary Figure M1). We did find evidence of systematically different concentrations of certain proteins at some time points (Table 2), notably BCA-1 at 1wk and 6CKine at T0. We detected only two proteins with different trajectories (Table 3; Figure 3): BCA-1 and IL-22 ( $p < 0.05$ ), both with higher concentrations in the PGD+ group, but otherwise similar trajectories. Several proteins (MCP-1, MIP-1 $\beta$ , IL-6, G-CSF, GRO $\alpha$ , IL-8, IL-10, and IL-1RA) associated with PC1 spiked at T0 and then regressed toward their pre-LTx levels in both groups (Figure 3).



**TABLE 2 Results of Kolmogorov–Smirnov tests of differences between undetected and severe primary graft dysfunction cases in protein concentrations in plasma at single time points.**

| Time | Protein    | $n_0$ | $n_3$ | Statistic | p-value |
|------|------------|-------|-------|-----------|---------|
| pre  | BCA-1      | 9     | 8     | 0.667     | 0.02024 |
| 2h   | 6CKine     | 9     | 8     | 0.778     | 0.00255 |
| 2h   | IL-22      | 9     | 8     | 0.778     | 0.00428 |
| 2h   | IL-16      | 9     | 8     | 0.750     | 0.01119 |
| 2h   | MCP-4      | 9     | 8     | 0.542     | 0.04977 |
| 24h  | 6CKine     | 9     | 8     | 0.778     | 0.00428 |
| 24h  | IL-6       | 9     | 8     | 0.764     | 0.00831 |
| 24h  | IL-22      | 9     | 8     | 0.667     | 0.02024 |
| 24h  | MCP-1      | 9     | 8     | 0.667     | 0.02024 |
| 24h  | IL-15      | 9     | 8     | 0.639     | 0.04689 |
| 24h  | BCA-1      | 9     | 8     | 0.639     | 0.04689 |
| 72h  | I-309      | 9     | 8     | 0.764     | 0.00831 |
| 72h  | PDGF-AA    | 9     | 8     | 0.667     | 0.02024 |
| 72h  | IL-12p40   | 9     | 8     | 0.556     | 0.02941 |
| 72h  | BCA-1      | 9     | 8     | 0.653     | 0.03357 |
| 72h  | IL-3       | 9     | 8     | 0.556     | 0.04689 |
| 72h  | IL-6       | 9     | 8     | 0.639     | 0.04689 |
| 1wk  | BCA-1      | 9     | 8     | 0.875     | 0.00140 |
| 1wk  | TRAIL      | 9     | 8     | 0.750     | 0.01119 |
| 1wk  | PDGF-AB/BB | 9     | 8     | 0.653     | 0.03357 |
| 1wk  | MIP-1δ     | 9     | 8     | 0.653     | 0.03357 |

Analytes are listed by sample time point first and in order of statistical evidence, up to unadjusted  $p < 0.05$ . See [Supplementary Table 1](#) for details on the full set of analytes. BCA-1, B-cell-attracting chemokine 1 (CXCL13); 6CKine, secondary lymphoid-tissue chemokine (CCL21); IL-22, interleukin-22; IL-16, interleukin-16; MCP-4, monocyte chemoattractant protein 4 (CCL13); IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein 1 (CCL2); IL-15, interleukin-15; I-309, T-lymphocyte-secreted protein I-309 (CCL1); PDGF-AA, platelet-derived growth factor-AA; IL-12p40, interleukin-12 p40 subunit; IL-3, interleukin-3; TRAIL, TNF-related apoptosis-inducing ligand; PDGF-AB/BB, platelet-derived growth factor-AB/BB; MIP-1δ, macrophage inflammatory protein-1 delta (CCL15).

## Perfusate protein discrimination of PGD

The same protein panel used for plasma analysis was used to assess perfusate solutions collected at the time of transplantation. Two proteins were consistently highly expressed in perfusate: FGF-2 and MIP-1δ. The remainder formed one higher- and one lower-concentration cluster ([Figure 4A](#)). The cases did not clearly cluster and three outliers were detected (S, P, and I). Clustering did not correlate with PGD grade. A direct comparison of all cases ([Figure 4B](#)) suggests that organs with higher analyte levels preceded PGD+, but we found no evidence that any single protein discriminated between PGD– and PGD+ ([Figure 4C](#)) ([Table 4](#)). Of 66 proteins with sufficient data, only two tests yielded  $p < 0.01$  (IL-17A and IL-1RA), a number expected by chance.

## BAL protein discrimination of PGD

BAL also did not clearly cluster cases or discriminate PGD groups ([Figure 5A](#)). However, higher concentrations generally correspond to PGD+ ([Figure 5B](#)). This pattern was statistically

evidenced for several proteins ([Table 5](#)): IL-1RA ( $p < 0.01$ ), Fractalkine, BCA-1, Eotaxin, IL-12p40, IL-6, IP-10, and M-CSF ( $p < 0.05$ ). Unlike the other sample types, PCA on all 71 proteins showed a clear separation between PGD– and PGD+ cases ([Figure 5C](#)), indicating that much of the variation in cytokine profiles was associated with PGD.

## Predictive modelling of PGD

The lack of strong associations between analyte concentrations and the PGD group using classical statistics led us to consider machine learning as an alternative approach. Keeping with classical statistics, machine learning models showed that BAL fluid is the specimen type with the greatest predictive value. [Supplementary Tables 2, 3](#) in the Methodological Supplement compare model performance across each predictor set. [Supplementary Table 2](#) presents the highest AUROC. To assess the sensitivity of these results to parameter tuning and moderate expectations for out-of-box performance, AUROCs in [Supplementary Table 3](#) are averaged from all models using near-optimal parameter settings. Given this, we next built a reduced model



TABLE 3 Results of multivariate Crámer tests of differences between undetected and severe primary graft dysfunction cases in protein concentrations across all time points.

| Protein | $n_0$ | $n_3$ | Statistic | p-value |
|---------|-------|-------|-----------|---------|
| BCA-1   | 42    | 42    | 5.007     | 0.0050  |
| IL-22   | 42    | 42    | 4.876     | 0.0240  |
| IL-15   | 42    | 42    | 1.646     | 0.0609  |
| 6CKine  | 42    | 42    | 1.892     | 0.0829  |
| Eotaxin | 42    | 42    | 0.850     | 0.0899  |

Analytes are listed in order of statistical evidence, up to unadjusted  $p < 0.1$ . See [Supplementary Table 1](#) for details on the full set of analytes. Abbreviations; BCA-1, B-cell-attracting chemokine 1 (CXCL13); IL-22, interleukin-22; IL-15, interleukin-15; 6CKine, secondary lymphoid-tissue chemokine (CCL21); Eotaxin, eosinophil chemotactic protein 1 (CCL11).

using only BAL data. After excluding NN models for worst performance and difficulty of importance measurement, we used a custom cumulative importance measure and a formalized “elbow method” cutoff to select candidate biomarkers (I-309, Fractalkine, IL-12p40, IL-5, FGF-2, PDGF-AA, IL-1RA, M-CSF, IL-6, BCA-1, IP-10, and Eotaxin). Using regularized logistic regression analysis of these 12 analytes, four (I-309, IL-5, PDGF-AA, and Eotaxin) were eliminated ([Figure 6A](#)). Subjectively, we selected three analytes, IL-1Ra, Fractalkine, and BCA-1, to determine their potential as candidates for predicting severe PGD. [Figure 6B](#) shows the link function and its values taken at all cases, showing that the model accurately predicts PGD in every case. A PCA of the 16 cases used to optimize the BAL models on these analytes showed that they clearly separate PGD subgroups ([Figure 6C](#)). As we had exhausted the available cases in the nested CV process, used to obtain optimal models, we could not further validate this model. Therefore, we used classical diagnostics and randomized quantile residuals [37] to evaluate goodness of fit. These identified some influential outliers. The same clinical predictor pool was used consistently across all models that included clinical data, either alone or in combination with biological specimen data. To assess the importance of specific clinical factors, optimized AUROCs for logistic regression models on a single clinical predictor each are reported in the Methodological Supplement ([Supplementary Figure S4](#)). These analyses were performed to contextualize the relative contribution of clinical variables compared with multiplex cytokine measurements.

## Robustness checks

To enhance the rigor of our approach we performed several robustness checks, as detailed in the methodological supplement.

## Discussion

Here we sought to identify donor and recipient inflammatory risk factors for the development of PGD to characterize its pathophysiology using a “hypothesis-free” approach. Our 71-analyte multiplex panel allowed us to examine previously reported markers of PGD while achieving unbiased discovery of new potential mechanistic/therapeutic targets and/or novel biomarkers. We sampled multiple biological sites from each recipient and obtained results using the same assay and data analysis methodology, allowing us to compare the utility of each

sample site against each other without bias from batch variability. It is important to distinguish the two analytical aims of this study. First, we performed associative analyses across plasma, perfusate, and BAL to identify cytokines linked to PGD status or peri-transplant temporal changes. Second, we used predictive modeling to compare the relative utility of different specimen types for discrimination of severe PGD and to prioritize a reduced set of candidate BAL analytes. Thus, the longitudinal plasma analyses were intended primarily to define peri-transplant inflammatory patterns and persistence of candidate signals over time, whereas predictive modeling focused on early specimen types most relevant to acute PGD classification. Our analysis demonstrated the presence of previously undescribed factors associated with PGD in these different biological samples and determined that T0 BAL had the strongest PGD predictive value.

To our knowledge, no previous human studies have investigated cytokine levels in static donor lung preservation solution. In keeping with a recent heart transplant multiplex study of heart preservation solutions [38], we identified a large array of detectable factors in lung perfusate, as 66 of the 71 cytokines were detected. Of the 66 identified cytokines, only IL-17a was detectably different between PGD groups. Our plasma studies provide insight into cytokine level changes across the transplant process and determine whether signatures of cytokines are associated with severe PGD. Fifteen analytes were detectably different in PGD+ vs. PGD-plasma at individual time points. Amongst these were IL-6 and MCP-1, which have been previously reported to be elevated in the plasma of PGD patients [5, 8–11]. While 15 analytes were detectably changed, only two, BCA-1 and IL-22, were detectably elevated in PGD at all timepoints, using multivariate approaches. To our knowledge, these cytokines have not been reported as plasma PGD biomarkers thus far and may indicate that PGD patients have increased early B cell signaling [39, 40]. Finally, we utilized T0 BAL as a window into the local lung microenvironment. A large array of analytes was present in BAL. Of the 71 analytes assessed, we demonstrated that eight (IL-1RA, Fractalkine, BCA-1, Eotaxin, IL-12p40, IL-6, IP-10, and M-CSF) were detectably elevated in PGD.

Using classical statistics, we identified novel analytes that have not been previously described or linked mechanistically in PGD. A secondary goal of our study was to explore whether our multiplex approach could optimize the identification of biomarkers that predict severe PGD. While most biomarker discovery studies use only statistical association tests or univariable prediction models to identify candidates, a growing number use multivariable models,



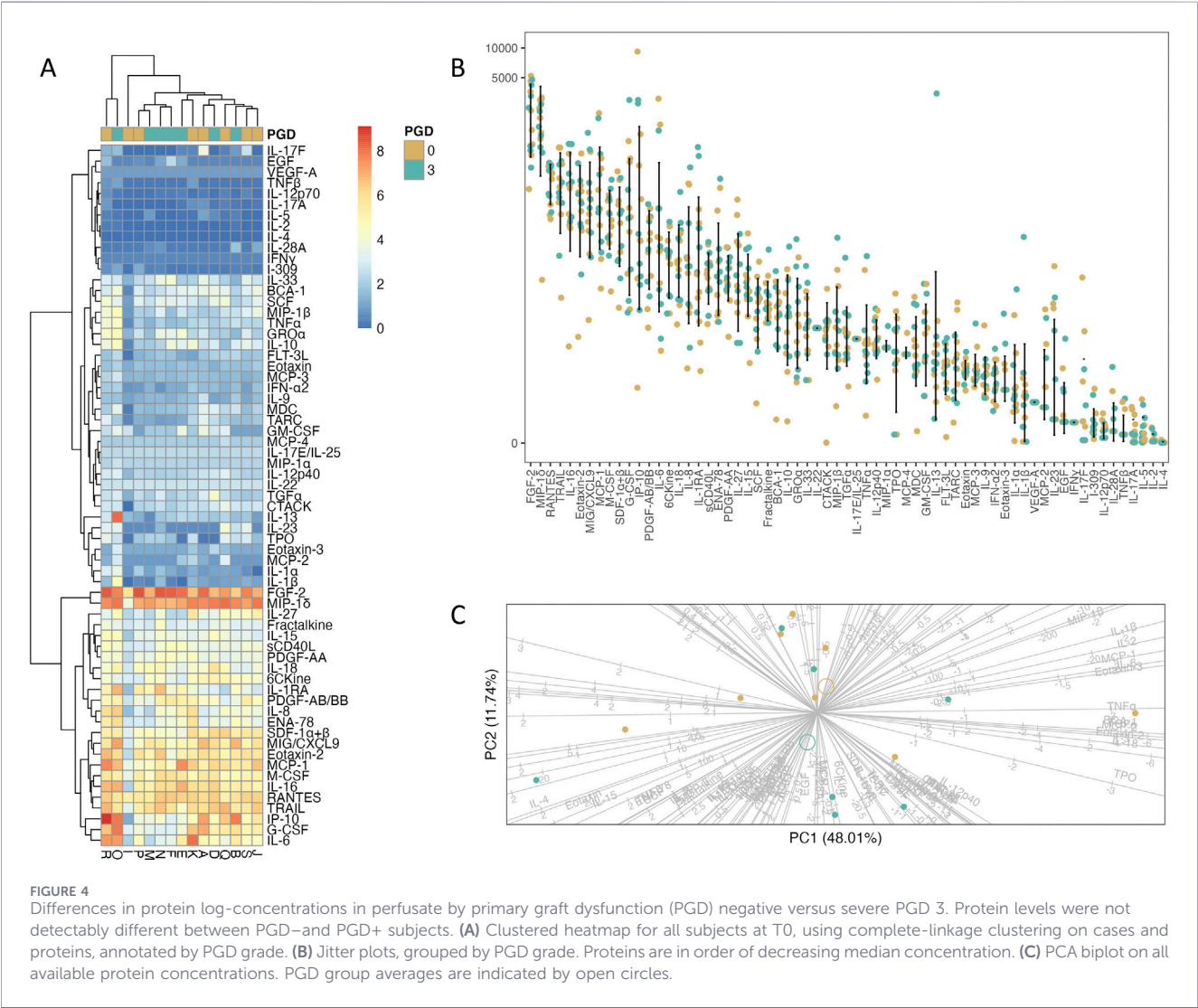
FIGURE 3

Differences in protein log-concentrations in plasma by PGD grade. Mean concentration and 1-standard deviation intervals across all time points, grouped by PGD grade. The panels are in order of increasing multivariate Cramér test unadjusted p-value and marked: \*\*\* $p < 0.0001$ ; \*\* $p < 0.001$ ; \* $p < 0.01$ ;  $p < 0.05$ . See the Methodological Supplement for a table of p-values. Note that tests detected differences in BCA-1 and IL-22 between PGD- and PGD+, though this number (2) is expected by chance. Irrespective of PGD grade, cytokines and chemokines peaked 24h post-transplantation in response to ischemia reperfusion injury.

TABLE 4 Results of Kolmogorov–Smirnov tests of differences between undetected and severe primary graft dysfunction cases in protein concentrations in perfusate.

| Protein | <i>n</i> <sub>0</sub> | <i>n</i> <sub>3</sub> | Statistic | p-value |
|---------|-----------------------|-----------------------|-----------|---------|
| IL-17A  | 8                     | 7                     | 0.625     | 0.0357  |
| IL-1RA  | 8                     | 7                     | 0.607     | 0.0870  |

Analytes are listed in order of statistical evidence, up to unadjusted *p* < 0.1. See [Supplementary Table 1](#) for details on the full set of analytes. Abbreviations; IL-17A, interleukin-17A; IL-1RA, interleukin-1 receptor antagonist.



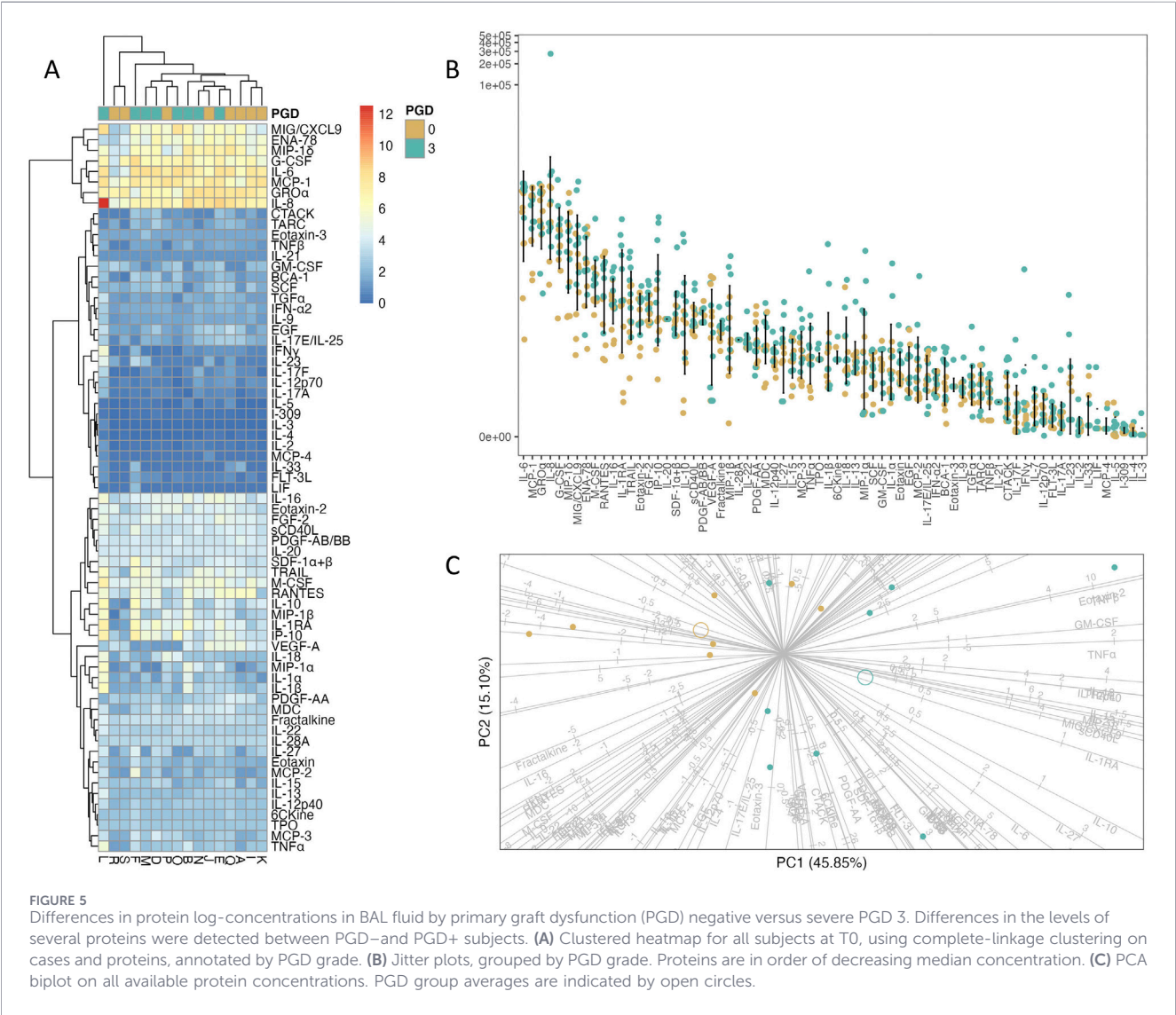
often internally cross-validated [41]. For example, Wolf *et al* [42] and Berra *et al* [43] used proteins measured in BAL by mass spectrometry or Western blot, in concert with FEV, to build machine learning models that successfully stratified the risk of CLAD development. To our knowledge, no multivariable studies have utilized patient-matched biospecimens from different sites to test multiplex data and sample types. Here we show that our multivariate predictive modeling comparisons indicated the optimum sample to analyze for its capacity to predict PGD onset, in our sample types, was BAL collected 2hrs post-transplant (T0). Using this exploratory reduction strategy, we prioritized IL-1Ra, CXCL13/BCA-1, and Fractalkine as

candidate BAL analytes associated with severe PGD. Using a LASSO analysis to assess the predictive power of analytes in T0 BAL specimen we identified three cytokines, IL-1Ra, BCA-1, and Fractalkine, as strong candidates that accurately predict PGD while minimizing model deviance. Except for IL-1Ra, these cytokines have not been previously correlated to PGD development. Interestingly, BCA-1 levels were also detectably elevated in plasma, both pre- and post-transplantation. Given the mechanism of action of BCA-1 [44, 45], our findings are interesting in light of recent studies implicating B cells in lung transplant IRI [46–48]. While not investigated in lung IRI or lung inflammation, Fractalkine has been shown to play important roles

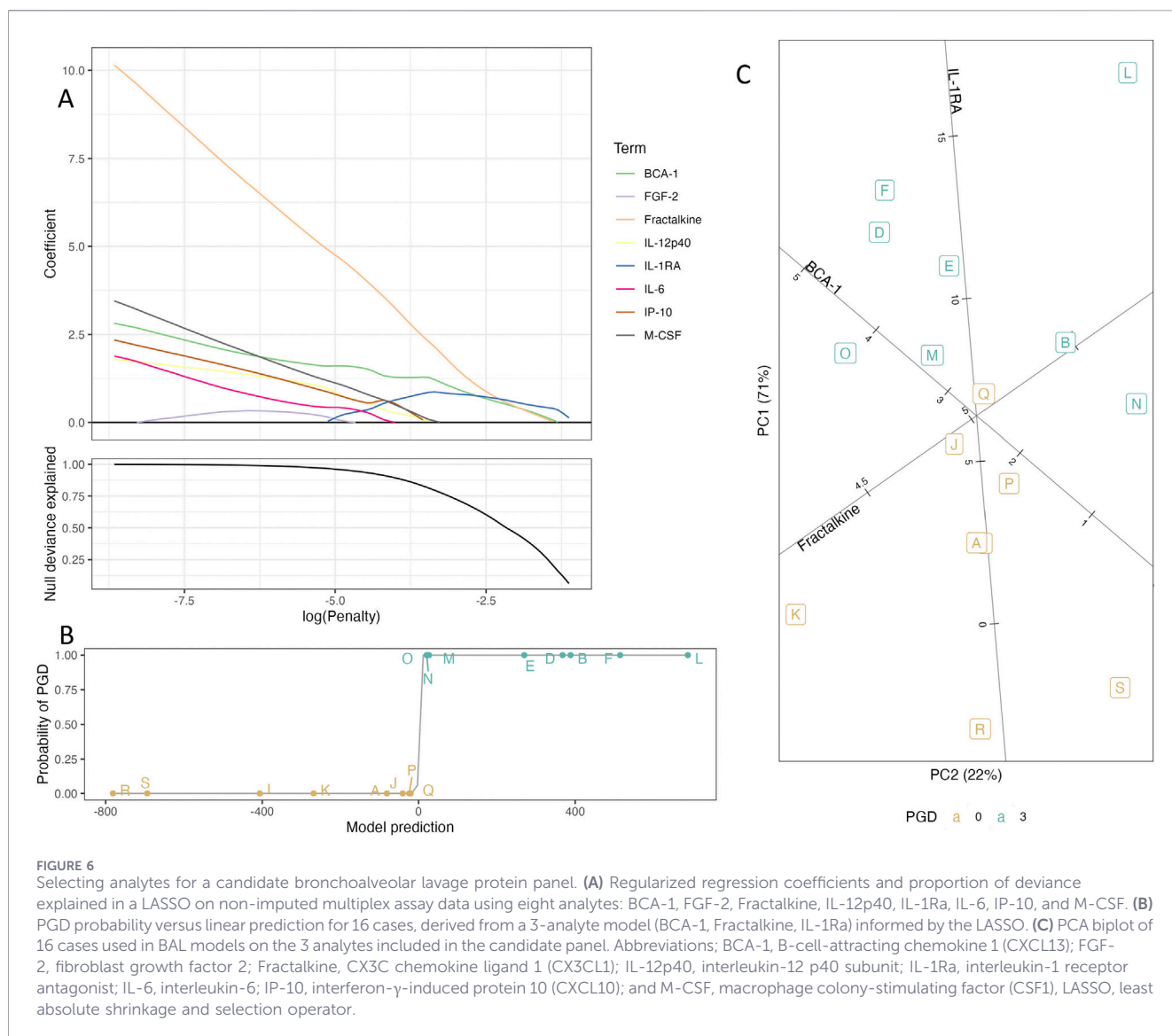
TABLE 5 Results of Kolmogorov–Smirnov tests of differences between undetected and severe primary graft dysfunction cases in protein concentrations in BAL fluid.

| Protein     | $n_0$ | $n_3$ | Statistic | p-value |
|-------------|-------|-------|-----------|---------|
| IL-1RA      | 8     | 8     | 0.875     | 0.00249 |
| Fractalkine | 8     | 8     | 0.750     | 0.01010 |
| BCA-1       | 8     | 8     | 0.750     | 0.01865 |
| Eotaxin     | 8     | 8     | 0.750     | 0.01865 |
| IL-12p40    | 8     | 8     | 0.750     | 0.01865 |
| IL-6        | 8     | 180   | 0.750     | 0.01865 |
| IP-10       | 8     | 8     | 0.750     | 0.01865 |
| M-CSF       | 8     | 8     | 0.750     | 0.01865 |

Analytes are listed in order of statistical evidence, up to unadjusted  $p < 0.05$ . See [Supplementary Table 1](#) for details on the full set of analytes. Abbreviations; BCA-1, B-cell-attracting chemokine 1 (CXCL13); 6CKine, secondary lymphoid-tissue chemokine (CCL21); IL-22, interleukin-22; IL-16, interleukin-16; MCP-4, monocyte chemoattractant protein 4 (CCL13); IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein 1 (CCL2); IL-15, interleukin-15; I-309, T-lymphocyte-secreted protein I-309 (CCL1); PDGF-AA, platelet-derived growth factor-AA; IL-12p40, interleukin-12 p40 subunit; IL-3, interleukin-3; TRAIL, TNF-related apoptosis-inducing ligand; PDGF-AB/BB, platelet-derived growth factor-AB/BB; MIP-1 $\delta$ , macrophage inflammatory protein-1 delta (CCL15).







in the context of cardiac IRI [49–51] and inflammation [52, 53]. Taken together, our findings support the need for future functional studies investigating the roles of IL-1Ra, BCA-1, and Fractalkine to fully evaluate the significance of these mediators in PGD.

Recent studies have utilized cytokine rapid testing to identify and stratify patients at risk of hospitalization upon presenting with respiratory distress [54]. Given the early post-transplant signal of IL-1Ra, BCA-1, and Fractalkine, observed in BAL, these findings raise the possibility that a focused cytokine panel could ultimately contribute to early PGD risk stratification. Our predictive modeling passed multiple data robustness checks. However, the present study is not sufficient to establish clinical utility, and any point-of-care application will require external validation, calibration testing, and assessment in broader cohorts that include intermediate and fluctuating PGD phenotypes. As such, these markers should not yet be considered a validated predictive panel but rather a biologically plausible and clinically testable candidate signature for follow-up studies.

Previous multiplex cytokine studies in cardiac transplantation showed similar large-scale cytokine changes at different time points post-transplant and note, as we have, new target cytokines [38]. A major difference in our study, beyond its lung focus, was the inclusion of patients with different early graft outcomes. By incorporating PGD+ and PGD-patients, we were able to not only map cytokine changes across sample type and time but further identified novel analytes that could have mechanistic and predictive value for the discrimination of PGD. To promote dichotomization in our machine learning models, we included patients that were either PGD 0 or 3 at all timepoints (24, 48, and 72 h). We acknowledge the accepted PGD construct is to collapse the 0–72 h grades 1–3 into PGD3 at 48 or 72h vs. no PGD3 at 48 or 72 h<sup>1</sup>. However, we believe our approach optimized its discovery and predictive ability given the relatively small sample size. Nevertheless, future studies are needed to validate the analytes we discovered using a larger sample cohort and accepted PGD diagnostic construct.



This study has several important limitations. Most notably, the cohort size was small relative to the dimensionality of the multiplex and modeling analyses, increasing the risk of model instability and overfitting. Accordingly, the very high predictive performance observed in some combined-feature models, including near-perfect AUROC values, should not be interpreted as evidence of a clinically ready classifier but rather as support for this framework as an exploratory approach to prioritize candidate biomarkers and informative specimen types. We focused our studies on the extremes of PGD, with our two groups split between PGD0 and PGD3 at all timepoints. We recognize that this approach does not capture the full clinical spectrum of PGD nor establish how identified markers would perform in intermediate phenotypes. Evaluation of PGD grades 1–2 and temporally dynamic trajectories will require substantially larger cohorts and will be the focus of future validation studies.

Baseline differences between PGD groups may also have influenced the observed biological signals. Although available clinical predictors were evaluated individually and in combination with biological data, and no single measured clinical variable performed comparably to the BAL cytokine signal, residual confounding cannot be excluded. In addition, several relevant donor and preservation variables, including ischemic time, donor PaO<sub>2</sub>/FiO<sub>2</sub>, ventilator settings, and flush volume, were unavailable in the de-identified tissue bank dataset, limiting our ability to assess their contribution to group differences.

Taken together, these considerations emphasize that the identified BAL analytes, including IL-1Ra, CXCL13/BCA-1, and Fractalkine, should be regarded as hypothesis-generating candidates. Validation in larger, independent, prospectively collected cohorts with more complete donor and perioperative annotation will be required.

In conclusion, we used matched perfusate, plasma, and BAL biospecimens to define inflammatory signatures associated with severe PGD after LTx. Across specimen types, early BAL demonstrated the clearest relationship with PGD status and identified BCA-1, Fractalkine, and IL-1Ra as candidate analytes for further study, supporting their potential relevance to PGD pathophysiology and the need for future mechanistic investigation. These findings should be interpreted cautiously given the limited cohort size, incomplete availability of certain donor and preservation variables, and the exploratory nature of the analyses. Nonetheless, this study provides a framework for integrating multiplex profiling with multivariable machine learning approaches to nominate biologically relevant, testable biomarkers and mechanistic targets for future translational PGD studies.

## 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.

## References

- Christie JD, Bellamy S, Ware LB, Lederer D, Hadjiladis D, Lee J, et al. Construct validity of the definition of primary graft dysfunction after lung transplantation. *The J Heart Lung Transplant* (2010) 29(11):1231–9. doi: 10.1016/j.healun.2010.05.013
- Huang HJ, Yusef RD, Meyers BF, Walter MJ, Mohanakumar T, Patterson GA, et al. Late primary graft dysfunction after lung transplantation and bronchiolitis obliterans syndrome. *Am J Transplant Official J Am Soc Transplant Am Soc Transpl Surgeons* (2008) 8(11):2454–62. doi: 10.1111/j.1600-6143.2008.02389.x

## Ethics statement

The studies involving humans were approved by University of Florida Institutional Review Board IRB#202200536. 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

Research concept and design: JCB, DN, and CA. Data acquisition, analysis, and interpretation: JCB, DN, LL, HM, BG, MR, AS, CL, AE, and CA. Manuscript drafting/writing: DN, JCB, and CA. 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.2026.15874/full#supplementary-material>

3. Daud SA, Yusen RD, Meyers BF, Chakinala MM, Walter MJ, Aloush AA, et al. Impact of immediate primary lung allograft dysfunction on bronchiolitis obliterans syndrome. *Am J Respir Crit Care Med* (2007) 175(5):507–13. doi: 10.1164/rccm.200608-1079OC
4. Kreisel D, Krupnick AS, Puri V, Guthrie TJ, Trulock EP, Meyers BF, et al. Short- and long-term outcomes of 1000 adult lung transplant recipients at a single center. *The J Thorac Cardiovasc Surg* (2011) 141(1):215–22. doi: 10.1016/j.jtcvs.2010.09.009
5. Moreno I, Vicente R, Ramos F, Vicente JL, Barberá M. Determination of Interleukin-6 in lung transplantation: association with primary graft dysfunction. *Transplant Proc* (2007) 39(7):2425–6. doi: 10.1016/j.transproceed.2007.07.056
6. Chacon-Alberty L, Kanchi RS, Ye S, Hochman-Mendez C, Daoud D, Coarfa C, et al. Plasma protein biomarkers for primary graft dysfunction after lung transplantation: a single-center cohort analysis. *Scientific Rep* (2022) 12(1):16137. doi: 10.1038/s41598-022-20085-y
7. Mathur A, Baz M, Staples ED, Bonnell M, Speckman JM, Hess PJ, et al. Cytokine profile after lung transplantation: cor-relation with allograft injury. *The Ann Thorac Surg* (2006) 81(5):1844–50. doi: 10.1016/j.athoracsur.2005.11.053
8. Hoffman SA, Wang L, Shah CV, Ahya V, Surname G, Pochettino A, et al. Plasma cytokines and chemokines in primary graft dysfunction post-lung transplantation. *Am J Transplant Official J Am Soc Transplant Am Soc Transpl Surgeons* (2009) 9(2):389–96. doi: 10.1111/j.1600-6143.2008.02497.x
9. Bharat A, Kuo E, Steward N, Aloush A, Hachem R, Trulock EP, et al. Immunological link between primary graft dysfunction and chronic lung allograft rejection. *The Ann Thorac Surg* (2008) 86(1):189–97. doi: 10.1016/j.athoracsur.2008.03.073
10. Shah RJ, Bellamy SL, Localio AR, Wickersham N, Diamond JM, Weinacker A, et al. A panel of lung injury biomarkers enhances the definition of primary graft dysfunction (PGD) after lung transplantation. *The J Heart Lung Transplant* (2012) 31(9):942–9. doi: 10.1016/j.healun.2012.05.001
11. Bonneau S, Landry C, Bégin S, Adam D, Villeneuve L, Clavet-Lanthier MÈ, et al. Correlation between neutrophil extracellular traps (NETs) expression and primary graft dysfunction following human lung transplantation. *Cells* (2022) 11(21):3420. doi: 10.3390/cells11213420
12. Allen JG, Lee MT, Weiss ES, Arnaoutakis GJ, Shah AS, Detrick B. Preoperative recipient cy-tokine levels are associated with early lung allograft dysfunction. *The Ann Thorac Surg* (2012) 93(6):1843–9. doi: 10.1016/j.athoracsur.2012.02.041
13. Christie JD, Robinson N, Ware LB, Plotnick M, De Andrade J, Lama V, et al. Association of protein C and type 1 plasminogen activator inhibitor with primary graft dysfunction. *Am J Respir Crit Care Med* (2007) 175(1):69–74. doi: 10.1164/rccm.200606-827OC
14. Frick AE, Verleden SE, Ordies S, Sacreas A, Vos R, Verleden GM, et al. Early protein expression profile in bronchoalveolar lavage fluid and clinical outcomes in primary graft dysfunction after lung transplantation. *Eur J Cardio-Thoracic Surg* (2020) 58(2):379–88. doi: 10.1093/ejcts/ezaa043
15. Verleden SE, Martens A, Ordies S, Neyrinck AP, Van Raemdonck DE, Verleden GM, et al. Immediate post-operative broncho-alveolar lavage IL -6 and IL -8 are associated with early outcomes after lung transplantation. *Clin Transplant* (2018) 32(4):e13219. doi: 10.1111/ctr.13219
16. DerHovannessian A, Weigt SS, Palchevskiy V, Shino MY, Sayah DM, Gregson AL, et al. The role of TGF- $\beta$  in the association between primary graft dysfunction and bronchiolitis obliterans syndrome. *Am J Transplan- Tation: Official J Am Soc Transplant Am Soc Transpl Surgeons*. (2016) 16(2):640–9. doi: 10.1111/ajt.13475
17. Machuca TN, Cypel M, Yeung JC, Bonato R, Zamel R, Chen M, et al. Protein expression profiling predicts graft Per-formance in clinical Ex Vivo lung perfusion. *Ann Surg* (2015) 261(3):591–7. doi: 10.1097/SLA.0000000000000974
18. Ghaidan H, Stenlo M, Niroomand A, Mittendorfer M, Hirdman G, Gvazava N, et al. Reduction of primary graft dysfunction using cytokine adsorption during organ preservation and after lung transplantation. *Nat Commun* (2022) 13(1):4173. doi: 10.1038/s41467-022-31811-5
19. Snell GI, Yusen 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. *The J Heart Lung Transplant* (2017) 36(10):1097–103. doi: 10.1016/j.healun.2017.07.021
20. Martinu T, Koutsokera A, Benden C, Cantu E, Chambers D, Cypel M, et al. International society for heart and lung transplantation consensus statement for the standardization of bronchoalveolar lavage in lung transplantation. *The J Heart Lung Transplant The Official Publ Int Soc Heart Transplant* (2020) 39(11):1171–90. doi: 10.1016/j.healun.2020.07.006
21. R Core Team. *R: A Language and Environment for Statistical Computing* (2021).
22. Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, et al. Welcome to the tidyverse. *J Open Source Softw* (2019) 4(43):1686. doi: 10.21105/joss.01686
23. Kuhn M, Wickham H. *Tidymodels: A Collection of Packages for Modeling and Machine Learning Using Tidyverse Principles* (2020).
24. Wickham H, Bryan J. *Readxl: Read Excel Files* (2023).
25. Tierney N, Cook D, McBain M, Fay C, O'Hara-Wild, Hester J, et al. *Naniar: Data Structures, Summaries, and Visualisations for Missing Data* (2023).
26. Tierney N, Hughes S, Averick M, Lee S, Wang E, Crane N, et al. *Visdat: Preliminary Visualisation of Data* (2023).
27. Kolde R. *Pheatmap: Pretty Heatmaps* (2019).
28. Wei T, Simko V, Levy M. *Corrplot: Visualization of a Correlation Matrix* (2021).
29. Brunson JC, Paul E. *Ordr: A 'Tidyverse' Extension for Ordinations and Biplots* (2022).
30. Dahl DB, Scott D, Roosen C. *Xtable: Export Tables to Latex or HTML* (2019).
31. Greenwell BM, Boehmke B. *Vip: Variable Importance Plots* (2023).
32. Pedersen TL. *Tidygraph: A Tidy API for Graph Manipulation* (2024).
33. Pedersen TL. *Ggraph: An Implementation of Grammar of Graphics for Graphs and Networks* (2024).
34. Galili T, Benjamini Y, Simpson G. *Dendextend: Extending 'Dendrogram' Functionality in R* (2023).
35. Kaplan J, Schlegel B. *Fastdummies: Fast Creation of Dummy (Binary) Columns and Rows from Categorical Variables* (2023).
36. Baringhaus L, Franz C. On a new multivariate two-sample test. *J Multivariate Anal* (2004) 88(1):190–206. doi: 10.1016/S0047-259X(03)00079-4
37. Dunn PK, Smyth GK. Randomized quantile residuals. *J Comput Graphical Stat* (1996) 5(3):236–44. doi: 10.1080/10618600.1996.10474708
38. Ledwoch N, Wiegmann B, Chichelnitskiy E, Wandrer F, Kühne JF, Beushausen K, et al. Identification of distinct secretory patterns and their regulatory networks of ischemia versus reperfusion phases in clinical heart transplantation. *Cytokine* (2022) 149:155744. doi: 10.1016/j.cyto.2021.155744
39. Mao Y, Wang M, Zhou Q, Jin J, Peng W, Wu J, et al. CXCL10 and CXCL13 expression were highly Up-regulated in peripheral blood mononuclear cells in acute rejection and poor response to anti-rejection therapy. *J Clin Immunol* (2011) 31(3):414–8. doi: 10.1007/s10875-010-9500-8
40. Arshad T, Mansur F, Palek R, Manzoor S, Liska V. A double edged sword role of Interleukin-22 in wound healing and tissue regeneration. *Front Immunol* (2020) 11:2148. doi: 10.3389/fimmu.2020.02148
41. Halloran K, Mackova M, Parkes MD, Hirji A, Weinkauff J, Timofte IL, et al. The molecular features of chronic lung allograft dysfunction in lung transplant airway mucosa. *The J Heart Lung Transplant* (2022) 41(12):1689–99. doi: 10.1016/j.healun.2022.08.014
42. Wolf T, Oumeraci T, Gottlieb J, Pich A, Brors B, Eils R, et al. Proteomic bronchiolitis Obliterans syndrome risk monitoring in lung transplant recipients. *Transplantation* (2011) 92(4):477–85. doi: 10.1097/TP.0b013e318224c109
43. Berra G, Farkona S, Mohammed-Ali Z, Kotlyar M, Levy L, Clotet-Freixas S, et al. Association between renin-angiotensin system and chronic lung allograft dysfunction. *Eur Respir J* (2021) 58:2002975. doi: 10.1183/13993003.02975-2020
44. Legler DF, Loetscher M, Roos RS, Clark-Lewis I, Baggiolini M, Moser B. B cell-attracting chemokine 1, a human CXC chemokine expressed in lymphoid tissues, selectively attracts B lymphocytes via BLR1/CXCR5. *The J Exp Med* (1998) 187(4):655–60. doi: 10.1084/jem.187.4.655
45. Jenh CH, Cox MA, Hipkin W, Lu T, Pugliese-Sivo C, Gonsiorek W, et al. Human B cell-attracting chemokine 1 (BCA-1; CXCL13) is an agonist for the human CXCR3 receptor. *Cytokine* (2001) 15(3):113–21. doi: 10.1006/cyto.2001.0923
46. Farahnak K, Bai YZ, Yokoyama Y, Morkan DB, Liu Z, Amrute JM, et al. B cells mediate lung ischemia/reperfusion injury by recruiting classical monocytes via synergistic B cell receptor/TLR4 signaling. *The J Clin Invest* (2024) 134(6):e170118. doi: 10.1172/JCI170118
47. Li W, Gauthier JM, Higashikubo R, Hsiao HM, Tanaka S, Vuong L, et al. Bronchus-associated lymphoid tissue-resident Foxp3+ T lymphocytes prevent antibody-mediated lung rejection. *The J Clin Invest* (2019) 129(2):556–68. doi: 10.1172/JCI122083
48. Tanaka S, Gauthier JM, Fuchs A, Li W, Tong AY, Harrison MS, et al. IL-22 is required for the induction of bronchus-associated lymphoid tissue in tolerant lung allografts. *Am J Transplant Official J Am Soc Transplant Am Soc Transpl Surgeons* (2020) 20(5):51–61. doi: 10.1111/ajt.15701
49. Grosse GM, Tryc AB, Dirks M, Schuppner R, Pflugrad H, Lichtinghagen R, et al. The temporal dynamics of plasma fractalkine levels in ischemic stroke: association with clinical severity and outcome. *J Neuroinflammation* (2014) 11:74. doi: 10.1186/1742-2094-11-74
50. Kanzawa T, Tokita D, Saiga K, Yamakawa T, Ishigooka H, Fukuda H, et al. Role of Fractalkine-CX3CR1 axis in acute rejection of mouse heart allografts subjected to ischemia reperfusion injury. *Transpl Int Official J Eur Soc Organ Transplant* (2022) 35:10157. doi: 10.3389/ti.2022.10157
51. Loh SX, Ekinci Y, Spray L, Jeyalan V, Olin T, Richardson G, et al. Fractalkine signalling (CX3CL1/CX3CR1 axis) as an emerging target in coronary artery disease. *J Clin Med* (2023) 12(14):4821. doi: 10.3390/jcm12144821
52. Arend WP. The balance between IL-1 and IL-1Ra in disease. *Cytokine and Growth Factor Rev* (2002) 13(4-5):323–40. doi: 10.1016/s1359-6101(02)00020-5
53. Griffith JW, Faustino LD, Cottrell VI, Nepal K, Hariri LP, Chiu RSY, et al. Regulatory T cell-derived IL-1Ra suppresses the innate response to respiratory viral infection. *Nat Immunol* (2023) 24(12):2091–107. doi: 10.1038/s41590-023-01655-2
54. Husain S, Sage AT, Del Sorbo L, Cypel M, Martinu T, Juvet SC, et al. A biomarker assay to risk-stratify patients with symp-toms of respiratory tract infection. *The Eur Respir J* (2022) 60(6):2200459. doi: 10.1183/13993003.00459-2022



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# Modeling the cost-effectiveness of introducing donation after controlled circulatory death for heart transplantation in France

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Heart transplantation following donation after controlled circulatory death (DCD) could alleviate the global shortage of organs. We assessed the cost-effectiveness of this strategy and outlined the factors enabling an efficient DCD heart program. Two strategies were compared: donation after brain death (DBD) using static cold storage vs. DBD + DCD transplantation, where DCD hearts are preserved with a normothermic *ex situ* perfusion system. Costs (€; 2024 value) and outcomes (quality-adjusted life years (QALYs)) were modelled through a 15-year time horizon. Our main assumption was that DCD would increase transplant access without affecting waitlist mortality, with equivalent post-transplant outcomes. With a 20% annual increase in transplant activity, the DBD + DCD strategy resulted in a mean incremental gain of 0.14 QALYs at an additional cost of €20,618 compared with DBD alone. The incremental cost-effectiveness ratio was €146,373 per QALY gained. An 8% relative reduction in waitlist mortality (i.e., four additional lives saved annually among 500 new waitlisted patients) would make the DCD heart program cost-effective at €100,000 per QALY. Lower consumable costs would further improve cost-effectiveness. Decision-makers should consider these findings in light of the disease severity and the persistent shortage of heart donors that could be alleviated with DCD.

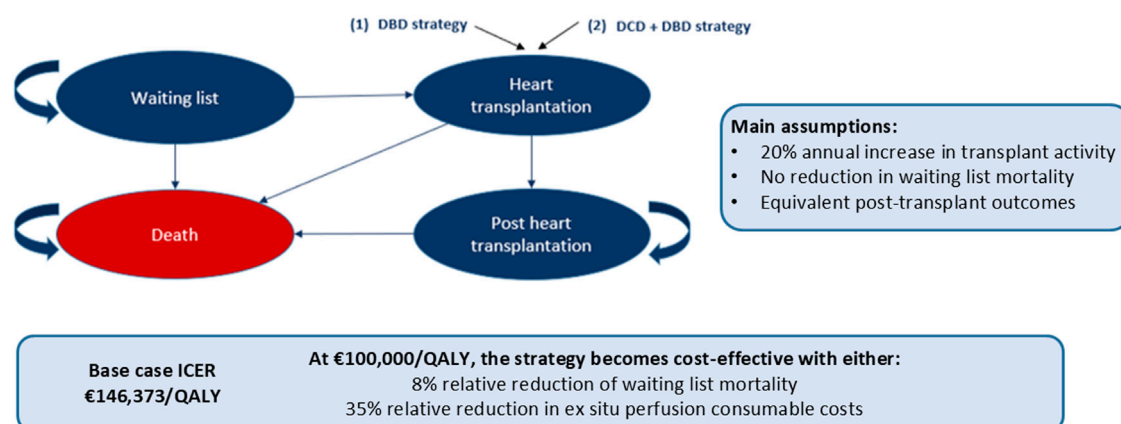
### KEYWORDS

cost effectiveness, donation after controlled circulatory death (DCD), donor pool expansion, heart transplantation, policy

## Introduction

Heart transplantation typically relies on the use of brain-dead donor hearts, which constitutes the main source of donation [1]. However, continuous large imbalances between organ supply and demand for heart transplantations has led to the investigation of new strategies to expand the donor pool [2]. Among these strategies, transplantation with donor hearts after controlled circulatory death has been of particular interest in recent years [3–9]. Increasing consideration of this strategy has been driven by the evolution of technologies, such as *ex situ* organ perfusion systems, allowing for reanimation of the heart after circulatory death and for evaluation of its suitability for transplantation [10]. Heart

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GRAPHICAL ABSTRACT

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transplantation using circulatory-dead donor hearts has been proved non-inferior to standard care transplantation using brain-dead donor hearts in terms of risk-adjusted survival at 6 months in a prospective randomized controlled trial [6]. A protocol to assess the feasibility of heart transplantation following Donation after controlled Circulatory Death (DCD) was conducted in France (Protocol PFS20-004, French Agency of Biomedicine) [11]. However, data on the cost-effectiveness of this donor pool expansion strategy are lacking. Indeed, DCD heart procurements are expensive (54,000 euro per normothermic *ex-situ* perfusion consumable) but may improve outcomes for patients with end-stage heart failure by increasing their chances to receive a graft with comparable post-transplantation outcomes [12]. Herein, we sought to model the cost effectiveness of introducing DCD heart transplantation alongside the current standard practice of Donation after Brain Death (DBD) with static cold storage from the French healthcare system perspective and to outline the factors enabling an efficient DCD heart program.

## Materials and methods

A cohort discrete-time state transition model with a time-inhomogeneous Markov process was developed [13]. In these decision models, a hypothetical cohort of individuals transition over discrete time periods (i.e., cycles) between mutually exclusive health states representing the clinical course of the disease [14]. The time inhomogeneous approach relaxes the Markov assumption, allowing transitions, rewards, or both to vary over time [13]. A cost utility analysis framework was used to compare costs and effects (expressed as quality-adjusted life years or QALYs) from the French healthcare

system perspective between standard care and the innovative strategy [15]. The model was built and run in R (version 4.2.2) within the R Studio software and using the R package “heemod” [16]. We followed the Consolidated Health Economic Evaluation Reporting Standards (CHEERS) reporting guidelines [17].

## Population

The modelled population consisted of adult patients (age  $\geq 18$  years) with end-stage heart failure who were eligible for a first heart transplantation and excluded patients awaiting multi-organ transplantations.

## Comparators

Two strategies were compared. The standard care strategy (DBD strategy) consists of performing transplantations using hearts from brain-dead donors, preserved via static cold storage, which remains the most widespread approach in France. The innovative strategy (DCD + DBD strategy) consists of performing DCD heart transplantations alongside the standard DBD approach. It involves the use of DCD hearts in accordance with the protocol in France (Protocol PFS20-004, French Agency of Biomedicine) [11]. In brief, individuals become donors after controlled withdrawal of life-sustaining treatment, followed by cardiac arrest and circulatory death; the direct procurement procedure is then undertaken, with the heart preserved using a normothermic *ex situ* perfusion system. This approach was developed to introduce heart retrieval within the existing French DCD protocol based on abdominal normothermic regional perfusion (NRP) for abdominal organ procurement.



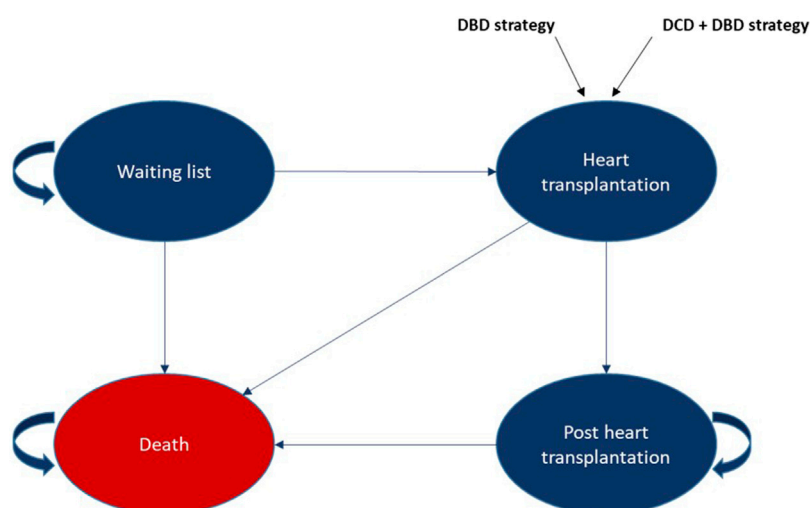


**FIGURE 1**  
Health state transition diagram. DCD: donation after circulatory death; DBD: donation after brain death. The health state transition diagram consisted of four distinct health states: waiting list, heart transplantation, post heart transplantation, and death. The heart transplantation state is a temporary state that an individual must leave after one cycle. The cycle duration was set to 1 month and time horizon to 15 years.

## Model structure

The model structure was adapted from previously published cost-effectiveness models in end-stage heart failure [18, 19] and in the context of liver transplantation, where strategies aimed at expanding the donor pool were evaluated [20, 21]. It consisted of four distinct health states (Figure 1). The cycle duration was set to 1 month, and half cycle correction was performed using the life table method [22]. A time horizon of 15 years was chosen due to deep uncertainty beyond that period and based on French data from the French Agency of Biomedicine. Most patients are transplanted or deceased within 3 years from registration on the waiting list, and the median survival after transplantation is roughly 12 years [23]. Costs and QALYs were discounted at 2.5% according to French recommendations [24].

## Model parameters and assumptions

Crude epidemiological, cost, and utility estimates used to parametrize the model are presented in Table 1.

### Transition probabilities

Short and long-term outcomes after heart transplantation were considered equivalent between the two strategies, consistent with the findings of the DCD Heart Trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03831048) number: NCT03831048) and early U.S. experience with a three-year follow-up [6, 25]. In order to assess efficiency in a French context, we mainly used epidemiological and survival data from the French Agency of Biomedicine [23]. For the post-transplantation period, individual patient data were reconstructed from a published survival curve of recipients after a first heart transplantation (period 2004 – June 2022) [26]. We then fitted a log-normal parametric model after comparing the goodness of fit of different models and their plausibility when compared to empirical

data and derived monthly probabilities for each model cycle (Supplementary Figures 1a–c; Supplementary Table 1). As no published data exists on the potential of DCD to increase the French heart-donor pool, an estimate based on an analysis of the United Network for Organ Sharing (UNOS) deceased donor database 2005–2014 was used [27]. The study reports a 30% projected annual increase with DCD heart transplantation but acknowledges possible overestimation, as some DCD hearts may be deemed unsuitable after functional assessment. A more conservative 20% annual increase was therefore modeled. This assumption was also considered consistent with the French transplantation context, where DCD heart transplantation is expected to begin with a pilot phase and to be progressively implemented across heart transplant centers. Uncertainty around this parameter was explored in the deterministic sensitivity analysis, using a 15%–25% range.

While the increase of available grafts should improve the probability of transplantation in waitlisted candidates, it is unclear whether expanding the donor pool with DCD would reduce waiting list mortality [12, 28]. Therefore, the assumption made would be conservative in the main analysis and implementing DCD would only increase transplant probability without affecting mortality (worst case scenario).

### Costs

Given the scarcity of economic information available for outpatient care in this indication, the costs considered were limited to those in a hospital setting, which were supposed to be the main cost drivers. Costs were valued from the healthcare system perspective according to French guidelines, which focuses solely on healthcare production and accounts for all monetary costs of healthcare, regardless of who bears the cost [24, 29]. For the pre-transplantation period, costs were extracted from a previous work which assessed the economic burden of patients awaiting heart



TABLE 1 Parameters considered in the model.

| Parameter                                                                  | Estimate* | Range*          | Distribution** | Source                                         |
|----------------------------------------------------------------------------|-----------|-----------------|----------------|------------------------------------------------|
| <b>Transition probabilities</b>                                            |           |                 |                |                                                |
| Probability (%) of HT at 1-year post listing                               | 61.6      | 49.3–73.9       | Beta           | French agency of biomedicine                   |
| Probability (%) of death at 1-year post listing                            | 10.2      | 8.2–12.2        | Beta           | French agency of biomedicine                   |
| Survival after first HT (median in months)                                 | 146.7     | 140.5–152.2     | Lognormal      | French agency of biomedicine                   |
| Annual increase of transplant activity with DCD (%)                        | 20        | 15–25           | Triangle       | Jawitz et al, 2020                             |
| <b>Costs (€ 2024 price year)</b>                                           |           |                 |                |                                                |
| Mean cost per patient from WL to 1-year post listing                       | 16,614    | 10,797–25,771   | Gamma          | Atfeh et al, 2025                              |
| Mean cost per patient of HT surgery with static cold storage               | 148,958   | 119,166–178,750 | Gamma          | DRG 27C054 - French national cost study (2022) |
| Cost of normothermic <i>ex-situ</i> perfusion consumable (including taxes) | 54,000    | 43,200–64,800   | Gamma          | Unitary purchase price                         |
| Mean cost per patient in the first year after HT                           | 13,722    | 10,695–16,749   | Gamma          | CUPIDON study (NCT02602691)                    |
| Mean cost per patient in the second year after HT                          | 7,625     | 5,943–9,307     | Gamma          | CUPIDON study (NCT02602691)                    |
| Mean cost per patient in the third year after HT and beyond                | 3,919     | 3,054–4,784     | Gamma          | CUPIDON study (NCT02602691)                    |
| <b>Utilities</b>                                                           |           |                 |                |                                                |
| On the waiting list                                                        | 0.44      | 0.17–0.71       | Beta           | Emin et al, 2016                               |
| When transplanted                                                          | 0.74      | 0.47–1          | Beta           | Emin et al, 2016                               |
| Post-HT                                                                    |           |                 |                |                                                |
| First year                                                                 | 0.82      | 0.64–1          | Beta           | CUPIDON study (NCT02602691)                    |
| 1.5 years                                                                  | 0.81      | 0.63–0.99       | Beta           | CUPIDON study (NCT02602691)                    |
| 2 years                                                                    | 0.80      | 0.62–0.98       | Beta           | CUPIDON study (NCT02602691)                    |
| 2.5 years                                                                  | 0.79      | 0.61–0.97       | Beta           | CUPIDON study (NCT02602691)                    |
| 3 years and beyond                                                         | 0.76      | 0.58–0.94       | Beta           | CUPIDON study (NCT02602691)                    |

WL, waiting list; HT, heart transplantation; DCD, donation after controlled circulatory death.

\*Estimates and ranges were adjusted to 1-month cycle lengths in the model when necessary. Ranges were used for the deterministic sensitivity analysis.

\*\*Distributions used in the probabilistic sensitivity analysis. Method of moments was used to estimate parameter distributions.

transplantation [30]. The cost of DBD transplantation in standard care was based on the Diagnosis Related Group 27C054 and was valued using the French National Cost Study, which produces the closest valuation to the hospital production cost [24]. The cost of DCD transplantation was computed as that of DBD transplantation, plus the cost of using an *ex-situ* perfusion system; its cost being the unit purchase price of consumables of €54,000 including taxes (under the manufacturer's business model, the equipment was provided free of charge). The total cost of heart transplantation for the innovative strategy was then calculated in proportion to the annual increase in the volume of heart transplantations performed and the ratio between DBD and DCD heart transplantations. For the post-transplantation period, cost data were extracted from the French CUPIDON study [31] (ClinicalTrials.gov identifier NCT02602691), covering individual patient costs from 6 to 36 months after transplantation. The first 6 months were extrapolated from the monthly costs between months 6 and 12 reported in the CUPIDON study. They were also assumed equivalent between the two strategies, in line with the clinical assumptions, and to remain stable beyond 36 months, consistent with published cost-effectiveness models in this indication [18, 19]. All costs were expressed in euros (to convert to US dollars, multiply by 1.16) at 2024 price year and adjusted for inflation based on the

French National Institute of Statistics and Economic Studies (INSEE) Consumer Price Indices of the healthcare products and services [32].

## Utilities

After conducting a literature search, we retrieved a systematic review of health state utilities in patients with heart failure [33]. As no data were available in France, utility scores on the waiting list and at the time of transplantation were extracted from a cross-sectional survey of the United Kingdom Cardiothoracic Transplant Audit with administration of EQ-5D questionnaires [34]. We chose this study because it was the only one to provide utility scores on a population of end-stage heart failure patients eligible for transplantation, defined on the basis of registration on the waiting list. Although cross-country differences in general population preferences may exist, France and the United Kingdom are culturally and economically comparable; therefore, UK-based utilities were considered reasonable proxies for France. For the DCD + DBD strategy, in the base case analysis, no utility decrement was applied to the temporary transplantation state in relation to the potential increased risk of primary graft dysfunction (PGD) with circulatory-dead donor hearts [35]. It was expected to be marginal given the ratio between DBD and

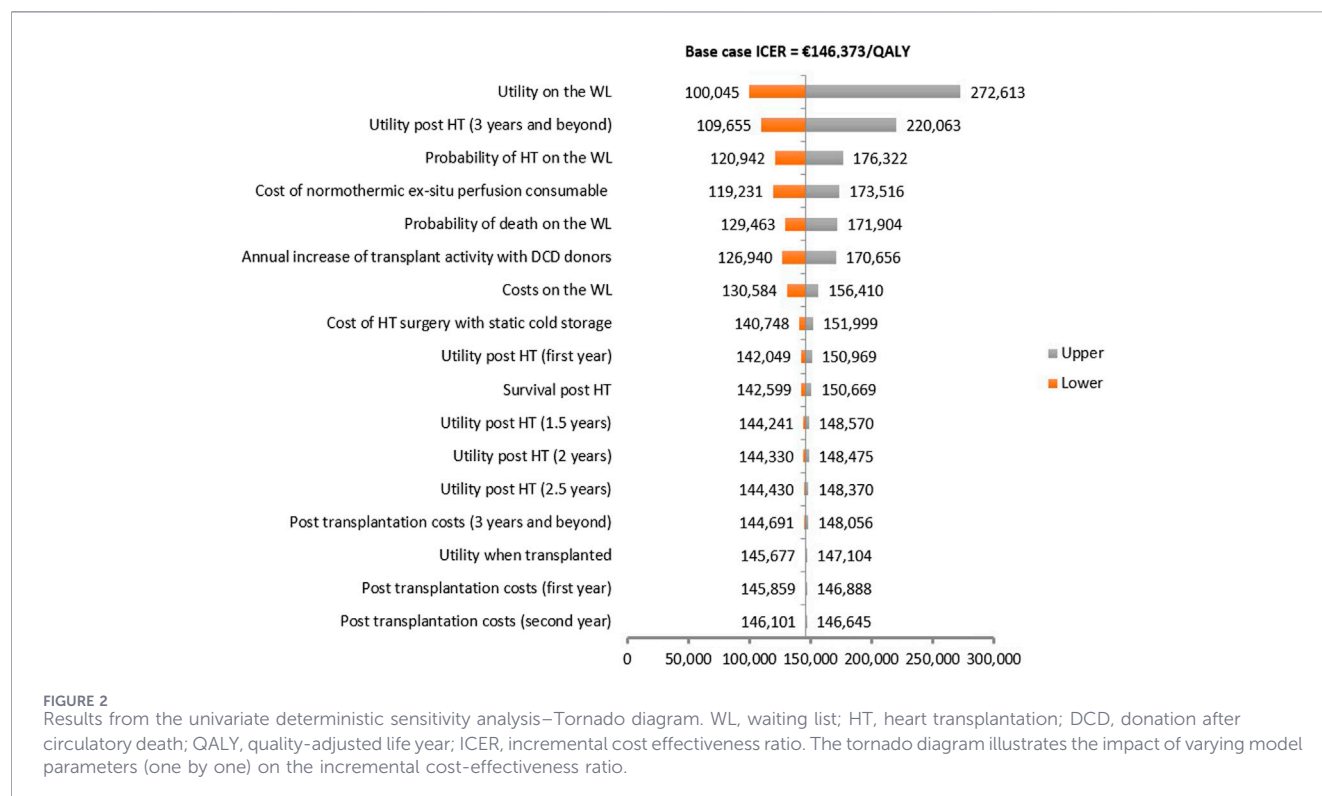


TABLE 2 Results from the scenario analyses.

| Scenario                                                    | Incremental cost (€) | Incremental QALY | ICER (€/QALY) |
|-------------------------------------------------------------|----------------------|------------------|---------------|
| Base case                                                   | 20,618               | 0.14             | 146,373       |
| Scenario 1: 4.5% discount rate applied                      | 20,651               | 0.13             | 162,133       |
| Scenario 2: No discount rate applied                        | 20,588               | 0.16             | 127,015       |
| Scenario 3: Increased primary graft failure impact          | 24,441               | 0.13             | 180,763       |
| Scenario 4: Gamma distribution for post-transplant survival | 20,622               | 0.14             | 147,246       |
| Scenario 5: 10-year time horizon                            | 20,440               | 0.11             | 192,933       |
| Scenario 6: 20-year time horizon                            | 20,775               | 0.17             | 121,462       |

QALY, quality-adjusted life year; ICER, incremental cost effectiveness ratio.

DCD heart transplantations and the relative frequency of these adverse events [36, 37]. A worst-case scenario related to PGD was tested further. Utility scores after heart transplantation were extracted from the French CUPIDON study. To obtain the number of QALYs, utility scores were multiplied by the number (or fractions) of life years gained.

## Analyses

A deterministic base-case analysis was performed using the point estimates of each model parameter. Model outputs were computed to an incremental cost-effectiveness ratio (ICER) expressed as a cost per QALY gained (€/QALY).

A univariate deterministic sensitivity analysis (DSA) was performed in order to test the robustness of the estimation of our ICER. Parameter values were varied one at a time based on the input's source (95%

confidence interval or standard error) or set to a specific range if not available (Table 1). Results of the DSA were presented in a tornado diagram. Scenario analyses were performed to explore the impact of certain model settings and assumptions. Scenarios 1 and 2 tested the application of a discount rate of 4.5% and 0% respectively, according to French guidelines [24]. Scenario 3 modeled a 10% disutility and a 20% cost increase (based on expert opinion) to the heart transplantation state in the DCD + DBD strategy to reflect a pessimistic PGD assumption with DCD, potentially affecting both costs and patient outcomes significantly. Scenario 4 explored the impact of an alternative, more pessimistic, but still fairly well fitted parametric survival distribution (i.e., gamma). Finally, different time horizons were assessed in scenario 5 and 6 (respectively 10 and 20 years).

A probabilistic sensitivity analysis (PSA), running 1000 Monte Carlo simulations, was performed to account for statistical uncertainty. Distributions were assigned according to the type of

parameter. The PSA results were presented on a cost-effectiveness plane and using a cost-effectiveness acceptability curve to represent the probability that the innovative strategy is cost-effective according to different willingness-to-pay (WTP) thresholds (using the R package “BCEA” [38]).

Finally, two independent threshold analyses were performed to assess, holding all other parameters constant: (1) the required change in waiting list mortality with DCD implementation for the innovative strategy to be cost-effective at given thresholds and (2) the reduction in consumable costs for the innovative strategy to be cost-effective at given thresholds.

## Model validation

Face validity of the model (structure, inputs, and outputs) and assumptions were assessed by health economists and clinical experts in heart transplantation. Internal validation included structured debugging, extreme value testing, and verification of all calculations. Markov traces are presented in [Supplementary Figure 3](#).

## Results

Over a 15-year time horizon, patients in the DBD strategy experienced 5.53 QALYs at a cost of €184,110 whereas patients in the DCD + DBD strategy experienced 5.67 QALYs at a cost of €204,728. These outcomes resulted in an incremental gain of 0.14 QALYs at an incremental cost of €20,618, producing an ICER of €146,373 per QALY gained. The results of the DSA are presented in [Figure 2](#). The main drivers of the ICER estimate were the utility of patients on the waiting list and after 3 years post-transplantation. Results from the scenario analyses were reported in [Table 2](#). The application of a utility decrement and an increase in the cost of heart transplantation in a pessimistic scenario of major impact of PGD with DCD resulted in an ICER of €180,763 per QALY at 15 years.

Results of the PSA are presented in [Figures 3, 4](#). The mean probabilistic ICER of the DCD + DBD strategy compared to DBD alone was €142,423 per QALY with a mean incremental cost of €20,609 (95% credible interval: €11,220–€37,453) and a mean incremental QALY gain of 0.14 (95% credible interval: –0.03–0.3). The innovative strategy reached 50% and 80% chances of being cost-effective respectively at a WTP of €139,200 and €261,000 per QALY.

The independent threshold analyses are presented in [Figures 5, 6](#) and can be interpreted according to different willingness-to-pay thresholds. For example, taking a WTP of €100,000 per QALY, the DCD + DBD strategy is cost-effective if (1) an 8% relative reduction in waiting list mortality is reached with the donor pool expansion (i.e. 0.8% absolute reduction, from 10.2% to 9.4% probability of death 1 year after listing) and (2) a 35% relative reduction in consumable costs is negotiated (i.e., moving from €54,000 to €35,500 per consumable).

## Discussion

To our knowledge, this is the first study assessing the cost-effectiveness of implementing heart transplantation following DCD,

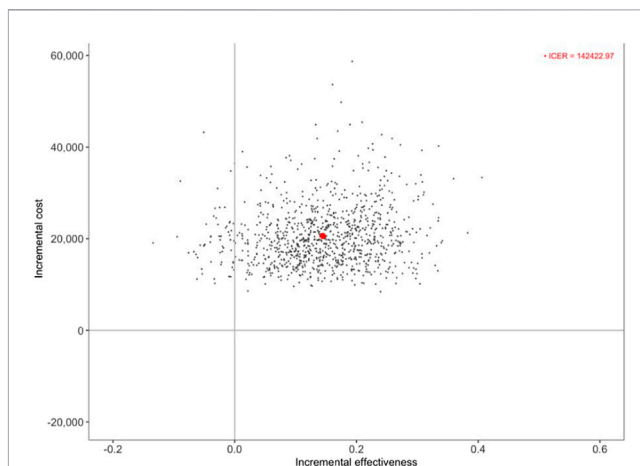


**FIGURE 3**  
Results from the probabilistic sensitivity analysis - Cost-effectiveness plane. ICER: incremental cost effectiveness ratio. Each point represents a simulated ICER from the probabilistic sensitivity analysis (1000 simulations). The mean probabilistic ICER was €142,423 per QALY.

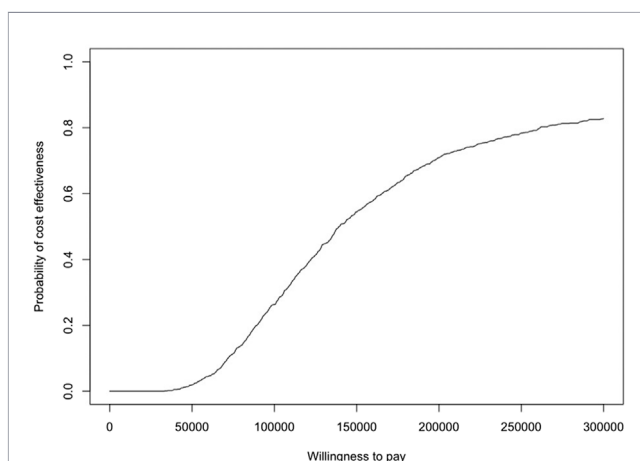


**FIGURE 4**  
Results from the probabilistic sensitivity analysis - Cost-effectiveness acceptability curve. The cost-effectiveness acceptability curve represents the probability of cost-effectiveness according to different willingness-to-pay thresholds. The innovative strategy reached 50% and 80% chances of being cost-effective, respectively, at a willingness to pay of €139,200 and €261,000 per QALY.

according to a protocol with direct procurement procedure (i.e., involving an *ex-situ* perfusion machine).

The assumptions underlying our model structure and parameters are primarily drawn from the existing literature in this area: our main hypothesis is that the introduction of DCD heart transplantations could increase the odds of a candidate to receive a graft [12, 28], while achieving outcomes comparable to standard care transplantation [3–6].

These assumptions are further supported by recent international experience, including long-term Australian data showing comparable post-transplant survival between DCD and DBD recipients up to 10 years using direct procurement followed by *ex-situ* machine perfusion [39].

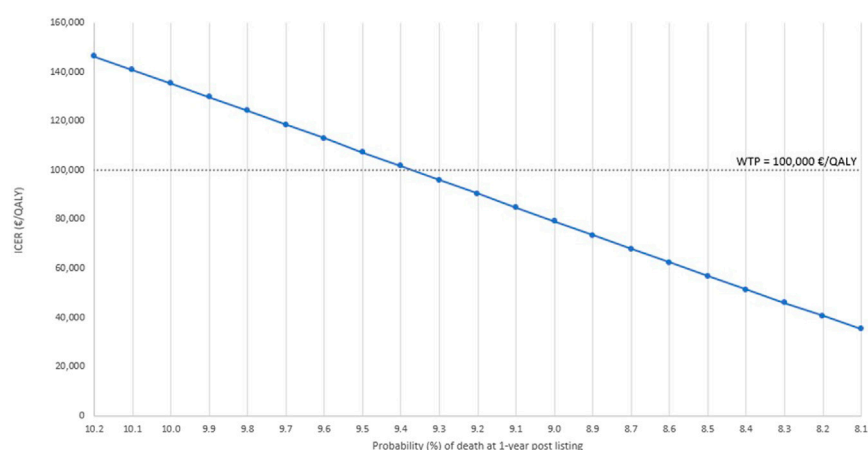


FIGURE 5

Results from the threshold analysis - Waiting list mortality. The figure shows, holding all other parameters constant, the required change in waiting list mortality with DCD implementation for the innovative strategy to be cost-effective at given willingness-to-pay thresholds. For example, taking a willingness-to-pay of €100,000 per QALY, the DCD + DBD strategy is cost-effective if a 0.8% reduction in waiting list mortality is reached with the donor pool expansion (i.e., moving from 10.2% to 9.4% probability of death 1 year after listing).

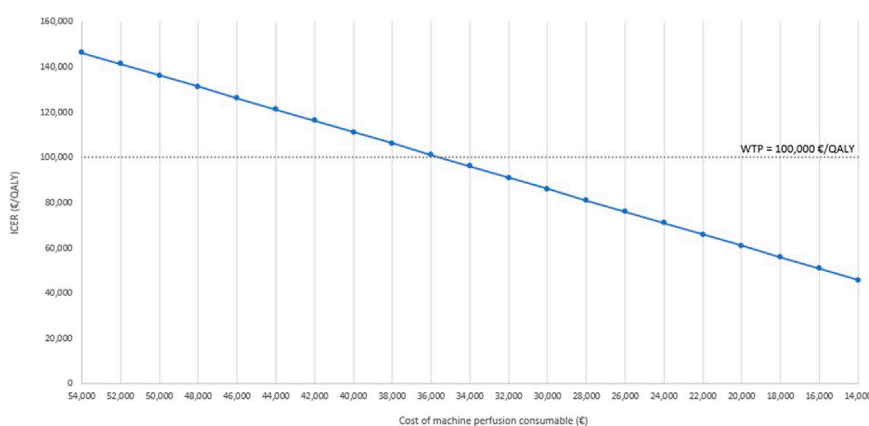


FIGURE 6

Results from the threshold analysis - Machine perfusion consumable costs. The figure shows, holding all other parameters constant, the required reduction in consumable costs for the innovative strategy to be cost-effective at given willingness-to-pay thresholds. For example, taking a willingness-to-pay of €100,000 per QALY, the DCD + DBD strategy is cost-effective if a 35% reduction in consumables costs is negotiated (i.e., moving from €54,000 to €35,500 per consumable).

For aspects where evidence was limited or uncertain, we adopted a conservative approach in our assumptions. In particular, we assumed that an increase in the supply of donors would not affect the mortality risk on the waiting list in the base case analysis. Sensitivity analyses were also performed. We specifically tested a pessimistic alternative scenario in which PGD, a commonly feared complication with DCD [36, 37], had a major impact on costs and quality of life during the heart transplantation state in the DCD + DBD strategy, which led to an ICER of €180,763 per QALY gained over 15 years. Also, if implementing DCD heart transplantations were to reduce waiting list mortality, the strategy would offer greater value for money. In our model, an 8% relative reduction of probability of death 1 year after listing corresponded to an ICER of €100,000 per QALY. In France, an average of 500 patients are newly registered on the waiting list each year,

which would translate to approximately four additional lives saved per year for the DCD program to be cost-effective at this willingness-to-pay threshold. Additionally, lowering consumable costs could further improve the cost-effectiveness of DCD heart transplantation.

This health economic evaluation aims to provide valuable insights for decision-making in countries where DCD heart transplantation procedures are not yet authorized and/or reimbursed. In some countries such as France, no predefined cost-effectiveness threshold is yet used as a formal decision-making criterion [40]. In practice, decisions are often influenced implicitly by both the cost-effectiveness ratio and the clinical severity or burden associated with the condition [41]. End-stage heart failure is an advanced stage of disease known to be associated with poor prognosis and impaired quality of life [34, 42, 43]. Given the persistent and worldwide shortage of heart donors [44], it may

be regarded as falling within the scope of unmet (or partially unmet) medical needs [45]. These aspects therefore also need to be considered in the decision-making process. To help the decision, threshold analyses were also provided. They could serve as a foundation for value-based negotiations between decision-makers and manufacturers to support fair (yet sustainable) pricing of machine perfusion consumables. They could also help align heart transplantation practices with value-based objectives for efficient DCD heart programs, including reducing waiting list mortality, as this parameter appears to be pivotal for the cost-effectiveness of the strategy.

Direct procurement followed by *ex-situ* machine perfusion is not the only approach currently used for DCD heart transplantation [1]. Alternative strategies, particularly thoraco-abdominal NRP followed by static cold storage, have demonstrated favorable outcomes, notably in the recent Spanish national experience [8].

Direct procurement followed by *ex-situ* machine perfusion was considered in the present analysis as this approach was developed to introduce heart retrieval within the existing French DCD protocol based on abdominal NRP for abdominal organ procurement [11]. Therefore, it should be acknowledged that our findings are specific to this procedure, reflecting the French setting, and should not be extrapolated to strategies based on thoraco-abdominal NRP, which involve different procurement procedures and resource requirements.

Nevertheless, from an international perspective, countries such as Australia, the United States, and the United Kingdom initially implemented direct procurement followed by *ex-situ* machine perfusion for DCD hearts without combining heart procurement with abdominal NRP. Considering the favorable outcomes associated with abdominal-NRP for liver and kidney transplantations in the French experience [46, 47], recent efforts to promote the international standardization of DCD practices involving the use of NRP [48], and the health economic considerations outlined in the present analysis, our findings may provide useful information for healthcare systems considering the integration of abdominal NRP alongside direct heart procurement and *ex-situ* machine perfusion.

The main strength of this study was its use of epidemiological and survival data from the French organ transplantation agency to parametrize the model. Also, individual patient cost data in a French context were used as model inputs, retrieved from a cost of illness study and a randomized controlled trial with cost data collection (i.e., they were not reconstituted based on practice recommendations). Economic models are, by definition, simplifications of the reality, but we implemented a time-inhomogeneous Markov process to the decision model to help reflect the clinical course of the disease and associated costs and outcomes. Moreover, the model could be adapted to support the evaluation of DCD implementation in different healthcare settings globally.

Our study does have limitations. One limitation is that we could not perform subgroup analyses based on patient characteristics and pathways, which would have been interesting given the inherent heterogeneity of this population [18]. Due to the scarcity of data in this indication, we had to assume the candidate population at the entry of the model as homogenous. It might be possible that specific populations with different levels of graft priority slightly differ in

terms of transplantation access, cost, and consequences. The analysis was limited to hospital costs and may therefore underestimate total costs, although hospital care was assumed to be the main cost driver in this indication. Additional implementation costs related to logistics, coordination, and learning curves were not explicitly modeled due to limited available data, but uncertainty related to procedure costs was partially explored in the DSA. Indeed, these costs remain difficult to estimate accurately in the French setting, given the very recent authorization of DCD heart transplantation in the country (March 2026). Accurately estimating these additional costs would require dedicated prospective micro-costing analyses of the procedure, which were beyond the scope of this early economic evaluation. However, we assumed that DCD heart procurement would not adversely affect abdominal organ procurement, consistent with the recently reported French national feasibility study, in which adding heart retrieval in the setting of abdominal NRP did not compromise the procurement of other organs [11]. Another limit is the absence of utility estimates on the pre-transplant period and after 3 years in a French context, which would be interesting to collect as being main drivers of uncertainty in the DSA.

Finally, in the present model, the impact of donor pool expansion was captured among listed candidates. However, increasing the availability of donor hearts may affect listing practices and outcomes in patients with advanced heart failure who are currently not listed because of persistent donor shortage. These broader effects were not modelled due to their inherent uncertainty but may further improve the overall benefits associated with DCD heart transplantation.

Future research should aim to evaluate the potential change of the candidates' waiting list mortality risk with the implementation of DCD procedures. Organizational aspects (perfusionists, coordination of extended heart procurement, etc.) should also be investigated in real-world settings. A budget impact analysis could be of interest to assess the sustainability of this strategy for healthcare payers.

In conclusion, our results suggest that, under the assumption of a 20% annual increase in transplant activity—improving the odds of a candidate receiving a graft without affecting waitlist mortality and assuming equivalent post-transplant outcomes—implementing DCD heart transplantation alongside DBD would result in an ICER of €146,373 per QALY gained over a 15-year time horizon from the French healthcare system perspective. Lowering consumable costs and reducing waitlist mortality are key factors enabling an efficient DCD heart program.

## Data availability statement

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

## Author contributions

Conceptualization: JA, PG, and LH. Methodology: JA, PG, JG, LS, MP, and LH. Data curation: JA. Formal analysis: JA. Supervision: LH. Validation: PG, JG, LS, MP, and LH. Writing – original draft:



JA. Writing – review and editing: JA, PG, JG, LS, MP, and LH. 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.

## References

- Kharawala A, Nagraj S, Seo J, Pargaonkar S, Uehara M, Goldstein DJ, et al. Donation after circulatory death heart transplant: current state and future directions. *Circ Heart Fail* (2024) 17(7):e011678. doi: 10.1161/CIRCHEARTFAILURE.124.011678
- Jacob S, Garg P, Wadiwala I, Yazji JH, Alomari M, Alamouti-Fard E, et al. Strategies for expanding donors pool in heart transplantation. *Rev Cardiovasc Med* (2022) 23(8):285. doi: 10.31083/j.rcm2308285
- Messer S, Page A, Axell R, Berman M, Hernández-Sánchez J, Colah S, et al. Outcome after heart transplantation from donation after circulatory-determined death donors. *J Heart Lung Transpl Off Publ Int Soc Heart Transpl* (2017) 36(12):1311–8. doi: 10.1016/j.healun.2017.10.021
- Chew HC, Iyer A, Connellan M, Scheuer S, Villanueva J, Gao L, et al. Outcomes of donation after circulatory death heart transplantation in Australia. *J Am Coll Cardiol* (2019) 73(12):1447–59. doi: 10.1016/j.jacc.2018.12.067
- Messer S, Cernic S, Page A, Berman M, Kaul P, Colah S, et al. A 5-year single-center early experience of heart transplantation from donation after circulatory-determined death donors. *J Heart Lung Transpl* (2020) 39(12):1463–75. doi: 10.1016/j.healun.2020.10.001
- Schroder JN, Patel CB, DeVore AD, Bryner BS, Casalinova S, Shah A, et al. Transplantation outcomes with donor hearts after circulatory death. *N Engl J Med* (2023) 388(23):2121–31. doi: 10.1056/NEJMoa2212438
- Kenny LA, Armstrong L, Berman M, Brierley J, Crossland D, Dark J, et al. Heart transplantation and donation after circulatory death in children. A review of the technological, logistical and ethical framework. *Transpl Int* (2025) 38:13801. doi: 10.3389/ti.2025.13801
- Pérez-Blanco A, González-Vilchez F, González-Costello J, Royo-Villanova M, Miñambres E, Cuenca JJ, et al. DCDD heart transplantation with thoraco-abdominal normothermic regional perfusion and static cold storage: the experience in Spain. *Am J Transpl* (2025) 25(7):1526–39. doi: 10.1016/j.ajt.2025.02.006
- Le Dorze M, Charpentier J, Cheisson G, Couret D, Ducos G, Zuber B. cDCDD and heart procurement: challenges from a French critical care perspective. *Transpl Int* (2025) 38:14779. doi: 10.3389/ti.2025.14779
- Pahuja M, Case BC, Molina EJ, Waksman R. Overview of the FDA's circulatory system devices panel virtual meeting on the TransMedics organ care system (OCS) heart - portable extracorporeal heart perfusion and monitoring system. *Am Heart J* (2022) 247:90–9. doi: 10.1016/j.ahj.2022.02.003
- Akamkam A, Gaillard M, Dang Van S, Gautier-Renard G, Chu Van E, Armand A, et al. Direct procurement and *ex-situ* assessment of donor hearts after controlled circulatory death: a French national pilot study. *Arch Cardiovasc Dis* (2026) 119(6-7):441–51. doi: 10.1016/j.acvd.2025.11.011
- Hess NR, Hong Y, Yoon P, Bonatti J, Sultan I, Serna-Gallegos D, et al. Donation after circulatory death improves probability of heart transplantation in waitlisted candidates and results in post-transplant outcomes similar to those achieved with brain-dead donors. *J Thorac Cardiovasc Surg* (2024) 167(5):1845–60.e12. doi: 10.1016/j.jtcvs.2023.09.012
- Alarid-Escudero F, Krijkamp E, Enns EA, Yang A, Hunink MGM, Pechlivanoglou P, et al. A tutorial on time-dependent cohort state-transition models in R using a cost-effectiveness analysis example. *Med Decis Mak Int J Soc Med Decis Mak* (2023) 43(1):21–41. doi: 10.1177/0272989X221121747
- Briggs A, Claxton K, Sculpher M. *Decision Modelling for Health Economic Evaluation*. Oxford University Press (2006).
- Drummond MF, Sculpher MJ, Claxton K, Stoddart GL, Torrance GW. In: *Methods for the Economic Evaluation of Health Care Programmes 4th Ed*. Oxford: Oxford University Press (2015).
- Filipović-Pierucci A, Zarca K, Durand-Zaleski I. Markov models for health economic evaluation: the R package heemod. *ArXiv E-Prints. R package version 1.0.2*. 1702.03252 (2017).
- Husereau D, Drummond M, Augustovski F, de Bekker-Grob E, Briggs AH, Carswell C, et al. Consolidated health economic evaluation reporting standards 2022 (CHEERS 2022) statement: updated reporting guidance for health economic evaluations. *Value Health J Int Soc Pharmacoeconomics Outcomes Res* (2022) 25(1):3–9. doi: 10.1016/j.jval.2021.11.1351
- Long EF, Swain GW, Mangi AA. Comparative survival and cost-effectiveness of advanced therapies for end-stage heart failure. *Circ Heart Fail* (2014) 7(3):470–8. doi: 10.1161/CIRCHEARTFAILURE.113.000807
- Alba AC, Alba LF, Delgado DH, Rao V, Ross HJ, Goeree R. Cost-effectiveness of ventricular assist device therapy as a bridge to transplantation compared with nonbridged cardiac recipients. *Circulation* (2013) 127(24):2424–35. doi: 10.1161/CIRCULATIONAHA.112.000194
- Handley TJ, Arnow KD, Melcher ML. Despite increasing costs, perfusion machines expand the donor pool of livers and could save lives. *J Surg Res* (2023) 283:42–51. doi: 10.1016/j.jss.2022.10.002
- Webb AN, Lester ELW, Shapiro AMJ, Eurich DT, Bigam DL. Cost-utility analysis of normothermic machine perfusion compared to static cold storage in liver transplantation in the Canadian setting. *Am J Transpl* (2022) 22(2):541–51. doi: 10.1111/ajt.16797
- Barendregt JJ. The life table method of half cycle correction: getting it right. *Med Decis Mak Int J Soc Med Decis Mak* (2014) 34(3):283–5. doi: 10.1177/0272989X13519863
- Agence de la Biomédecine. *Rapport Médical Et Scientifique*. Saint-Denis La Plaine (2023).
- Haute Autorité de Santé. *Choices in Methods for Economic Evaluation*. Saint Denis La Plaine, France: HAS (2020). p. 115.
- Bakhtiyar SS, Sakowitz S, Mallick S, Curry J, Benharash P. Heart transplantation after donation after circulatory death: early United States experience. *Ann Thorac Surg* (2024) 118(2):484–93. doi: 10.1016/j.athoracsur.2024.05.013
- Liu N, Zhou Y, Lee JJ. IPDfromKM: reconstruct individual patient data from published Kaplan-Meier survival curves. *BMC Med Res Methodol* (2021) 21(1):111. doi: 10.1186/s12874-021-01308-8
- Jawitz OK, Raman V, DeVore AD, Mentz RJ, Patel CB, Rogers J, et al. Increasing the United States heart transplant donor pool with donation after circulatory death. *J Thorac Cardiovasc Surg* (2020) 159(5):e307–e309. doi: 10.1016/j.jtcvs.2019.09.080
- Gernhofer YK, Bui QM, Powell JJ, Perez PM, Jones J, Batchinsky AI, et al. Heart transplantation from donation after circulatory death: impact on waitlist time and transplant rate. *Am J Transpl* (2023) 23(8):1241–55. doi: 10.1016/j.ajt.2023.04.026
- Kim DD, Silver MC, Kunst N, Cohen JT, Ollendorf DA, Neumann PJ. Perspective and costing in cost-effectiveness analysis, 1974–2018. *Pharmacoeconomics* (2020) 38(10):1135–45. doi: 10.1007/s40273-020-00942-2

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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.2026.17149/full#supplementary-material>

30. Atfeh J, Guerre P, Sebbag L, Pozzi M, Huot L. Economic burden and healthcare trajectories of patients awaiting heart transplantation in a French tertiary center. *Transpl Int* (2025) 38:13703. doi: 10.3389/ti.2025.13703
31. Sebbag. *ALLOMAP cost-effectiveness for Heart Transplant Patients in France. CUPIDON Results and Impact*. London, United Kingdom: ESOT Congr (2025).
32. Dunn A, Grosse SD, Zuvekas SH. Adjusting health expenditures for inflation: a review of measures for health services research in the United States. *Health Serv Res* (2018) 53(1):175–96. doi: 10.1111/1475-6773.12612
33. Di Tanna GL, Urbich M, Wirtz HS, Potrata B, Heisen M, Bennisson C, et al. Health state utilities of patients with heart failure: a systematic literature review. *Pharmacoeconomics* (2021) 39(2):211–29. doi: 10.1007/s40273-020-00984-6
34. Emin A, Rogers CA, Banner NRSteering Group. UK cardiothoracic transplant audit. Quality of life of advanced chronic heart failure: medical care, mechanical circulatory support and transplantation. *Eur J Cardio-thorac Surg* (2016) 50(2):269–73. doi: 10.1093/ejcts/ezw054
35. Giovannico L, Fischetti G, Parigino D, Savino L, Di Bari N, Milano AD, et al. Veno-arterial extracorporeal membrane oxygenation (VA-ECMO) support in new era of heart transplant. *Transpl Int* (2024) 37:12981. doi: 10.3389/ti.2024.12981
36. Cho PD, Kim ST, Zappacosta H, White JP, McKay S, Biniwale R, et al. Severe primary graft dysfunction in heart transplant recipients using donor hearts after circulatory death: united States experience. *J Heart Lung Transpl* (2025) 44(5):760–9. doi: 10.1016/j.healun.2024.11.027
37. Ayer A, Truby LK, Schroder JN, Casalnova S, Green CL, Bishawi MA, et al. Improved outcomes in severe primary graft dysfunction after heart transplantation following donation after circulatory death compared with donation after brain death. *J Card Fail* (2023) 29(1):67–75. doi: 10.1016/j.cardfail.2022.10.429
38. Baio G. BCEA: a R package to perform bayesian cost-effectiveness analysis. *Value Health J Int Soc Pharmacoeconomics Outcomes Res* (2014) 17(7):A550. doi: 10.1016/j.jval.2014.08.1793
39. Joshi Y, Scheuer S, Chew H, Villanueva J, Soto C, MacLean C, et al. Long-term outcomes from a decade of donation after circulatory death heart transplantation in Australia. *JACC Heart Fail* (2026):103168. doi: 10.1016/j.jchf.2026.103168
40. Ghabri S. Could or should we use cost-effectiveness thresholds in the French value-based pricing process for new drugs? *Pharmacoeconomics* (2024) 42(8):823–7. doi: 10.1007/s40273-024-01393-9
41. Annemans LJP. Innovative public price mechanisms for market access of innovative medicines: a marriage between cost-effectiveness, medical need, and budget impact. *Value Health J Int Soc Pharmacoeconomics Outcomes Res* (2023) 26(3):400–1. doi: 10.1016/j.jval.2023.01.006
42. Xanthakis V, Enserro DM, Larson MG, Wollert KC, Januzzi JL, Levy D, et al. Prevalence, neurohormonal correlates, and prognosis of heart failure stages in the community. *JACC Heart Fail* (2016) 4(10):808–15. doi: 10.1016/j.jchf.2016.05.001
43. Rose EA, Gelijns AC, Moskowitz AJ, Heitjan DF, Stevenson LW, Dembitsky W, et al. Long-term use of a left ventricular assist device for end-stage heart failure. *N Engl J Med* (2001) 345(20):1435–43. doi: 10.1056/NEJMoa012175
44. Lewis A, Koukoura A, Tsianos GI, Gargavanis AA, Nielsen AA, Vassiliadis E. Organ donation in the US and Europe: the supply vs demand imbalance. *Transpl Rev Orlando Fla* (2021) 35(2):100585. doi: 10.1016/j.trre.2020.100585
45. Vreman RA, Heikkinen I, Schuurman A, Sapede C, Garcia JL, Hedberg N, et al. Unmet medical need: an introduction to definitions and stakeholder perceptions. *Value Health J Int Soc Pharmacoeconomics Outcomes Res* (2019) 22(11):1275–82. doi: 10.1016/j.jval.2019.07.007
46. Savier E, Lim C, Rayar M, Orlando F, Boudjema K, Mohkam K, et al. Favorable outcomes of liver transplantation from controlled circulatory death donors using normothermic regional perfusion compared to brain death donors. *Transplantation* (2020) 104(9):1943–51. doi: 10.1097/TP.0000000000003372
47. Savoye E, Legeai C, Branchereau J, Gay S, Riou B, Gaudez F, et al. Optimal donation of kidney transplants after controlled circulatory death. *Am J Transpl* (2021) 21(7):2424–36. doi: 10.1111/ajt.16425
48. Miñambres E, Berman M, Antonini MV, Campo-Cañaveral De La Cruz JL, Croome K, Feltrin G, et al. Normothermic regional perfusion (NRP) use in controlled donation after circulatory determination of death (cDCDD): results of the European Society for Organ Transplantation Bucharest consensus conference. *Transpl Int* (2026) 39:16391. doi: 10.3389/ti.2026.16391



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# Sequential dual-organ living donation: selection, operative approach, and early donor outcomes at a Canadian center

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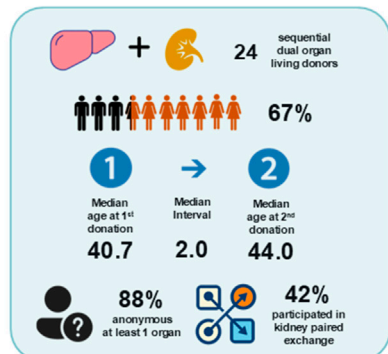
Sequential dual-organ living donation, defined as donation of a kidney and a partial liver graft by the same individual in separate operations, represents a rare extension of living donor transplantation. A donor-focused analysis was performed to characterize the donor population, the operative considerations posed by a second donor operation, and early donor outcomes at a single high-volume center. A retrospective review of all dual-organ living donors at our institute was performed. Twenty-four donors were identified (n = 10 liver-first and n = 14 kidney-first). The median interval between donations was 2 years (IQR 1.1–4.2). Twelve donors donated both organs anonymously, nine donated one organ anonymously and directed the other, and three directed both donations. Anonymous donation is a strong motivator in this unique population. Ten donors (42%) participated in kidney paired donation. Liver donations (left lateral segment (42%), left lobe (25%), and right lobe (33%)) were performed by an open approach. Laparoscopic nephrectomy was performed in all cases except two prior liver donors. Liver-after-kidney donors (n = 14) did not develop renal dysfunction after donation. Prior organ donation does not usually require modification of surgical technique for the second procedure. Dual-organ living donation is rare but can be safely performed independent of sequence.

### KEYWORDS

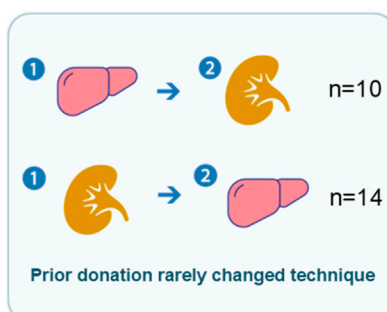
donor safety and outcomes, living donor hepatectomy, living donor nephrectomy, living donor transplantation, Sequential dual organ donation

## Sequential Dual Organ Living Donation: Selection, Operative Approach and Early Donor Outcomes at a Canadian Center

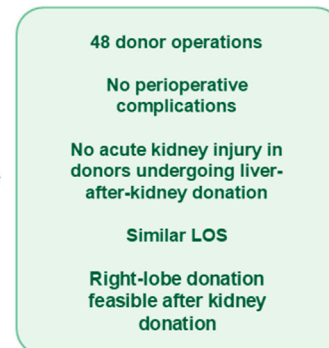
### Largest Single Center Case Series



### Two Feasible Sequences



### Early Outcomes



**TAKE-HOME: Safe in selected donors and irrespective of sequence; selection and planning is important.**



GRAPHICAL ABSTRACT

Goh, et al. Transpl. Int. 2026  
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## Introduction

Living donation plays an important role in facilitating timely access to high-quality liver and kidney grafts for those in need of transplantation. However, unlike deceased donation, it requires a healthy individual to assume the operative risks for the benefit of another, making donor safety the fundamental constraint on the expansion of living donor transplantation. The ethical framework established at the Vancouver Forum [1] together with the Declaration of Istanbul on Organ Trafficking and Transplant Tourism anchor the principles of all living donation and set the standard against organ trafficking, tourism, and exploitation. Any extension of living donation must therefore remain consistent with these principles wherever it is practiced.

Within the living donation landscape, a small number of living donors extend this commitment to donate a kidney at one time and a partial liver graft at another or *vice versa*. This practice, termed sequential dual-organ living donation, is uncommon but important. It challenges assumptions derived from single-organ donation and requires renewed attention to ethical considerations of “double equipoise,” donor selection, timing between operations, and risk communication [2].

Emerging registry [3] and cohort data [4–9] report acceptable donor-specific outcomes. These reports have described both sequences (liver-first donation followed by kidney donation or

*vice versa*) and outline perioperative outcomes that are acceptable in well-selected donors and within highly experienced teams. They also highlight the need for careful preoperative assessment, meticulous intraoperative technique, and structured early postoperative monitoring [10].

However, these reports have not included detailed information about surgical considerations and operative approach towards the second organ donation in prior donors. Furthermore, some authors have raised concerns about the safety of liver-after-kidney donation and have suggested that the amount of liver resected should be minimized in prior kidney donors to decrease the risk of perioperative renal dysfunction [10].

Herein, we present a donor-specific analysis and sequence comparison (liver-after-kidney and kidney-after-liver donation) of the largest single-center cohort of sequential dual-organ living donors reported to date from a high-volume North American living donation program. We describe (i) the demographic characteristics and donation patterns, directed versus non-directed, of these donors; (ii) the operative considerations posed by a second donor operation; and (iii) short-term perioperative donor outcomes.

## Methods

### Study design and setting

A retrospective review of all sequential dual-organ living donors at the Ajmera Transplant Centre, Toronto General Hospital, University Health Network (UHN), Toronto, Ontario, Canada was performed. The study period began at program inception and extended through December 2025. All living donors

**Abbreviations:** FLR, Functional liver remnant; GRWR, Graft-to-recipient weight ratio; KPD, Kidney paired donation; NSAIDs, Non-steroidal anti-inflammatory drugs; LOS, Length of stay; IQR, Interquartile range; UHN, University Health Network; LLS, Left lateral segment (hepatectomy); LL, Left lobe (hepatectomy); RL, Right lobe (hepatectomy).

provided written informed consent for data collection under protocols approved by the UHN Research Ethics Board.

This study was designed as a donor-focused analysis. Sequential dual-organ living donors were defined as individuals who completed two separate living donor operations: one living donor hepatectomy (left lateral segment, left lobe, or right lobe) and one living donor nephrectomy (left or right sided, laparoscopic or open). Outcomes of interest (detailed below) included donor demographic characteristics, the nature and sequence of each donation, the interval between them, participation in kidney paired donation, operative approach, perioperative complications, length of stay, and perioperative renal biochemistry. Recipient characteristics and post-transplant recipient outcomes were not collected as the focus of this study was to evaluate the perioperative outcomes of sequential dual donors in relation to their sequence of donation.

## Protocol for selection – donor hepatectomy and donor nephrectomy

None of the donors in our cohort expressed a desire to donate two organs at the outset. The sequence of organ donation was therefore determined by individual donors without input from our team, and they were evaluated for either liver or kidney donation first based upon their initial presentation as willing liver or kidney donors, respectively. Evaluation for the subsequent living organ donation was conducted sequentially and independently of the first at the request of the donor. Evaluations for liver and kidney donation were conducted according to standard protocols, as previously described [11–13]. The medical, nursing, and psychosocial teams performing the evaluation of liver and kidney donors at our center are separate and distinct. The surgeons involved in living liver donation procedures and living kidney donation procedures are also distinct except for one surgeon who performs both.

Selection of which kidney to donate was driven by relative function. If function was symmetric, the anatomically less complex kidney was chosen. For liver donation, the type of hepatectomy was selected to maximize the donor's functional liver remnant (FLR) and provide an adequate graft-to-recipient weight ratio (GRWR). If the left lobe (LL) or left lateral segment (LLS) anatomy was not favorable for donation, or if a suitable recipient for a LL/LLS graft was not available within the donor's donation timeline, then right lobe donation was considered. Prior organ donation was not a factor in determining the laterality of liver or kidney donation.

Voluntary participation in Canada's national kidney paired donation (KPD) program was offered to all anonymous nondirected kidney donors as well as directed living kidney donors who were found to be incompatible with their intended recipients.

## Operative procedures

All living donor hepatectomies were performed through an upper midline incision. Strict intravenous fluid restriction was implemented during all donor hepatectomy procedures to minimize central venous pressure, regardless of prior kidney

donation status. Blood pressure targets were achieved with vasopressor administration until parenchymal transection was completed, after which resuscitation with intravenous crystalloids was initiated and vasopressors were rapidly weaned off. All living donor nephrectomies were performed laparoscopically, except in two in whom a planned open mini-incision was undertaken because of anticipated adhesions related to prior hepatectomies.

## Data collection

Variables included donor demographics and age at each donation, donor body mass index (BMI), comorbidities (presence of diabetes or hypertension), order of donation (liver-first vs. kidney-first), interval between donations, nature of each donation (directed vs. anonymous), participation in KPD, surgical approach and side/segment donated, length of stay, perioperative complications for each procedure, and perioperative renal biochemistry.

## Statistical analysis

Descriptive statistics were used for demographics and operative characteristics. Continuous variables were summarized using medians and interquartile ranges as appropriate. Between-group comparisons were assessed using the Mann-Whitney U test. Statistical significance was defined *a priori* as  $p < 0.05$ .

## Results

### Cohort characteristics

We identified 24 sequential dual-organ living donors during the study period, representing approximately 0.5% of all living donors in our center. Donors were a median of 40.7 years old at the time of their first donation (IQR 29.9–48.2 years) and 44.0 years old at the time of their second donation (IQR 32.5–49.8 years). The median interval between donations was 2.0 years (IQR 1.1–4.2 years). A majority of the donors were female (67%). Median donor BMI was 24.3 (IQR 22.8–27.6). None of the donors had hypertension or diabetes mellitus. Detailed cohort characteristics are summarized in Tables 1, 2.

### Donation sequence and nature of donation

Of the cohort, 42% (10/24) underwent liver donation first followed by kidney donation. The remainder of the cohort (14/24) donated a kidney first and subsequently underwent living donor hepatectomy. A high proportion of donors (88%,  $n = 21$ ) performed at least one donation anonymously. Twelve living donors donated both organs in an anonymous nondirected fashion, nine donated one organ anonymously and directed the other, and three directed both donations. Twelve of 24 kidney donations were anonymous nondirected while 21 of 24 liver donations were anonymous nondirected. Ten out of 24 donors (42%) participated in KPD, thereby enabling additional transplants. Ten donors underwent living kidney donation at another transplant center in Canada



TABLE 1 Characteristics of all dual living donors in this study.

| Year of 1st donation | 1st organ | Age at 1st donation (years) | Age at 2nd donation (years) | Interval (years) | Sex | BMI  | Liver type | Approach Incision | Liver recipient       | Liver LOS (days) | Kidney side | Approach Incision          | Kidney Recipient      | KPD | Kidney LOS (days) |
|----------------------|-----------|-----------------------------|-----------------------------|------------------|-----|------|------------|-------------------|-----------------------|------------------|-------------|----------------------------|-----------------------|-----|-------------------|
| 2013                 | Kidney    | 40.0                        | 49.8                        | 9.8              | F   | 22.3 | RL         | Open midline      | Anonymous nondirected | 6                | L           | Laparoscopic pfannenstiel* | Anonymous nondirected | N   | 3                 |
| 2014                 | Liver     | 41.4                        | 42.6                        | 1.1              | M   | 19.2 | LLS        | Open midline      | Directed              | 8                | L           | Open Mini-incision         | Directed              | N   | 5                 |
| 2014                 | Kidney    | 20.3                        | 21.6                        | 1.3              | M   | 23.7 | LLS        | Open Midline      | Anonymous nondirected | 4                | L           | Laparoscopic pfannenstiel* | Anonymous nondirected | N   | 2                 |
| 2015                 | Liver     | 19.8                        | 25.1                        | 5.3              | F   | 28.3 | LLS        | Open Midline      | Anonymous nondirected | 5                | L           | Laparoscopic pfannenstiel  | Anonymous nondirected | Y   | 3                 |
| 2016                 | Kidney    | 30.1                        | 40.0                        | 9.9              | F   | 27.8 | RL         | Open Midline      | Anonymous nondirected | 5                | L           | Laparoscopic pfannenstiel* | Directed              | Y   | 3                 |
| 2016                 | Liver     | 43.4                        | 45.3                        | 1.8              | F   | 24.2 | LL         | Open Midline      | Anonymous nondirected | 6                | L           | Laparoscopic pfannenstiel  | Anonymous nondirected | N   | 4                 |
| 2016                 | Kidney    | 37.5                        | 40.0                        | 2.5              | F   | 26.4 | LLS        | Open Midline      | Directed              | 5                | R           | Laparoscopic pfannenstiel  | Directed              | N   | 4                 |
| 2019                 | Liver     | 55.6                        | 57.3                        | 1.7              | M   | 25.2 | LLS        | Open midline      | Anonymous nondirected | 4                | L           | Laparoscopic pfannenstiel  | Anonymous nondirected | Y   | 3                 |
| 2019                 | Kidney    | 37.4                        | 42.8                        | 5.4              | M   | 35.3 | LL         | Open midline      | Anonymous nondirected | 5                | L           | Laparoscopic pfannenstiel* | Directed              | N   | 3                 |
| 2019                 | Liver     | 29.4                        | 31.9                        | 2.5              | M   | 22.9 | RL         | Open midline      | Anonymous nondirected | 4                | R           | Open Mini-incision         | Directed              | N   | 2                 |
| 2020                 | Liver     | 20.0                        | 23.9                        | 3.8              | M   | 27.1 | RL         | Open midline      | Anonymous nondirected | 6                | L           | Laparoscopic pfannenstiel  | Anonymous nondirected | N   | 4                 |
| 2020                 | Kidney    | 26.3                        | 30.4                        | 4.1              | M   | 24.3 | RL         | Open midline      | Anonymous nondirected | 4                | L           | Laparoscopic pfannenstiel  | Anonymous nondirected | N   | 2                 |
| 2020                 | Kidney    | 48.6                        | 53.2                        | 4.6              | F   | 26.4 | LL         | Open midline      | Anonymous nondirected | 4                | L           | Laparoscopic pfannenstiel* | Anonymous nondirected | N   | 2                 |
| 2020                 | Liver     | 53.5                        | 54.4                        | 0.9              | F   | 21.5 | LLS        | Open midline      | Anonymous nondirected | 6                | L           | Laparoscopic pfannenstiel  | Anonymous nondirected | Y   | 4                 |

(Continued)

TABLE 1 Continued

| Year of 1st donation | 1st organ | Age at 1st donation (years) | Age at 2nd donation (years) | Interval (years) | Sex | BMI  | Liver type | Approach Incision | Liver recipient       | Liver LOS (days) | Kidney side | Approach Incision          | Kidney Recipient      | KPD | Kidney LOS (days) |
|----------------------|-----------|-----------------------------|-----------------------------|------------------|-----|------|------------|-------------------|-----------------------|------------------|-------------|----------------------------|-----------------------|-----|-------------------|
| 2021                 | Kidney    | 47.7                        | 49.7                        | 2.0              | F   | 22.6 | LLS        | Open midline      | Anonymous nondirected | 5                | L           | Laparoscopic pfannenstiel  | Directed              | Y   | 4                 |
| 2021                 | Liver     | 23.9                        | 24.5                        | 0.6              | F   | 23.6 | LLS        | Open midline      | Anonymous nondirected | 4                | L           | Laparoscopic pfannenstiel  | Anonymous nondirected | Y   | 2                 |
| 2022                 | Liver     | 44.5                        | 46.1                        | 1.6              | F   | 23.7 | LLS        | Open midline      | Anonymous nondirected | 4                | L           | Laparoscopic pfannenstiel  | Directed              | Y   | 2                 |
| 2022                 | Kidney    | 48.8                        | 49.9                        | 1.1              | F   | 27.4 | LL         | Open midline      | Anonymous nondirected | 4                | L           | Laparoscopic pfannenstiel* | Directed              | N   | 2                 |
| 2022                 | Kidney    | 53.1                        | 55.4                        | 2.3              | F   | 31.6 | RL         | Open midline      | Directed              | 7                | L           | Laparoscopic McBurney*     | Directed              | N   | 1                 |
| 2022                 | Kidney    | 30.8                        | 32.6                        | 1.9              | F   | 18.7 | LL         | Open midline      | Anonymous nondirected | 4                | L           | Laparoscopic pfannenstiel* | Directed              | N   | 2                 |
| 2022                 | Liver     | 43.1                        | 45.2                        | 2.1              | F   | 24.3 | RL         | Open midline      | Anonymous nondirected | 7                | L           | Laparoscopic pfannenstiel  | Directed              | Y   | 5                 |
| 2023                 | Kidney    | 32.0                        | 33.0                        | 1.0              | F   | 31.6 | LLS        | Open midline      | Anonymous nondirected | 4                | L           | Laparoscopic McBurney*     | Anonymous nondirected | Y   | 1                 |
| 2023                 | Kidney    | 51.6                        | 52.3                        | 0.7              | F   | 20.5 | RL         | Open midline      | Anonymous nondirected | 5                | L           | Laparoscopic McBurney*     | Anonymous nondirected | Y   | 3                 |
| 2024                 | Kidney    | 48.1                        | 49.0                        | 0.9              | M   | 33.2 | LL         | Open midline      | Anonymous nondirected | 5                | R           | Laparoscopic pfannenstiel  | Directed              | N   | 3                 |

\*denotes donors who underwent kidney donation at another center prior to liver donation at our center. LLS: left lateral segment. LL: left lobe. RL: right lobe. KPD: kidney paired donation. LOS: length of stay. BMI: body mass index.

TABLE 2 Baseline characteristics of all sequential dual living organ donors.

| Characteristics                 | Donors (n = 24)            |
|---------------------------------|----------------------------|
| Sex                             |                            |
| Male                            | 8 (33%)                    |
| Female                          | 16 (67%)                   |
| Liver first, followed by kidney | 10 (42%)                   |
| Kidney first, followed by liver | 14 (58%)                   |
| Age at first donation           | 40.7 years (IQR 29.9–48.2) |
| Age at second donation          | 44.0 years (IQR 32.5–49.8) |
| Interval between donation       | 2.0 years (IQR 1.1–4.2)    |
| Age at liver donation           | 42.9 years (IQR 32.1–49.8) |
| Liver donation type             |                            |
| Right lobe                      | 8 (33%)                    |
| Left lobe                       | 6 (25%)                    |
| Left lateral segment            | 10 (42%)                   |
| Liver recipient type            |                            |
| Anonymous nondirected           | 21 (87%)                   |
| Directed                        | 3 (13%)                    |
| Liver donation approach         |                            |
| Open                            | 24 (100%)                  |
| Age at kidney donation          | 41.3 years (IQR 30.6–48.2) |
| Kidney donation type            |                            |
| Right                           | 3 (12%)                    |
| Left                            | 21 (88%)                   |
| Kidney donation approach        |                            |
| Open                            | 2 (8%)                     |
| Laparoscopic                    | 22 (92%)                   |
| Kidney recipient type           |                            |
| Anonymous nondirected           | 12 (50%)                   |
| Directed                        | 12 (50%)                   |
| Kidney paired donation          | 10 (42%)                   |

prior to seeking liver donation at our center; nine of ten sought anonymous nondirected liver donation, while one was directed. All liver donation procedures were performed at our center. No liver-first donors subsequently donated a kidney at another transplant center in Canada.

## Operative characteristics

Liver donations included left lateral segment hepatectomy (n = 10), left lobe hepatectomy (n = 6), and right lobe hepatectomy (n = 8). All hepatectomies were performed through an upper midline incision. As discussed earlier, graft selection was individualized. In the kidney-first cohort (n = 14), five donated an RL graft, four donated an LLS graft, and five donated an LL graft. In the liver-first cohort (n = 10), three donated an RL graft, six donated an LLS graft, and one donated an LL graft.

Kidney donations were predominantly left-sided (88%). The laparoscopic approach was used in all except two donors. The

first kidney-after-liver donor in our series underwent a planned open left nephrectomy due to anticipated adhesions from prior left lateral hepatectomy and lack of confidence about attempting a laparoscopic nephrectomy at an early stage in the evolution of our program. The only individual to donate a right kidney after right hepatectomy underwent a planned open right nephrectomy due to anticipated adhesions to the cut surface of the liver in the right upper quadrant that could complicate a laparoscopic approach.

## Perioperative outcomes and recovery

None of the donors experienced a perioperative complication in either of their two procedures. The median LOS after living donation of a liver graft was 5.5 days (IQR 4.0–6.0) in the liver-first cohort and 5.0 days (IQR 4.0–5.0) in the liver-second cohort (Figure 1A). The LOS did not differ significantly ( $p = 0.42$ ). The median length of stay (LOS) after living donation of a kidney graft was 2.5 days (IQR 2.0–3.0) in the kidney-first cohort and 3.5 days (IQR 2.3–4.0) in the kidney-second cohort (Figure 1B). This finding was not statistically significant ( $p = 0.07$ ). None of the donors experienced acute kidney injury post donation (Figure 2). The type of donor hepatectomy performed did not impact renal function in prior kidney donors.

## Discussion

### Donor characteristics and perioperative outcomes

This is the largest single-center series of 24 sequential dual-organ living donors reported to date. Our granular series describes a donor population that is young, predominantly female, uniformly free of hypertension and diabetes, and characterized by a high prevalence of anonymous donation.

Twenty-one of 24 donors (88%) performed at least one donation anonymously and 12 donated both organs anonymously. This reflects the systematic development of anonymous living liver donation at our center [14–16]. As such, this led a substantial proportion of our cohort to seek liver donation here after donating a kidney elsewhere.

Across 48 donor operations there were no perioperative complications, length of stay did not differ significantly by donation sequence, and none of the liver-after-kidney donors developed acute kidney injury. Five liver-after-kidney donors underwent right lobe donation without perioperative renal impairment despite highly restrictive intraoperative fluid administration, consistent with earlier reports of preserved renal function in this sequence [5, 10]. This does not support the suggestion that the volume of liver resected should be constrained in prior kidney donors [10]. However, the small sample size precludes an accurate estimation of risk, and these findings should be cautiously extrapolated to the broader population of prior living kidney donors.

These uniformly favorable outcomes should be interpreted in the context of an exceptionally healthy and highly selected donor cohort. All donors were fit and free of comorbidities. In donors

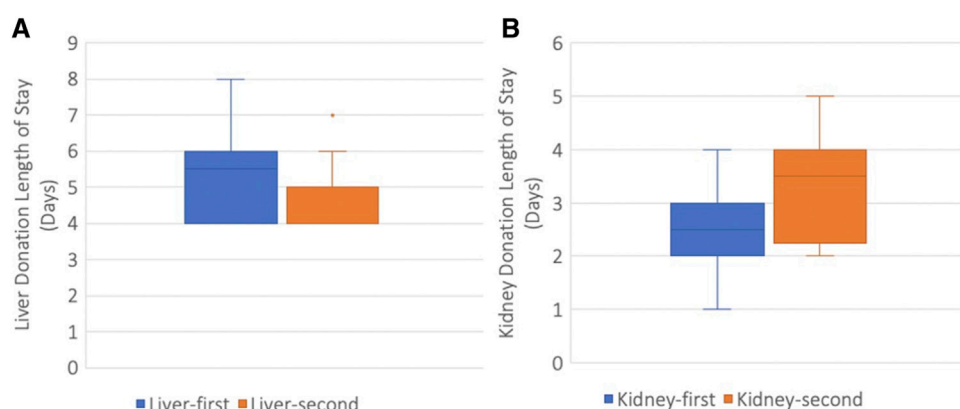


**FIGURE 1**  
Length of stay for donors who underwent (A) liver-first (n = 10) compared to liver-second (n = 14) living donation. Mann-Whitney U p = 0.42 and (B) kidney-first (n = 14) compared to kidney-second (n = 10) living donation. Mann-Whitney U p = 0.07.

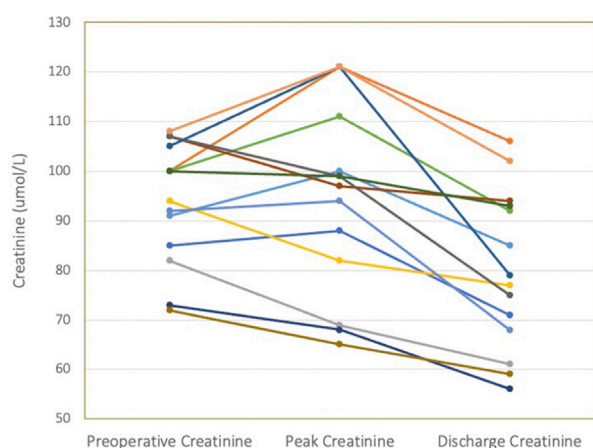


**FIGURE 2**  
Creatinine levels of living donors who underwent living liver donation after living kidney donation (n = 14). Friedman test p = < 0.001.

returning for a second donation, more stringent thresholds for fitness and risk should apply.

## Implications for donor selection, sequencing, and operative planning

Prior donor surgery rarely altered the operative approach to the second donation (kidney-first, n = 14; liver-first, n = 10). Laparoscopic nephrectomy was feasible in all prior liver donors except two, early in our experience, in whom a planned open mini-incision was undertaken for anticipated adhesions from ipsilateral prior hepatectomy with an unsuitable contralateral kidney.

Conversely, prior kidney donation did not influence the type of hepatectomy performed. Graft selection followed the usual principles of maximizing the donor's FLR while providing a graft of adequate graft-to-recipient weight ratio. Prior donation should

therefore inform operative planning and consent rather than constrain graft type or laterality.

No donor identified dual donation as an objective at first presentation. Each returned of their own accord. As such, the sequence observed reflects the order of presentation rather than the design of our program. For a candidate presenting with dual donation as an objective, our data do not support a prescriptive pathway.

Each sequence carries theoretical trade-offs: kidney-first places the lower-risk operation first, whereas liver-first avoids a complex hepatectomy in a donor with a solitary kidney. Sequence selection is best grounded in donor wishes, anatomy and physiologic reserve, recipient urgency, and center experience, with equipoise maintained across both options.

A median interval between donations of 2.0 years was observed in our cohort. Our current data at this point are insufficient to define a minimum interval policy between donations. However, a minimum interval of 6 months was considered appropriate to allow adequate physiological recovery and reassessment of psychosocial stability before a second donation.

Ten of 24 donors (42%) participated in kidney paired donation, so the yield from one individual's decision may extend beyond two transplants, and integration of living liver donors into liver-paired exchange could extend it further [17]. No consensus guidance exists for donors who return, and we would support standards addressing selection, minimum interval, consent for cumulative risk, and a registry able to audit the sequential donation journey.

## Limitations

Limitations include the retrospective design, modest sample size, and the lack of long-term quality of life and functional outcomes. Follow-up was confined to the perioperative period, so long-term renal and hepatic function and the cumulative long-term risk of two donor operations remain unaddressed. We did not examine psychosocial drivers, donor identity, or health-related quality of life, all of which shape attitudes to donation

[18], and our observations regarding anonymous donation are therefore descriptive rather than explanatory. This study was designed as a donor-specific analysis; therefore, recipient characteristics and post-transplant outcomes were not examined, nor was a comparator cohort of single-organ donors included. All of the above would be valuable in future multicenter studies to better contextualize the outcomes of sequential dual-organ donation.

## Conclusion

Sequential dual-organ living donation is rare but was completed safely in this cohort irrespective of sequence, and prior donation seldom required modification of operative technique. For programs contemplating sequential donation, our data support disciplined selection, thoughtful planning, and vigilant perioperative care. Early donor outcomes were reassuring, including preserved renal function after right lobe donation in prior kidney donors. The field now needs consensus guidance specific to these donors and registry infrastructure capable of following them beyond the perioperative period. The cumulative impact of these donors on transplant activity, including their role in paired donation chains, justifies the investment.

## 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 University Hospital Network Research Ethics Board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from all participants.

## References

1. Pruett TL, Tibell A, Alabdulkareem A, Bhandari M, Cronin DC, Dew MA, et al. The ethics statement of the Vancouver forum on the live lung, liver, pancreas, and intestine donor. *Transplantation* (2006) 81(10):1386–7. doi: 10.1097/01.tp.0000214976.36526.e3
2. Bababekov YJ, Pomfret EA. Ethical considerations for simultaneous vs sequential liver-kidney donation from the same live donor: donor(s) selection is key. *Gastroenterology* (2024) 166(3):365–8. doi: 10.1053/j.gastro.2023.11.021
3. Sidoti CN, Terlizzi K, Donnelly C, Jaffe IS, Motter JD, Philosophe B, et al. Second time around: increased rate of living donation from repeat organ donors. *Clin Transpl* (2025) 39(1):e70049. doi: 10.1111/ctr.70049
4. Nair A, Modaresi Esfeh J, Kwon CHD, Aucejo F, Quintini C, Hashimoto K. Living liver donation in previous kidney donors: a single-center experience. *Am J Transpl* (2020) 20(5):1461–2. doi: 10.1111/ajt.15815
5. Al Harakeh H, Emmanuel B, Hughes C, Tevar A, Steel JL, DiMartini A, et al. Sequential liver and kidney living donors: making the ultimate gift twice. *Clin Transpl* (2022) 36(8):e14750. doi: 10.1111/ctr.14750
6. Jenkins R, Hapuarachchy BL, Wells A, Shah M, Rached H, Wesson R, et al. Twice is just as nice: favorable donor outcomes in living donor liver transplantation after

## Author contributions

Participated in research design: SG, NS, SS, JL, MS, GS, BS, and AG. Participated in the writing of the paper: SG, NS, SS, JL, MS, GS, BS, and AG. Participated in the performance of the research: SG, NS, SS, IM, CS, TR, NG, JL, MS, GS, BS, MC, AG. Participated in data analysis: SG and AG. 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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previous kidney donation. *Am J Transplant* (2025) 25(8):S270. doi: 10.1016/j.ajt.2025.07.601

7. Steel JL, Trakumas TL, Tevar AL, Huerer ML, Ganesh S, Tillman E, et al. Post-donation outcomes for sequential liver and kidney donors versus single donor matched controls. *Am J Transplant* (2025) 25(8):S743. doi: 10.1016/j.ajt.2025.07.1753

8. Jackson WE, Kriss MS, Burton JR, Jr., Nydam TL, Conzen KD, Pomposelli JJ, et al. Reply to living liver donation in previous kidney donors: a single-center experience. *Am J Transpl* (2021) 21(1):435–7. doi: 10.1111/ajt.16153

9. Yankol Y, Karatas C, Kanmaz T, Kocak B, Kalayoglu M, Acarli K. Extreme living donation: a single center simultaneous and sequential living liver-kidney donor experience with long-term outcomes under literature review. *Turk J Surg* (2021) 37(3):207–14. doi: 10.47717/turkjsurg.2021.5387

10. Imai D, Rokop ZP, Yokoyama M, Sharma A, Mihaylov P, Powelson J, et al. Renal function in sequential living kidney-then-liver donors undergoing right lobe donation: a two-center case study. *Clin Transpl* (2025) 39(5):e70168. doi: 10.1111/ctr.70168



11. Sapisochin G, Goldaracena N, Laurence JM, Levy GA, Grant DR, Cattral MS. Right lobe living-donor hepatectomy-the Toronto approach, tips and tricks. *Hepatobiliary Surg Nutr* (2016) 5(2):118–26. doi: 10.3978/j.issn.2304-3881.2015.07.03
12. Richardson R, Connelly M, Dipchand C, Garg AX, Ghanekar A, Houde I, et al. Kidney paired donation protocol for participating donors 2014. *Transplantation* (2015) 99:S1–S88. doi: 10.1097/TP.0000000000000918
13. Lam NN, Dipchand C, Fortin MC, Foster BJ, Ghanekar A, Houde I, et al. Canadian society of transplantation and Canadian society of nephrology commentary on the 2017 KDIGO clinical practice guideline on the evaluation and care of living kidney donors. *Can J Kidney Health Dis* (2020) 7:2054358120918457. doi: 10.1177/2054358120918457
14. Reichman TW, Fox A, Adcock L, Wright L, Abbey SE, Levy G, et al. Anonymous living liver donation: donor profiles and outcomes. *Am J Transpl* (2010) 10(9):2099–104. doi: 10.1111/j.1600-6143.2010.03244.x
15. Cattral MS, Ghanekar A, Selzner N. Anonymous living donor liver transplantation: the altruistic strangers. *Gastroenterology* (2023) 165(6):1315–7. doi: 10.1053/j.gastro.2023.08.043
16. Goldaracena N, Jung J, Aravinthan AD, Abbey SE, Krause S, Pritlove C, et al. Donor outcomes in anonymous live liver donation. *J Hepatol* (2019) 71(5):951–9. doi: 10.1016/j.jhep.2019.06.027
17. Patel MS, Mohamed Z, Ghanekar A, Sapisochin G, McGilvray I, Sayed BA, et al. Living donor liver paired exchange: a North American first. *Am J Transpl* (2021) 21(1):400–4. doi: 10.1111/ajt.16137
18. Naibauer S, Branagan WT, Lehto S, Reynolds N, Mikulich-Gilbertson S, Page CE, et al. Attitudes towards living organ donation: a cross-sectional survey study. *Front Public Health* (2025) 13:1552393. doi: 10.3389/fpubh.2025.1552393



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# Whole pediatric liver graft in modified RAPID transplantation for unresectable colorectal liver metastases

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Liver transplantation has re-emerged as a therapeutic option for unresectable colorectal liver metastases (CRLM), but organ shortage and allocation concerns remain major limitations. The RAPID procedure combines auxiliary liver transplantation with delayed total hepatectomy, allowing small-graft use while the native liver provides temporary metabolic support. We report a modified RAPID transplantation, interpreted as a RAPID-derived staged auxiliary orthotopic liver transplantation (AOLT) strategy, using a whole pediatric graft in a 68-year-old woman with liver-only CRLM and sustained response to chemotherapy. The graft, from a 2-year-old donor (280 g; GRWR 0.4), had been declined by all pediatric centers due to non-standard infectious risk and was authorized by the Italian National Transplant Center within a regulated pathway. Favorable arterial anatomy preserved native right liver perfusion, while portal inflow management combined intentional surgical narrowing of the right portal vein with delayed portal vein embolization (PVE). At pre-PVE CT, graft volume was 350 cc (estimated GRWR 0.5); after PVE, it reached 580 cc (estimated GRWR 0.8), enabling completion hepatectomy at four weeks. At eight months, the patient had normal liver function and no recurrence. To our knowledge, this is the first reported use of a whole pediatric liver graft in modified RAPID transplantation.

### KEYWORDS

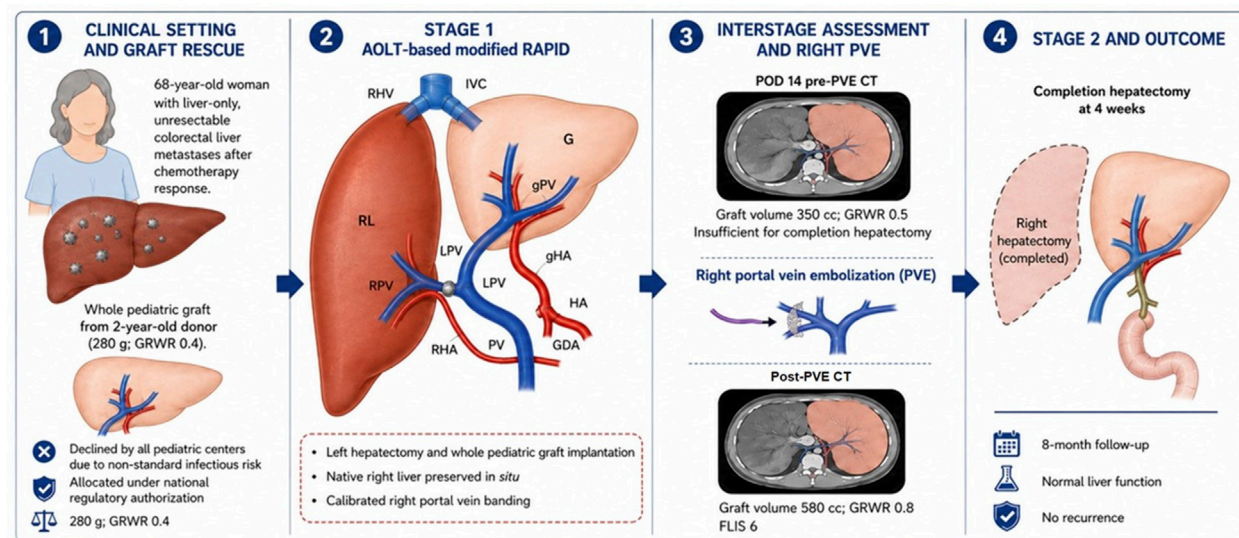
auxiliary orthotopic liver transplantation, colorectal liver metastases, modified RAPID transplantation, pediatric liver graft, transplant oncology

## Introduction

Liver transplantation (LT) for unresectable colorectal liver metastases (CRLM) has gained renewed interest after prospective experiences and the randomized TransMet trial demonstrated meaningful survival benefit in highly selected patients [1–4]. Nevertheless, the use of standard orthotopic LT for CRLM remains constrained by organ shortage, allocation ethics, and competition with established transplant indications. These limitations have stimulated increasing interest in transplant oncology strategies that may reduce the impact of CRLM transplantation on the conventional donor pool [5].

The RAPID (Resection and Partial Liver Transplantation with Delayed Total Hepatectomy) procedure was proposed to address some of these limitations by combining auxiliary liver transplantation with staged hepatectomy [6]. In the original

## Whole Pediatric Liver Graft in Modified RAPID Transplantation for Unresectable Colorectal Liver Metastases



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GRAPHICAL ABSTRACT

description, a small partial graft, typically segments 2–3, is implanted during the first stage while the native liver provides temporary metabolic support during graft hypertrophy and functional recovery. Completion hepatectomy is subsequently performed once adequate graft volume and function have been achieved. International consensus recommendations have recently been published to standardize patient selection and procedural conduct [7].

Published RAPID experiences have generally relied on split left lateral segment grafts obtained from deceased or living adult donors. This approach is consistent with the original rationale of using small grafts and preserving conventional allocation opportunities [8, 9]. However, reduced graft volume may pose technical and functional challenges, particularly in adult recipients, making careful inflow modulation, graft monitoring, and timing of completion hepatectomy essential.

The use of a whole pediatric liver graft instead of a conventional split left lateral segment graft has not been previously described in the RAPID setting. Because the present case used a whole graft and a sequential portal inflow strategy rather than complete right portal vein ligation at stage 1, it should be interpreted as a RAPID-derived staged auxiliary orthotopic liver transplantation strategy (AOLT) rather than canonical RAPID sensu stricto. A fundamental prerequisite for any such use is that it must not compete with pediatric transplant candidates. We report a case in which a whole pediatric graft, formally declined by all pediatric transplant centers due to non-standard infectious risk, was allocated under national regulatory authorization within an approved transplant oncology protocol for unresectable CRLM. This report focuses on technical feasibility, perioperative assessment, graft regeneration, intentional surgical portal inflow management, and allocation considerations.

## Methods

The present study was conducted within the RAPID–Padova institutional protocol for unresectable CRLM, approved by the local ethics committee and registered at [ClinicalTrials.gov](https://clinicaltrials.gov) (NCT04865471). Written informed consent was obtained from the patient for publication of the case and accompanying images.

Eligibility for transplantation was assessed by a multidisciplinary transplant oncology board. Selection criteria included unresectable liver-only CRLM, sustained response to systemic therapy, absence of extrahepatic progression, favorable tumor biology, and adequate performance status. Molecular tumor profile, radiological response, tumor marker trend, and prior resection of the primary tumor were all considered during evaluation. The availability of a rescue or otherwise non-utilized graft was not considered a reason to broaden oncological eligibility criteria.

Preoperative vascular anatomy was reviewed to assess feasibility of AOLT, preservation of native liver perfusion during the interstage period, and subsequent completion hepatectomy. Particular attention was paid to hepatic arterial anatomy, portal inflow configuration, venous outflow reconstruction, and biliary reconstruction options.

Donor graft allocation followed a stepwise process aligned with Italian national transplant regulations. The graft was formally offered to all pediatric transplant centers according to standard allocation priority. Only after exhaustion of all pediatric allocation options, and following assessment by an infectious disease specialist, was the graft considered for adult use within the modified RAPID strategy. Specific written authorization from the Italian National Transplant Center was obtained before graft acceptance. This authorization was protocol-specific and did not set a precedent for routine pediatric-to-adult graft allocation in oncological

transplantation. Donor infectious risk, blood culture results, respiratory microbiology, antibiotic coverage, and overall donor risk assessment were reviewed and documented before acceptance.

Perioperative feasibility was assessed according to technical completion of stage-1 modified RAPID transplantation, graft-to-recipient weight ratio (GRWR), portal inflow modulation strategy, Doppler ultrasound assessment, liver function during the interstage period, radiological graft volume, gadoxetic-acid functional liver imaging score (FLIS; range 0–6), completion hepatectomy, postoperative liver function, and early oncological follow-up.

## Results

### Recipient and oncological selection

A 68-year-old woman with no major comorbidities was diagnosed with adenocarcinoma of the splenic flexure and synchronous bilobar CRLM. Molecular profiling demonstrated RAS and BRAF wild-type disease. Systemic treatment with FOLFOX plus panitumumab achieved sustained radiological response and normalization of tumor markers. Despite favorable tumor biology and treatment response, liver resection was not feasible because of the initial bilobar distribution and insufficient future liver remnant.

After resection of the primary tumor, restaging confirmed liver-only disease without extrahepatic progression. The patient had Eastern Cooperative Oncology Group (ECOG) performance status 0 and was accepted for transplantation within the institutional protocol. Standard orthotopic LT was not considered a realistic option given the patient's oncological indication, the allocation priority framework applicable in Italy, and the absence of a suitable adult deceased donor split graft within a clinically acceptable timeframe.

A favorable vascular configuration was identified preoperatively, with a replaced right hepatic artery arising from the superior mesenteric artery and a common hepatic artery supplying the left liver. This anatomy was considered advantageous because it allowed preservation of arterial inflow to the native right liver during the interstage period while enabling direct arterial reconstruction of the graft at stage 1.

### Donor and graft allocation

A whole liver graft from a 2-year-old, 15-kg donor was considered for allocation. The donor had experienced post-anoxic encephalopathy after drowning and was classified as carrying non-standard infectious risk following infectious disease assessment, due to elevated inflammatory markers, bilateral pneumonia on imaging, and positive bronchoalveolar lavage cultures despite persistently negative blood cultures. Broad-spectrum antibiotic therapy with piperacillin/tazobactam had been initiated before procurement.

The graft was formally offered to all pediatric transplant centers in Italy in accordance with national allocation protocols. All contacted pediatric centers declined the graft, citing the non-standard infectious risk classification and its associated uncertainty in a pediatric recipient population. Only after exhaustion of all pediatric allocation options was the graft

considered for use within the modified RAPID strategy. Organ utilization in the adult recipient was authorized by the Italian National Transplant Center within the approved protocol and did not require modification of standard antimicrobial prophylaxis for liver transplantation.

### Stage-1 modified RAPID transplantation

Stage-1 modified RAPID transplantation consisted of left hepatectomy extended to the middle hepatic vein and auxiliary orthotopic implantation of the whole pediatric graft. The measured back-table graft weight was 280 g, corresponding to a GRWR of 0.4. The graft was preserved using University of Wisconsin solution, and cold ischemia time was 480 min.

Venous outflow reconstruction was performed using the recipient common trunk of the middle and left hepatic veins and the graft vena cava. The graft hepatic artery was anastomosed directly to the recipient common hepatic artery at the level of the gastroduodenal artery, while native right liver arterial inflow was preserved through the replaced right hepatic artery arising from the superior mesenteric artery. Portal anastomosis was performed between the native left portal branch and the graft portal vein. Portal inflow management was achieved by intentional surgical narrowing of the right portal vein rather than complete ligation, aiming to reduce abrupt hyperperfusion of the small pediatric graft while maintaining residual portal inflow to the native right liver. The degree of narrowing was guided by graft size, intraoperative macroscopic assessment of graft perfusion and congestion, and the need to preserve native right liver perfusion (Figures 1, 2). Biliary reconstruction was performed using Roux-en-Y hepaticojejunostomy. Doppler ultrasound confirmed patency of the portal and arterial anastomoses, hepatopetal portal flow, and preserved arterial signal. Quantitative portal flow and direct portal pressure measurements were not obtained.

### Interstage assessment and completion hepatectomy

Volumetric and functional kinetics during the interstage period and after completion hepatectomy are shown in Figure 3 and Supplementary Table S1. Recipient weight was approximately 70 kg. The measured back-table graft weight was 280 g, corresponding to a GRWR of 0.4. At the pre-PVE CT assessment, graft volume was 350 cc, corresponding to an estimated GRWR of 0.5. This was considered insufficient to proceed safely to completion hepatectomy. Therefore, right portal vein embolization was performed on POD 14 to further redirect portal inflow toward the graft and promote additional hypertrophy. Two weeks after PVE, graft volume had increased to 580 cc, corresponding to an estimated GRWR of 0.8. The post-PVE graft volume increase was 230 cc over 14 days, corresponding to 16.4 cc/day and a relative increase of approximately 65.7%. Liver function had also improved, with total bilirubin 2.1 mg/dL, AST 40 U/L, ALT 38 U/L, and INR 1.1. Hepatobiliary contrast-enhanced MRI confirmed preserved graft function, with a gadoxetic-acid functional liver imaging score (FLIS; range 0–6) of 6. Completion right hepatectomy was therefore performed 4 weeks after stage 1 (Figure 4).



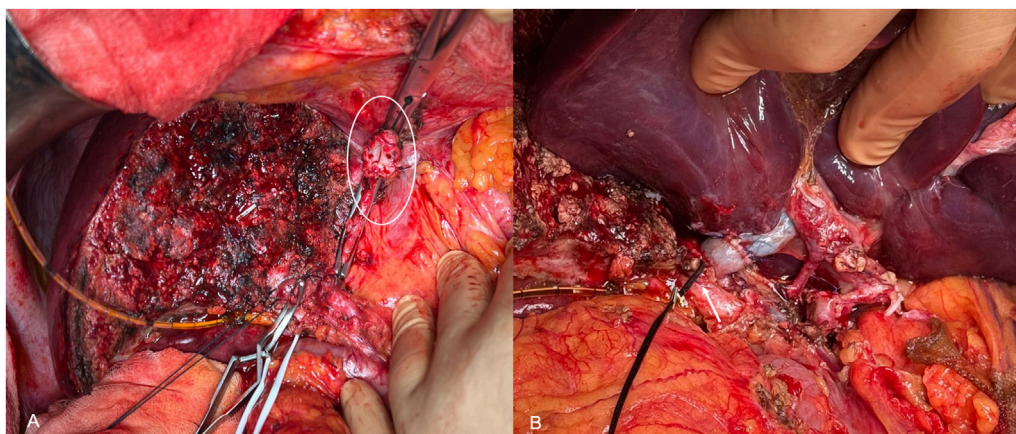


FIGURE 1

(A) Left hepatectomy extended to the middle hepatic vein; a venous cuff formed by the common trunk of the middle and left hepatic veins is shown above the clamp. (B) Whole pediatric liver graft implanted in an auxiliary orthotopic position. Arrow shows intentional surgical narrowing of the native right portal vein.

No post-hepatectomy liver failure occurred. Ascites production was initially moderate and resolved progressively during the first postoperative week. At 8-month follow-up, the patient was alive with normal liver function and no radiological evidence of disease recurrence.

## Discussion

This report describes the technical feasibility of using a whole pediatric liver graft in a modified RAPID transplantation strategy for unresectable CRLM. Its primary ethical premise is that the graft used in this case was not in competition with any pediatric transplant candidate: it had been formally declined by all contacted pediatric transplant centers due to non-standard infectious risk, and its allocation to an adult recipient occurred only under specific regulatory authorization from the Italian National Transplant Center within an approved institutional protocol. This report should therefore be interpreted as documenting the rescue use of an otherwise non-utilized graft, not as a proposal for routine pediatric-to-adult allocation in transplant oncology.

The terminology of the present procedure deserves clarification. Canonical RAPID, as defined in the original description and in recent consensus recommendations, relies on partial orthotopic liver transplantation using a small graft, typically segments 2–3, followed by delayed total hepatectomy after adequate graft volume and function have been achieved [6]. The present case preserved the staged logic of RAPID, namely auxiliary orthotopic graft implantation, temporary native liver support, graft hypertrophy, and delayed completion hepatectomy. However, it differed from canonical RAPID in two key aspects: the use of a whole pediatric liver graft rather than a partial graft, and the adoption of sequential portal inflow management rather than complete right portal vein ligation at stage 1. For this reason, the present procedure is better described as a RAPID-derived staged AOLT strategy rather than canonical RAPID *sensu stricto*.

The portal inflow strategy also differed from canonical RAPID. In the original RAPID concept, complete right portal vein ligation is intended to

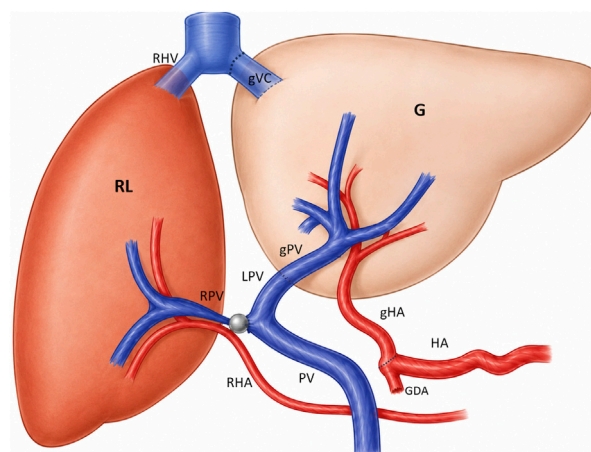
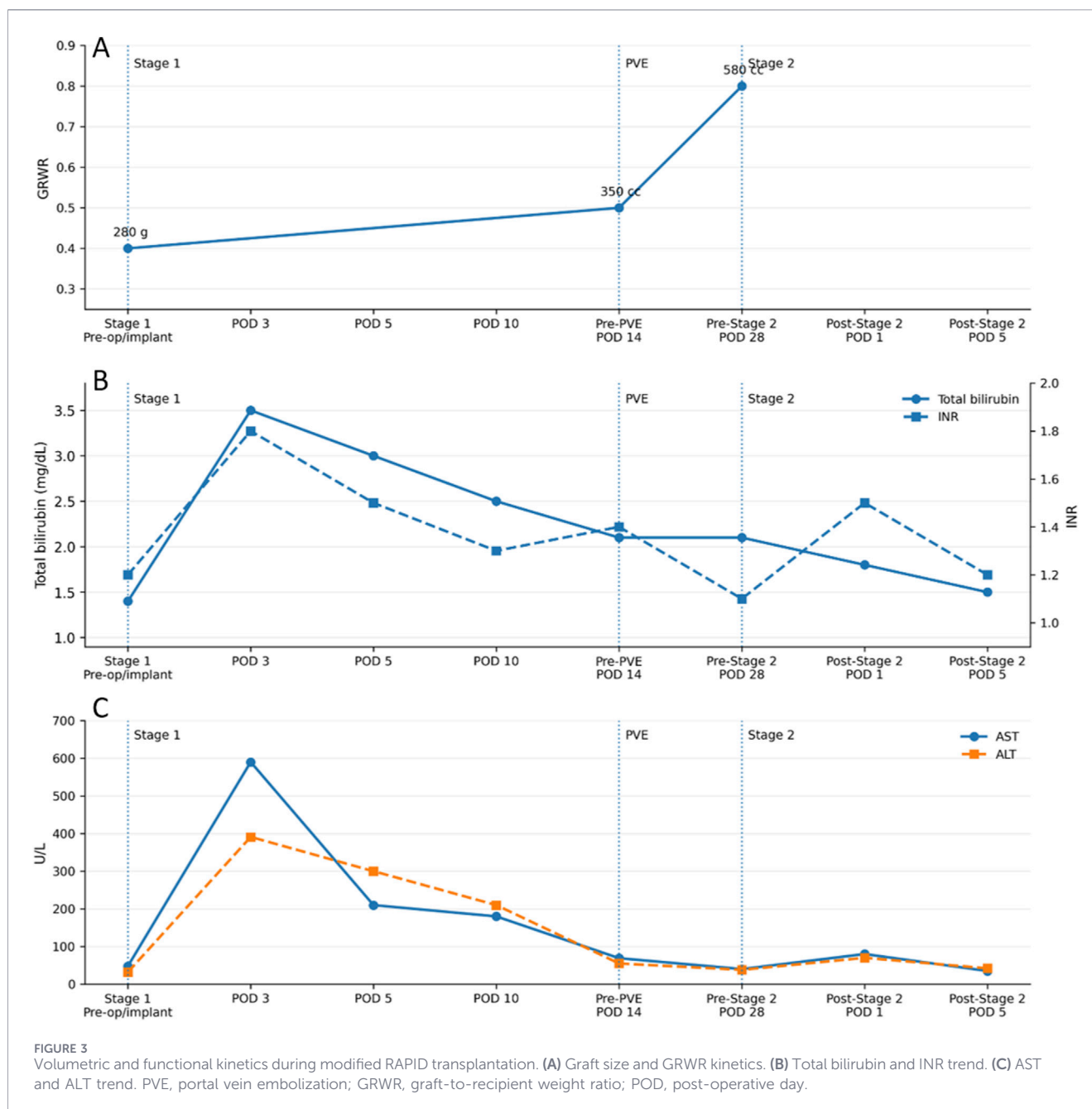


FIGURE 2

Schematic representation of stage-1 modified RAPID transplantation using a whole pediatric liver graft (G), within a RAPID-derived staged auxiliary orthotopic liver transplantation strategy. The native right liver (RL) is preserved with arterial inflow from a replaced right hepatic artery (RHA) arising from the superior mesenteric artery. The graft hepatic artery (gHA) is anastomosed to the recipient common hepatic artery (CHA), the graft portal vein (gPV) to the native left portal branch (LPV), and venous outflow is reconstructed using the recipient common trunk of the middle and left hepatic veins and the graft vena cava (gVC). Portal inflow is managed by intentional surgical narrowing of the right portal vein. Biliary reconstruction is performed using Roux-en-Y hepaticojejunostomy (not shown).

aggressively redirect portal flow toward the graft and stimulate rapid hypertrophy. In the present case, intentional surgical narrowing of the right portal vein was initially performed to reduce the risk of abrupt hyperperfusion of a small pediatric graft in an adult recipient. Delayed right PVE was then used as a second step to further redirect portal inflow and stimulate graft hypertrophy. This sequence may have combined small-for-size protection with delayed hypertrophic stimulation, although the lack of direct portal pressure and quantitative flow measurements prevents precise assessment of the relative contribution of each maneuver.





The availability of intermediate volumetric and functional assessment helps clarify the rationale for sequential inflow management. At the pre-PVE CT assessment, graft volume was 350 cc and estimated GRWR was 0.5, which was considered insufficient for safe completion hepatectomy. Delayed right PVE was therefore performed as a second step. Before stage 2, graft volume had increased to 580 cc and estimated GRWR to 0.8, with preserved functional assessment. The post-PVE increase of 230 cc over 14 days, corresponding to 16.4 cc/day and approximately 65.7% relative growth, supported the decision to proceed to completion hepatectomy. This sequence suggests that the initial narrowing strategy may have protected the small pediatric graft from abrupt portal hyperperfusion, whereas delayed PVE provided the additional hypertrophic stimulus required for stage 2. However, because direct

portal flow and pressure measurements were not obtained, the relative contribution of each maneuver cannot be precisely quantified.

The distinction between conventional LT for CRLM and modified RAPID transplantation is important. While the TransMet trial and prospective experiences support LT in selected patients with unresectable CRLM [3, 4], these data refer primarily to standard orthotopic transplantation [4]. RAPID and RAPID-derived strategies remain evolving approaches supported by limited clinical experience and recently published international consensus recommendations [7]. In this setting, objective documentation of graft volume and function before completion hepatectomy is essential, and the present case met both volumetric and functional criteria before proceeding to stage 2.

Several technical aspects merit discussion. First, favorable recipient arterial anatomy facilitated the procedure. The replaced

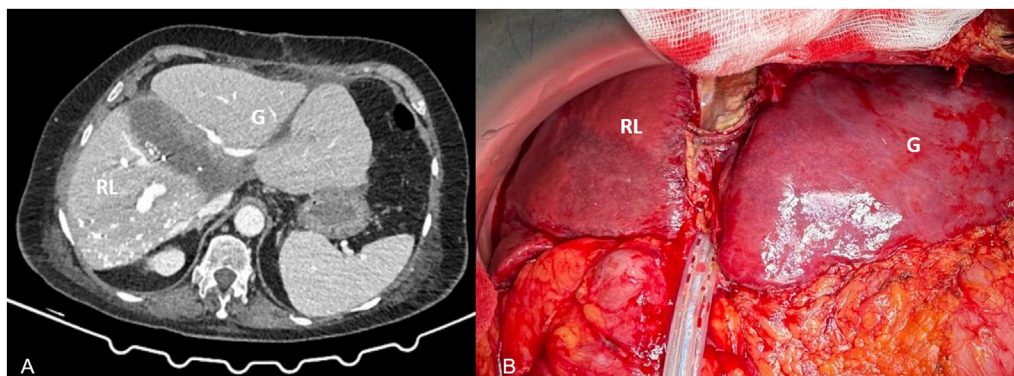


**FIGURE 4**  
Perioperative imaging and intraoperative findings. **(A)** Postoperative contrast-enhanced CT scan after right portal vein embolization showing marked hypertrophy of the pediatric graft following stage-1 modified RAPID transplantation. **(B)** Intraoperative view of the second stage before completion right hepatectomy. RL, right native liver; G, pediatric graft.

right hepatic artery from the superior mesenteric artery allowed arterial inflow to the native liver remnant to be maintained throughout the interstage period, while the recipient common hepatic artery was available for direct graft reconstruction. However, this anatomical configuration should be considered advantageous rather than mandatory. In other settings, end-to-side anastomosis of the graft artery to the common hepatic artery may be feasible, with only temporary clamping and preservation of arterial inflow through the gastroduodenal artery or collateral pathways. Therefore, a replaced right hepatic artery should not be interpreted as an absolute prerequisite for modified RAPID transplantation, although it may simplify arterial management.

Second, the use of a whole pediatric graft differs technically from a split left lateral segment graft. The intact hilar and caval structure of the whole pediatric graft may simplify certain aspects of back-table preparation and implantation. At the same time, smaller vascular diameters, susceptibility to hyperperfusion injury, and the inherently limited functional reserve of a pediatric graft in an adult recipient require meticulous surgical planning and postoperative surveillance [9]. Doppler ultrasound confirmed vascular patency in the present case, but quantitative portal flow and portal pressure measurements were not available.

From an ethical standpoint, this case satisfies the conditions that would be required to justify pediatric-to-adult graft allocation in this specific context: the graft was unavailable to pediatric recipients through formal exhaustion of the allocation protocol; its use was authorized by the national regulatory institution within a prospectively registered protocol; the recipient met rigorous oncological and functional selection criteria; and the procedure was conducted within a multidisciplinary oversight framework. Importantly, graft selection and recipient eligibility must remain entirely decoupled. The availability of a rescue or otherwise non-utilized graft must not broaden oncological indications, weaken recipient selection, or justify expansion beyond accepted transplant-oncology criteria. These conditions are not routinely met and are unlikely to recur frequently, which underscores that this experience does not support policy changes in pediatric organ allocation.

The main limitations of this report are its single-case design, short oncological follow-up of 8 months, and absence of direct portal pressure and quantitative portal flow measurements. No

conclusion regarding long-term oncological efficacy can be drawn from a single case with this follow-up duration. In addition, without direct hemodynamic measurements, it is not possible to determine whether immediate complete right portal vein ligation would have produced faster hypertrophy or allowed a shorter interstage interval. Publication bias inherent to case reports with favorable outcomes should also be acknowledged. Nevertheless, this case demonstrates the technical feasibility of whole pediatric graft rescue within a RAPID-derived staged AOLT strategy in one carefully selected patient under exceptional regulated allocation conditions. Future prospective registries should systematically collect hemodynamic, volumetric, functional, and oncological data to define the reproducibility and safety of this approach.

## Data availability statement

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

## Ethics statement

Written informed consent for publication of this case report and accompanying images was obtained from the recipient. For the deceased pediatric donor, consent/authorization for organ donation and use of anonymized donor-related information was provided by the donor's legal guardian/next of kin, in accordance with national regulations and institutional procedures.

## Author contributions

RM conceived and designed the study, performed the surgical procedure, analyzed the data, and wrote the manuscript. NG, ML, and FM participated in the surgical procedure and data collection. MC, GB, and MA contributed to patient management, data collection, and interpretation of clinical data. GM and DC contributed to imaging

analysis and interpretation. EG participated in data collection and manuscript preparation. GE supervised the study, contributed to research design, and critically revised the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

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## References

1. van der Geest LGM, Lam-Boer J, Koopman M, Verhoef C, Elferink MAG, de Wilt JHW. Nationwide trends in incidence, treatment and survival of colorectal cancer patients with synchronous metastases. *Clin Exp Metastasis* (2015) 32:457–65. doi: 10.1007/s10585-015-9719-0
2. Tomlinson JS, Jarnagin WR, DeMatteo RP, Fong Y, Kornprat P, Gonen M, et al. Actual 10-year survival after resection of colorectal liver metastases defines cure. *J Clin Oncol* (2007) 25(29):4575–80. doi: 10.1200/JCO.2007.11.0833
3. Dueland S, Grut H, Syversveen T, Hagness M, Line PD. Selection criteria related to long-term survival following liver transplantation for colorectal liver metastasis. *Am J Transpl* (2020) 20(2):530–7. doi: 10.1111/ajt.15682
4. 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, randomized controlled trial. *Lancet* (2024) 404(10458):1107–18. doi: 10.1016/S0140-6736(24)01595-2
5. Sapisochin G, Hibi T, Toso C, Man K, Berenguer M, Heimbach J, et al. Transplant oncology in primary and metastatic liver tumors: principles, evidence, and opportunities. *Ann Surg* (2021) 273(3):483–93. doi: 10.1097/SLA.0000000000004071
6. Line PD, Hagness M, Berstad AE, Foss A, Dueland S. A novel concept for partial liver transplantation in nonresectable colorectal liver metastases: the RAPID concept. *Ann Surg* (2015) 262(1):e5–e9. doi: 10.1097/SLA.0000000000001165
7. Malagò M., Line P.-D., Balci D., Raptis D., Nadalin S., Scatton O., et al. International consensus recommendations for the RAPID procedure in liver transplantation: the RAPID consensus ISLS 2023 Zurich collaborative. *Int J Surg* (2025) 111(4):2766–72. doi: 10.1097/JIS.0000000000002145
8. Nadalin S, Settmacher U, Rauchfuß F, Balci D, Königsrainer A, Line PD. RAPID procedure for colorectal cancer liver metastasis. *Int J Surg* (2020) 82S:93–6. doi: 10.1016/j.jisu.2020.03.078
9. Azoulay D, Feray C, Lim C, Salloum C, Conticchio M, Cherqui D, et al. A systematic review of auxiliary liver transplantation of small-for-size grafts in patients with chronic liver disease. *JHEP Rep* (2022) 4(4):100447. doi: 10.1016/j.jhepr.2022.100447

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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.2026.17124/full#supplementary-material>



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# Dual-clamp technique facilitating end-to-side suture anastomosis in murine cervical heart transplantation

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### KEYWORDS

end-to-side anastomosis, heart transplantation, microsurgical technique, mouse model, transplant model

Dear Editors,

Heterotopic cervical heart transplantation (HT) in mice is a widely used preclinical model in transplant immunology and chronic allograft vasculopathy research [1, 2]. Although the cuff technique is popular due to its simplicity, it compromises unilateral cerebral blood flow, requires custom-made cuffs, and carries a higher risk of thrombosis and vessel twisting. At our center, we favor manual end-to-side suture anastomosis, as it preserves the continuity of the common carotid artery (CCA) and external jugular vein (EJV), thereby preventing neurological complications and adhering to the 3Rs principle. Moreover, this technique avoids high-velocity arterial jets in the end-to-end grafts and ensures maximum comparability between studies using the abdominal HT model [3].

A major technical hurdle of this manual approach is that the CCA lies deep within the body and is widely separated from the EJV, making precise suturing highly challenging, especially in the confined cervical space. To overcome this, we apply a dual-clamp occlusion technique [4] (Figure 1A). Two microclamps are used: one placed at the proximal end and one at the distal end, each simultaneously clamping both the CCA and the EJV, physically bringing the two vessels into close proximity and effectively eliminating height mismatch (Figures 1B–E). Additionally, sterile, nonwoven pads moistened with cold saline are placed over the clamp handles. The gentle downward pressure not only keeps the graft cool but also elevates the deeper CCA to the superficial plane. This simple modification makes the artery-first anastomosis and the entire everting suture feasible.

Since implementing this refined technique in our established HT program (>1,000 cases since 2010), we have achieved a technical success rate of 83/87 (95.4%) in recent consecutive procedures. Technical success was defined as graft survival with maintained palpable contractility throughout the 100-day observation period (assessed daily by palpation). All 83 successful grafts remained functional during this period, free of thrombosis and other vascular complications. Although manual suturing is generally considered more technically demanding than the cuff method, the total operative time for the recipient (~25–30 min) was not prolonged. In fact, the anastomosis itself (15–20 min) was faster than cuff installation (20–30 min) [5]. In our institutional training curriculum for microsurgical transplantation, three surgeons with prior abdominal HT experience achieved consistent success in approximately 3 to 5 procedures, whereas three trainees without a microsurgical background required 50 to 70 operations to become proficient.



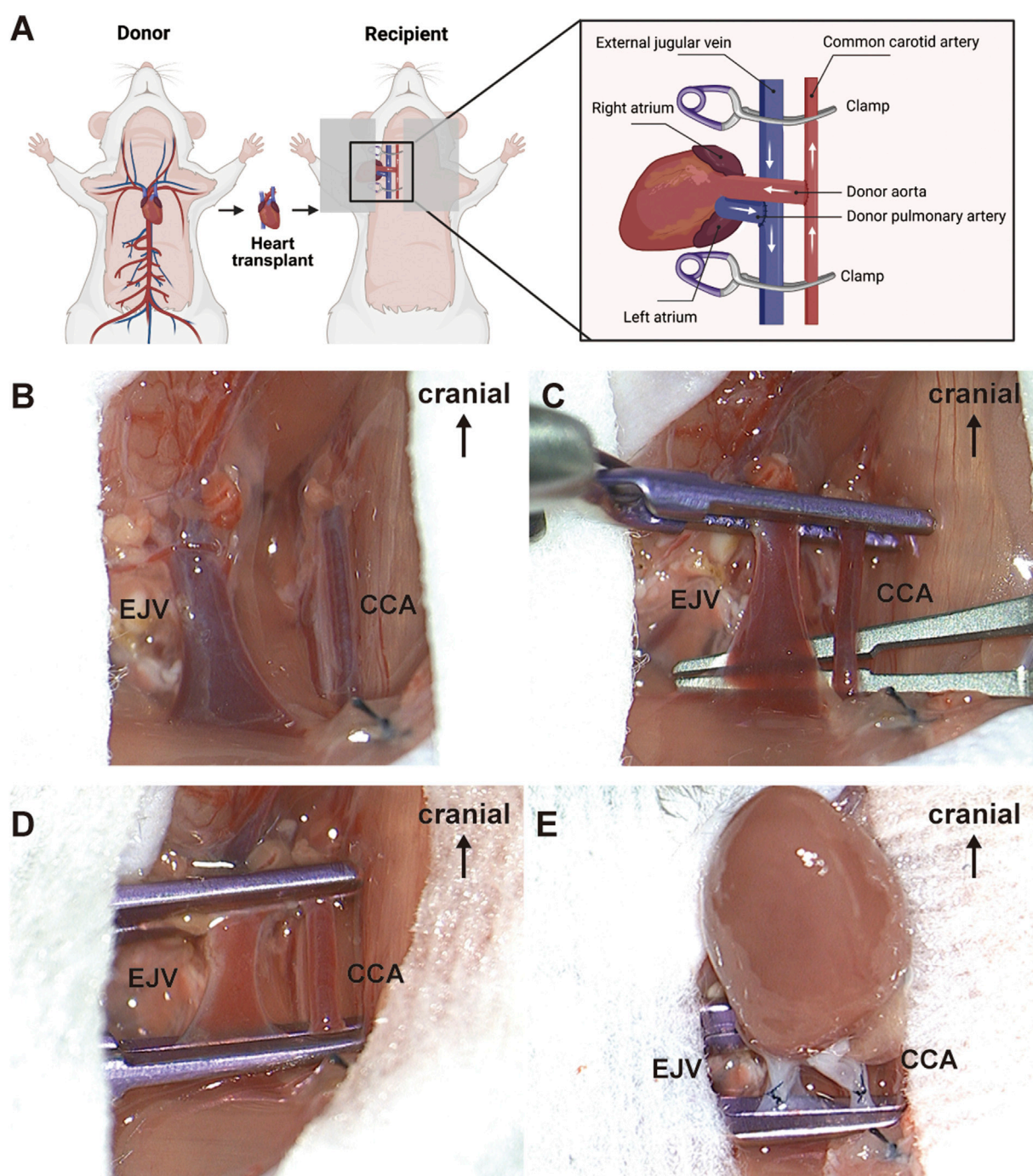


FIGURE 1

(A) Schematic diagram of end-to-side anastomosis using the dual-clamp vascular occlusion technique. (B) Isolate the recipient's CCA and EJV. (C) Clamp the CCA and EJV simultaneously with a microvascular clamp on the cranial side. (D) Clamp the CCA and EJV simultaneously with a microvascular clamp on the caudal side. (E) View of the allograft heart after completing all anastomoses. CCA, common carotid artery. EJV, external jugular vein.



While we acknowledge the single-center nature of this study and the lack of a prospective comparison with the cuff technique, we believe this technical modification provides a valuable refinement for cervical HT, particularly for microsurgeons with prior abdominal experience transitioning to the cervical model.

## 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 animal study was approved by the Animal Ethics Committee of Tongji Hospital. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

XH participated in performing surgery; XZ participated in writing the article. ZW participated in performing surgery and

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

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## Conflict of interest

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## References

1. Alegre ML, Atkinson C, Issa F, Valujskikh A, Zhang ZJ. Best practices of heart transplantation in mice. *Am J Transpl* (2025) 25:1820–9. doi: 10.1016/j.ajt.2025.04.012
2. Plenter RJ, Zamora MR, Grazia TJ. Four decades of vascularized heterotopic cardiac transplantation in the mouse. *J Invest Surg* (2013) 26:223–8. doi: 10.3109/08941939.2012.755238
3. Dun H, Sticco-Ivins M, Terada Y, Berning A, Lavine KJ, Kreisel D, et al. Cervical heterotopic heart transplantation in mice using a novel suture technique. *JHLT Open* (2024) 7:100164. doi: 10.1016/j.jhlto.2024.100164
4. Huang X, Xu X, Dai L, Wang Z, Cheng Q. Cervical heterotopic mouse heart transplantation using end-to-side anastomosis technique to study transplant immunology. *J Vis Exp* (2024) 233:e70704. doi: 10.3791/70704
5. Oberhuber R, Cardini B, Kofler M, Ritschl P, Oellinger R, Aigner F, et al. Murine cervical heart transplantation using the cuff technique. *J Vis Exp* (2014) 91:e51855. doi: 10.3791/50753



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