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Tremor and Tacrolimus Formulation

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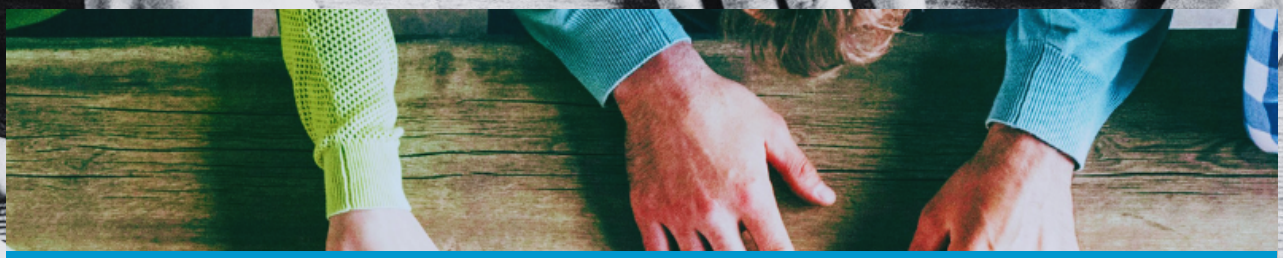

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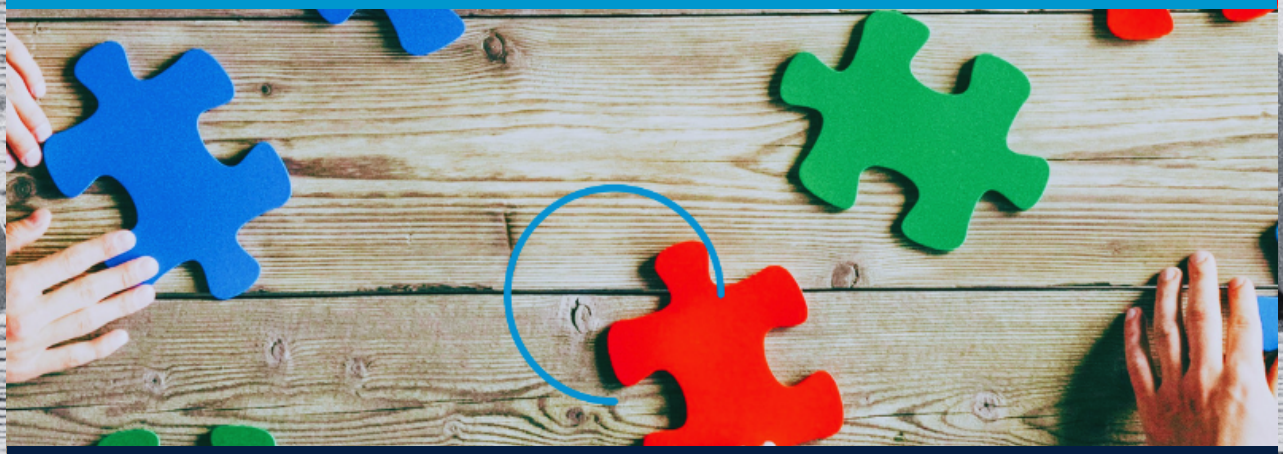

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TRANSPLANT TRIAL WATCH
published: 26 April 2024
doi: 10.3389/ti.2024.13111

Transplant Trial Watch

Simon R. Knight[1,2]* *and John M. O'Callaghan*[2,3]*

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Keywords: heart transplantation, randomised controlled trial, systematic review, living kidney donation, hemodynamic instability

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

RANDOMISED CONTROLLED TRIAL 1

Use of Intraoperative Haemoadsorption in Patients Undergoing Heart Transplantation: A Proof-of-Concept Randomized Trial.
by Nemeth, E., et al. ESC heart failure 2023 [record in progress].

Aims
This study aimed to investigate the role of intraoperative haemoadsorption in orthotopic heart transplant patients.

Interventions
Participants were randomised to receive either intraoperative haemoadsorption or standard care.

Participants
60 patients undergoing orthotopic heart transplantation.

Outcomes
The primary endpoint was early post-operative haemodynamic instability. Secondary endpoints were changes in procalcitonin (PCT) and C-reactive protein (CRP) levels post-operation, intraoperative change in mycophenolic acid (MPA) concentration, early allograft rejection, frequency of post-operative organ dysfunction, adverse immunological events, major complications, duration of ICU and in-hospital stay, and 1-year survival.

Follow-Up
1 year.

CET Conclusion
by John O'Callaghan

This is a very interesting, novel, RCT in heart transplantation. Heart recipients were randomised to standard care or to receive additional therapy with intra-operative hemoadsorption with the

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Cytosorb system from CytoSorbents, NJ, United States. The hemoadsorption cartridge was integrated into the cardiopulmonary bypass system and has been shown previously to remove cytokines, chemokines, bilirubin, myoglobin and plasma free haemoglobin. Patients were blinded to the treatment allocation, but clinical professionals were not. No sample size calculation could be done due to a lack of prior data on which to base it. The study found statistically significant differences across a range of outcomes, including the primary outcomes. Patients receiving hemoadsorption had a lower vasoactive-inotropic score, frequency of vasoplegic syndrome, risk of AKI, shorter median mechanical ventilation and median intensive care stay (by 3.5 days). The rates of cardiac allograft rejection, 30-day mortality and 1-year survival were similar between the groups, although it may have been too small to show differences in these outcomes. There were no device related complications.

Jadad Score
3.

Data Analysis
Modified intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
ClinicalTrials.gov—NCT03145441.

Funding Source
No funding received.

SYSTEMATIC REVIEW

Psychological Impact of Living Kidney Donation: A Systematic Review by the EAU-YAU Kidney Transplant Working Group.

by Cazauvieilh, V., et al. Transplant International 2023; 36: 11827.

Aims
This study aimed to examine the psychological effects of donating a kidney on living donors.

Interventions
A literature search was performed using Pubmed and Medline. Study screening and data extraction were performed by two independent reviewers. The ROBINS-I tool was used to assess the risk of bias.

Participants
23 studies were included in the review.

Outcomes
The main outcomes of interest included assessment of quality of life, anxiety/depression, regret of donation, psychological impact over failure of transplant/death, and consequence of donation on donor/recipient relationship.

Follow-Up
N/A.

CET Conclusion

by John O'Callaghan

This is an interesting, well-conducted, and well-written, systematic review in living donation that gives a good description of the complexity in the donor-recipient relationship and the psychological outcome for the donor. Two independent reviewers screened references, extracted data and performed the risk-of bias assessment, which is clearly presented. A broad search was done, albeit only within pubmed/medline. 23 studies were included, comprised of a total 2,732 donors. The authors give a detailed description of the studies in narrative review. There is quantitative evidence from 3 studies that quality of life is the same pre and post-donation, whilst another 4 studies found quantitative evidence of improved quality of life at 1 year post-donation. These studies indicate risk factors that may be predictive of decreased donor quality of life such as donor fatigue, anxiety, depression, lack of social support, the donor-recipient relationship and any complications for the recipient. Three studies found no evidence of an impact of socio-economic status on quality-of-life post-donation. In general, studies found that the relationship between donors and recipients remained unchanged or improved/became closer. Some donors expected that their role as a carer for the recipient would decrease after donation. If this did not happen, donors felt disappointed or frustrated. In the majority of cases, donors were satisfied and did not regret donation. Importantly it was clearly demonstrated that it was possible to regret donation oneself, but to still recommend it for others. All studies showed a low rate of regret. There was some evidence of correlation between regret and the recipient's outcome from the transplant, but evidence was conflicting. One interesting complexity highlighted by the study is that donors used conscious or unconscious strategies to influence the transplant team to select them as a donor. This may make it difficult to interpret the results of pre and post-donation comparisons. The authors also acknowledge the impact of social desirability bias, which may have affected donor responses to questionnaires.

Trial Registration
N/A.

Funding Source
Not reported.

CLINICAL IMPACT SUMMARY

by Simon Knight

Whilst the medical consequences of living kidney donation are largely understood through use of large-scale registry data, the psychosocial response to donor assessment and donation are less comprehensively documented. A wide variety of qualitative and quantitative approaches have been taken, often with conflicting findings. Previous systematic reviews have focussed mainly on qualitative studies using questionnaires to assess quality of life, anxiety and depression [1]. In an attempt to make more sense of the existing literature, working group of young academics from the European Association of Urology have undertaken a detailed systematic review of both qualitative and quantitative studies reporting the psychosocial impact of living kidney donation [2].

The group identified 8 qualitative and 15 quantitative studies, and due to heterogeneity in the instruments used undertook narrative analysis of the findings. Whilst quantitative studies demonstrated stable or improved quality of life with low levels of regret, the more detailed exploration afforded by qualitative approaches demonstrated a much more mixed, complex picture. Donation can often impact quality of life, particularly in donors that experience post-operative fatigue, and many donors experience post-operative anxiety and depression with some expressing a sense of abandonment following donation. These aspects seem particularly important in the presence of donor or recipient medical complications, highlighting the importance of regular follow-up in donors. Despite this, very few donors express regret and most would recommend the process.

An interesting aspect that comes out of the qualitative studies is the impact of the pre-donation phase, with some donors describing anxiety induced by the investigations and work-up process, in particular relating to the fear of being found unsuitable, and the length of the process. Some donors reported employing strategies to influence decisions, such as downplaying existing psychological illnesses and withholding medical information to improve their chances of being found suitable to donate. Again, this highlights this importance of a detailed workup for all donors, including psychological assessment where indicated by history or clinical concerns.

One limitation of the existing literature is that it is difficult to identify those subgroups most at risk of psychological complications from the donation process. A few studies report the impact of recipient complications, donor-recipient relationship or social support on outcomes, but data on other aspects such as donor age (particular younger donors) and donor complications are lacking.

Overall, this review is a well-conducted study that provides a very comprehensive summary of what we currently know about the psychosocial impact of living donation. It also helps to highlight areas for future research.

Clinical Impact

3/5.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

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

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REFERENCES

1. Clemens KK, Thiessen-Philbrook H, Parikh CR, Yang RC, Karley ML, Boudville N, et al. Psychosocial Health of Living Kidney Donors: A Systematic Review. *Am J Transplant* (2006) 6:2965–77. doi:10.1111/j.1600-6143.2006.01567.x
2. Cazauvieilh V, Moal V, Prudhomme T, Pecoraro A, Piana A, Campi R, et al. Psychological Impact of Living Kidney Donation: A Systematic Review by the EAU—YAU Kidney Transplant Working Group. *Transpl Int* (2023) 36:11827. doi:10.3389/ti.2023.11827

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FORUM
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How to Treat T Cell Mediated Rejection? -A Call for Action

Klemens Budde *†

Department of Nephrology, Charité Universitätsmedizin Berlin, Berlin, Germany

Keywords: survey, ESOT, T cell mediated rejection (TCMR), European practices, treatment landscape

A Forum discussing:

European Survey on Clinical Practice of Detecting and Treating T-Cell Mediated Kidney Transplant Rejection
by Koshy P, Furian L, Nickerson P, Zaza G, Haller M, De Vries AP and Naesens M (2024). Transpl. Int. 37:12283. doi: 10.3389/ti.2024.12283

Over the last decades early rejection rates decreased, the majority of T cell mediated rejections (TCMR) respond to treatment [1, 2], and Banff borderline category is the most frequent finding in early biopsies questioning the clinical relevance of TCMR today. However, severe TCMR may cause nephron loss and inferior outcomes and is associated with development of donor-specific antibodies (DSA) and ABMR [3–5]. Recent evidence suggests that TCMR contributed to 34% of graft losses, compared to 31% due to ABMR [6]. The fact that a single rejection episode, which responds to treatment is not associated with worse graft outcome [2] supports the need for effective TCMR therapies. Despite initial treatment response, 39% of patients have persistent borderline or TCMR after anti-rejection therapy [4] and ongoing inflammation is associated with inferior outcomes and sensitization [4, 5]. In addition, anti-rejection therapy has many side effects causing significant morbidity and even mortality [7]. Thus, there remains a high unmet medical need for better and less toxic treatments for TCMR.

Given the importance of rejection since the early days of transplantation it is surprising to find only sparse high-quality evidence for anti-rejection therapy [4, 7–9]. The use of steroids and lymphocyte depleting agents for anti-rejection therapy dates back to the sixties with approval before the introduction of the Banff classification, when rejection rates were around 50%. Despite low-level evidence all transplant physicians have made personal experiences that steroids and anti-lymphocyte preparations are very effective in the treatment of TCMR, which might explain the lack of randomized trials for TCMR therapy under tacrolimus and mycophenolate. Thus, our current approach, although successful, is outdated as all previous evidence comes from an era with a different maintenance immunosuppression, different organ quality, limited ability to detect sensitization, and even without a clear differentiation between TCMR and ABMR.

In order to advance the field, the transplant community needs to re-focus on TCMR, to describe current standard of care for diagnosis and treatment and to define relevant treatment goals. The paper of the ESOT working group [9] in the current issue of the journal is an important step in this direction. This manuscript reports the results of a survey of 129 experienced European kidney transplant professionals (mainly nephrologists) on the diagnosis and treatment of TCMR and borderline lesions. For TCMR diagnosis European experts rely on traditional biomarkers and biopsies classified according to the most recent Banff classification. Protocol biopsies are performed in 57.5% of centers, although only 36% perform protocol biopsies in all patients.

Contrary to US [10], and similar to Canada [11], treatment for TCMR appears rather homogeneous across Europe [9]. TCMR and borderline changes in indication biopsies are treated with a steroid pulse and depending on the severity of rejection followed by lymphocyte

Forum

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depleting agents as second line treatment. Treatment of rejection is more heterogeneous in protocol biopsies, especially for borderline changes in whom only 62% receive high dose steroids. European experts agree to assess treatment effect early, however timing and assessment of response differed. Most respondents rely on the evolution of renal function within 1–4 weeks, although a large proportion considered a second biopsy important to assess efficacy and steroid resistance.

The excellent survey and the straightforward analysis provide crucial information on the common practices in Europe for diagnosis and treatment of TCMR. Together with surveys from US and Canada [9–11] the data are extremely helpful for clinical care, research, policy making, regulatory authorities, pharma industry, and future clinical trials. The survey highlights the need for standardized definitions, e.g., for steroid refractory rejection or treatment response. ESOT, together with other stakeholders could start an initiative for such standardized definitions for use in clinical practice, research and regulatory demands extending previous publications [1, 12]. Updated guidelines for follow-up biopsies, a more precise description of anti-TCMR therapy (e.g., drug dosing for steroid pulse or lymphocyte depleting agents, steroid tapering and maintenance immunosuppression) as well recommendations for follow-up care are needed.

The survey demonstrates that borderline changes in indication and protocol biopsies are treated as rejection in most centers worldwide challenging the Banff classification and regulatory assessment [1], who do not consider borderline as rejection. Given the frequency of borderline changes, who have limited interobserver reproducibility and may depend on pathologist's experience, one could speculate that eventually too many patients are treated. Randomized interventional trials are desperately needed for borderline lesions as well as objective tools (e.g., molecular diagnostics [13]) to identify those borderline changes, who benefit from treatment.

Interestingly, 36% of centers are performing regular protocol biopsies in Europe without clear evidence for a clinical benefit of this invasive procedure [9, 14, 15]. How to assess treatment response in patients with stable graft function? Undoubtedly, protocol biopsies are useful for clinical research, but there is a definitive need for good clinical trials to demonstrate improved outcomes after protocol biopsies.

Today, TCMR is frequently detected in "surveillance" biopsies due delayed or slow graft function in marginal kidneys. Tubulointerstitial infiltrates are found together with other pathologies such as acute tubular necrosis, capillaritis, or sclerotic lesions, making it difficult to differentiate inflammation or rejection from other causes of graft dysfunction. The classical case of an isolated TCMR several weeks after transplant with rising creatinine and quick response to treatment has become rare under current immunosuppression. Today's pathology conference is characterized by mixed pathologies in marginal kidneys with delayed/slow function making it difficult to assess an adequate treatment response without "baseline" values. These complexities may explain some of the heterogeneity in the survey and we need better ways to define treatment response in patients with mixed pathologies with or without delayed graft function. Granular data on the evolution of renal function and on the molecular and histological resolution of TCMR are desperately needed. Future research and clinical trials for TCMR should include follow-up biopsies and innovative biomarkers to improve our understanding of TCMR.

In summary, the European survey provides important information on current practice for diagnosis and treatment of TCMR, identifies current limitations and unmet medical needs and calls for action to solve these fundamental problems after kidney transplantation.

AUTHOR CONTRIBUTIONS

KB drafted the concept, reviewed the data and wrote this manuscript.

CONFLICT OF INTEREST

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REFERENCES

1. Seron D, Rabant M, Becker JU, Roufosse C, Bellini MI, Böhmig GA, et al. Proposed Definitions of T Cell-Mediated Rejection and Tubulointerstitial Inflammation as Clinical Trial Endpoints in Kidney Transplantation. *Transpl Int* (2022) 35:10135. doi:10.3389/ti.2022.10135
2. McDonald S, Russ G, Campbell S, Chadban S. Kidney Transplant Rejection in Australia and New Zealand: Relationships Between Rejection and Graft Outcome. *Am J Transpl* (2007) 7(5):1201–8. doi:10.1111/j.1600-6143.2007.01759.x
3. Wu K, Budde K, Lu H, Schmidt D, Liefeldt L, Glander P, et al. The Severity of Acute Cellular Rejection Defined by Banff Classification Is Associated With Kidney Allograft Outcomes. *Transplantation* (2014) 97(11):1146–54. doi:10.1097/01.TP.0000441094.32217.05
4. Ho J, Okoli GN, Rabbani R, Lam OLT, Reddy VK, Askin N, et al. Effectiveness of T Cell-Mediated Rejection Therapy: A Systematic Review and Meta-Analysis. *Am J Transpl* (2022) 22(3):772–85. doi:10.1111/ajt.16907
5. Rampersad C, Balshaw R, Gibson IW, Ho J, Shaw J, Karpinski M, et al. The Negative Impact of T Cell-Mediated Rejection on Renal Allograft Survival in the Modern Era. *Am J Transpl* (2022) 22(3):761–71. doi:10.1111/ajt.16883
6. Mayrdorfer M, Liefeldt L, Wu K, Rudolph B, Zhang Q, Friedersdorff F, et al. Exploring the Complexity of Death-Censored Kidney Allograft Failure. *J Am Soc Nephrol* (2021) 32(6):1513–26. doi:10.1681/ASN.2020081215
7. Alasfar S, Kodali L, Schinstock CA. Current Therapies in Kidney Transplant Rejection. *J Clin Med* (2023) 12(15):4927. doi:10.3390/jcm12154927
8. Bamoulid J, Staeck O, Crépin T, Halleck F, Saas P, Brakemeier S, et al. Anti-Thymocyte Globulins in Kidney Transplantation: Focus on Current Indications and Long-Term Immunological Side Effects. *Nephrol Dial Transpl* (2017) 32(10):1601–8. doi:10.1093/ndt/gfw368

9. Koshy P, Furian L, Nickerson P, Zaza G, Haller M, De Vries AP, et al. European Survey on Clinical Practice of Detecting and Treating T-cell Mediated Kidney Transplant Rejection *Transpl Int* (2024) 37:12283. doi:10.3389/ti.2024.12283
10. Sood P, Cherikh WS, Toll AE, Mehta RB, Hariharan S. Kidney Allograft Rejection: Diagnosis and Treatment Practices in USA- A UNOS Survey. *Clin Transpl* (2021) 35(4):e14225. doi:10.1111/ctr.14225
11. Leblanc J, Subrt P, Paré M, Hartell D, Sénécal L, Blydt-Hansen T, et al. Practice Patterns in the Treatment and Monitoring of Acute T Cell-Mediated Kidney Graft Rejection in Canada. *Can J Kidney Health Dis* (2018) 5: 2054358117753616. doi:10.1177/2054358117753616
12. Bestard O, Thaunat O, Bellini MI, Böhmig GA, Budde K, Claas F, et al. Alloimmune Risk Stratification for Kidney Transplant Rejection. *Transpl Int* (2022) 35:10138. doi:10.3389/ti.2022.10138
13. Hruba P, Madill-Thomsen K, Mackova M, Klema J, Maluskova J, Voska L, et al. Molecular Patterns of Isolated Tubulitis Differ From Tubulitis With Interstitial Inflammation in Early Indication Biopsies of Kidney Allografts. *Sci Rep* (2020) 10(1):22220. doi:10.1038/s41598-020-79332-9
14. Rush D, Arlen D, Boucher A, Busque S, Cockfield SM, Girardin C, et al. Lack of Benefit of Early Protocol Biopsies in Renal Transplant Patients Receiving TAC and MMF: A Randomized Study. *Am J Transpl* (2007) 7(11):2538–45. doi:10.1111/j.1600-6143.2007.01979.x
15. Budde K, Liefeldt L, Slowinski T, Glander P. No Evidence for a Relationship Between Infiltrates in Renal Protocol Biopsies and Outcome. *Am J Transpl* (2007) 7(11):2637–8. doi:10.1111/j.1600-6143.2007.01920.x

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ORIGINAL RESEARCH
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Impact of Switching From Immediate- or Prolonged-Release to Once-Daily Extended-Release Tacrolimus (LCPT) on Tremor in Stable Kidney Transplant Recipients: The Observational ELIT Study

Magali Giral[1†], Philippe Grimbert[2†], Baptiste Morin[3], Nicolas Bouvier[4], Matthias Buchler[5], Jacques Dantal[1], Valérie Garrigue[6], Dominique Bertrand[7], Nassim Kamar[8], Paolo Malvezzi[9], Karine Moreau[10], Yoni Athea[3] and Yannick Le Meur[11†]*

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Once-daily extended-release tacrolimus (LCPT) exhibits increased bioavailability versus immediate-release (IR-TAC) and prolonged release (PR-TAC) tacrolimus. Improvements in tremor were previously reported in a limited number of kidney transplant patients who switched to LCPT. We conducted a non-interventional, non-randomized, uncontrolled, longitudinal, prospective, multicenter study to assess the impact of switching to LCPT on tremor and quality of life (QoL) in a larger population of stable kidney transplant patients. The primary endpoint was change in The Essential Tremor Rating Assessment Scale (TETRAS) score; secondary endpoints included 12-item Short Form Survey (SF-12) scores, tacrolimus trough concentrations, neurologic symptoms, and safety assessments. Subgroup analyses were conducted to assess change in TETRAS score and tacrolimus trough concentration/dose (C_0/D) ratio by prior tacrolimus formulation and tacrolimus metabolizer status. Among 221 patients, the mean decrease of TETRAS score after switch to LCPT was statistically significant ($p < 0.0001$ vs. baseline). There was no statistically significant difference in change in TETRAS score after switch to LCPT between patients who had received IR-TAC and those who had received PR-TAC before switch, or between fast and slow metabolizers of tacrolimus. The overall increase of C_0/D ratio post-switch to LCPT was statistically significant ($p < 0.0001$) and from baseline to either M1 or M3 (both $p < 0.0001$) in the mITT population and in all subgroups. In the fast metabolizers group, the C_0/D ratio crossed over the threshold of 1.05 ng/mL/mg after the switch to LCPT. Other neurologic symptoms tended to improve, and the SF-12 mental component summary score improved significantly. No new safety concerns were evident. In this observational study, all patients had a significant improvement of tremor, QoL and C_0/D

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ratio post-switch to LCPT irrespective of the previous tacrolimus formulation administered (IR-TAC or PR-TAC) and irrespective from their metabolism status (fast or slow metabolizers).

Keywords: extended-release tacrolimus, LCPT, immunosuppression, kidney transplantation, tremor, C_0/D ratio, fast metabolizer, quality of life

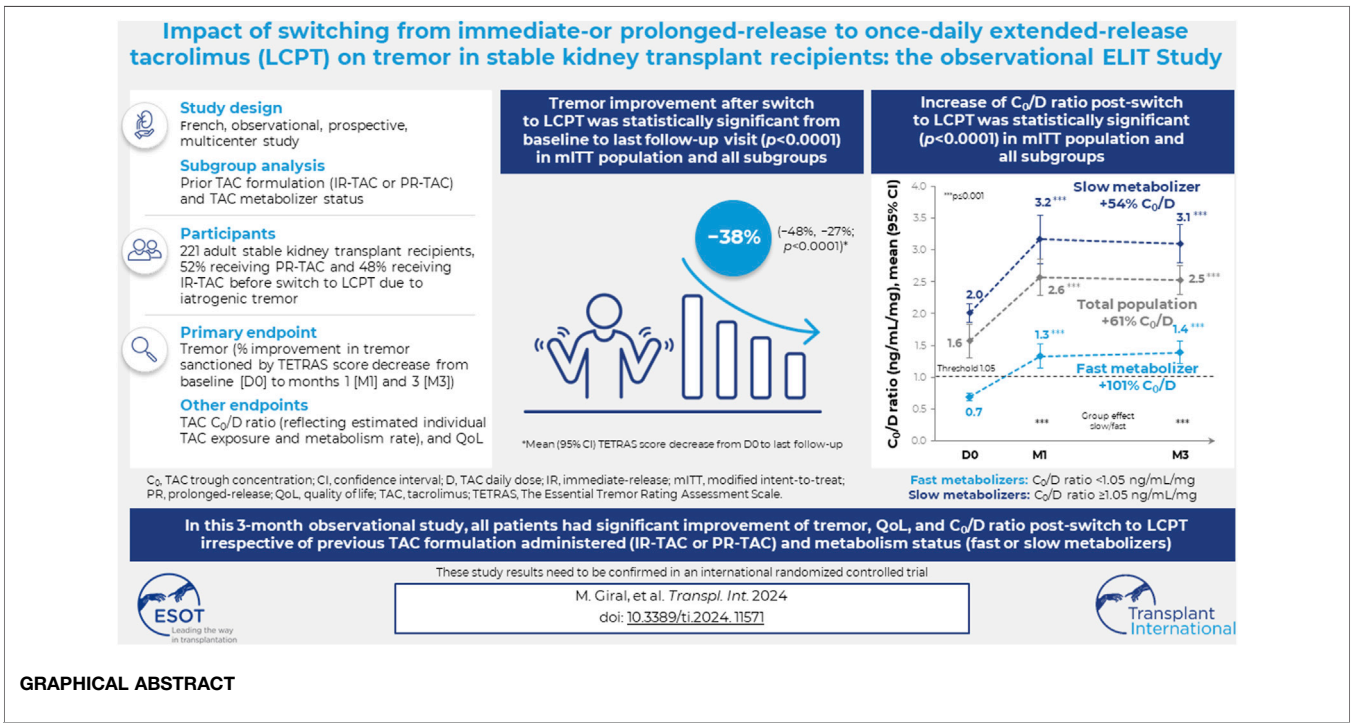
INTRODUCTION

Tacrolimus is currently the mainstay of immunosuppressive treatment in kidney transplant recipients [1, 2], and its use has contributed to improved 1-year graft survival rates, which are now approximately 95%–98% [3]. However, due to its narrow therapeutic range, strict monitoring of tacrolimus trough blood concentrations is required, as drug overexposure is often associated with increased toxicities, while underexposure may lead to graft rejection [4].

Calcineurin inhibitors (CNIs), including tacrolimus, are commonly associated with neurotoxicity [5, 6]. Because of their frequency and severity, neurologic symptoms are an important factor in morbidity and impaired quality of life (QoL) in kidney transplant recipients. One of the most frequently reported and disabling neurologic symptoms is tremor (observed in 34%–54% of tacrolimus recipients) [7, 8]. Although the pathogenesis is unknown, some observations suggest that the occurrence and severity of neurologic symptoms are correlated with tacrolimus plasma concentrations [9–11].

Tacrolimus is available as three formulations, each exhibiting a specific pharmacokinetic profile: immediate-release tacrolimus (IR-TAC), prolonged-release tacrolimus (PR-TAC), and extended-release tacrolimus (LCPT) [12].

LCPT has been developed using the MeltDose™ (Veloxis Pharmaceuticals) drug delivery technology that improves drug solubility and, thus, absorption. This feature, combined with a more distal release in the gastrointestinal tract, results in a significant increase in tacrolimus bioavailability with LCPT compared with IR-TAC and PR-TAC, an improvement in trough concentration/dose (C_0/D) ratio (trough tacrolimus blood concentration normalized by daily dose, which reflects estimated individual tacrolimus exposure and metabolism rate) [13], and may significantly reduce the maximum plasma concentration (C_{max}) [14] and/or the peak-to-trough fluctuations in blood drug concentrations [12, 14]. Hence, a 30% decrease in the daily dose required to achieve a similar systemic tacrolimus exposure and clinical efficacy has been observed with LCPT versus IR-TAC [14, 15]. In addition, LCPT has been shown to be at least as effective as IR-TAC in stable kidney transplant patients [16, 17], or as IR-TAC and PR-TAC in newly transplanted patients [18, 19], as measured by treatment failure rates at 6 and 12 months. The pre-dose concentration to daily dose (C_0/D) ratio of tacrolimus seems to be an appropriate tool for identifying patients at risk of



GRAPHICAL ABSTRACT

developing calcineurin-inhibitor toxicity such as rejection and lower renal function with increased risk of poor outcome after kidney transplantation [20–22]. A low tacrolimus concentration/dose ratio has been shown to increase the risk for the development of acute calcineurin inhibitor-induced nephrotoxicity [23].

The 7-day STRATO study of 38 stable kidney transplant recipients suggested that a switch in tacrolimus formulation from IR-TAC to LCPT resulted in a significant reduction in drug-induced tremor and a significant improvement in QoL [24]. The ELIT (*Evolution à Long terme des tremblements Iatrogènes de Tacrolimus* or Long-term Outcomes of Tacrolimus-induced Tremor) study was conducted, under real-life conditions, to further investigate whether kidney transplant patients may benefit from LCPT treatment, in terms of tremor improvement, tacrolimus dose reduction, C_0/D ratio improvement, clinical response, QoL, and safety. The primary study objective was to assess the change in tremor and the impact on daily activities after switching to LCPT.

MATERIALS AND METHODS

Study Design

The ELIT study was a non-interventional, non-randomized, uncontrolled, longitudinal, prospective, multicenter study that was conducted at 25 hospitals performing kidney transplants in France. The study was approved by the French Authority for computerized research data (Comité Consultatif sur le Traitement de l'Information en Matière de Recherche dans le domaine de la Santé, C.C.T.I.R.S.) and all subjects provided written consent for the use of their data for the purpose of this study.

Participants

Eligible patients were aged >18 years, had undergone their first kidney transplant <7.5 years prior to the study, had stable kidney function, had received tacrolimus for ≥8 weeks with the dose unchanged for ≥15 days, had tacrolimus trough blood concentrations of 4–15 ng/mL, and were presenting with tremor requiring treatment adjustment and had switched from IR-TAC or PR-TAC to LCPT, according to clinician judgement. Patients diagnosed with Parkinson's disease or any other neurologic syndrome potentially associated with tremor were excluded.

Treatment

Patients were treated at their attending clinician's discretion, and in accordance with product labelling. As such, no constraints were imposed on the dosages and administration schedules. The practical modalities of the switch to LCPT were also conducted at the discretion of the attending clinician.

Tacrolimus daily doses, trough blood concentrations, and any dosage adjustments were reported at each assessment (see below). In the event of treatment discontinuation, the date and the reason(s) for discontinuation were specified.

Outcomes and Assessments

All data were collected by each investigational site and recorded in an electronic case report form at three visits: baseline/Day 0 (D0), Month 1 (M1), and Month 3 (M3). Baseline/D0 corresponds to the day of switching from IR-TAC or PR-TAC to LCPT.

The primary endpoint was the percent improvement in The Essential Tremor Rating Assessment Scale (TETRAS) score [25] from baseline to the last follow-up visit. TETRAS scores were obtained at each study visit. This scale comprises 12 items, each scored from 0 to 4, and assesses the impact of tremors on a patient's activities of daily living. The total TETRAS score (ranging from 0 to 48) is the sum of the 12 items, with higher scores indicating more severe tremors.

The key secondary endpoint was patient health-related QoL, assessed using the 12-item Short Form Survey (SF-12) [26] at D0 and M3. The SF-12 is a 12-item questionnaire, providing two composite scores: a "physical component summary" score (including "physical functioning," "role-physical," "bodily pain" and "general health perceptions" scores) and a "mental component summary" score (including "vitality," "role-emotional," "social functioning," and "mental health" scores). All scores are standardized on a 0 to 100 scale, with 0 indicating the worst QoL.

Patient demographic and clinical characteristics were collected at baseline. At each visit, blood tacrolimus concentrations, LCPT dose, and neurologic symptoms were recorded, and standard safety assessments were conducted (e.g., adverse events [AEs] and laboratory tests, including blood cell count, biochemistry, liver function, kidney function, and lipid profile). C_0/D ratio was calculated for each patient by dividing the tacrolimus pre-dose concentration (C0) by the corresponding daily tacrolimus dose (D). Patients were categorized into two metabolizer groups based on a cut-off value of 1.05 ng/mL/mg at baseline: patients with a tacrolimus C_0/D ratio <1.05 ng/mL/mg were defined as fast metabolizers, while patients with a C_0/D ratio ≥1.05 ng/mL/mg were defined as slow metabolizers.

Patients were also categorized into two analysis subgroups: patients treated with IR-TAC as the last tacrolimus formulation prior to the switch to LCPT (the IR-TAC pretreated group) and patients treated with PR-TAC as the last tacrolimus formulation prior to the switch to LCP (the PR-TAC pretreated group).

Statistical Analyses

It was estimated that a total of 229 patients would be required to detect a change of ≥15% on the TETRAS scale, with an alpha risk of 5% and a beta risk of 10%, assuming a standard deviation (SD) of 70% for the improvement rate from baseline to the last follow-up visit. To account for 15% of observations being unusable or missing, it was estimated that data from 270 patients were required.

The efficacy analyses were performed on the modified intent-to-treat (mITT) population, which included all patients with at least one efficacy assessment. All patients who received at least one dose of LCPT were included in the safety analysis population.

Descriptive statistics were summarized as mean with SD, minimum, maximum, and median with interquartile range (IQR) for qualitative data, and number of patients with percentages for quantitative data. All statistical tests were carried out at a two-sided, 5% significance level.

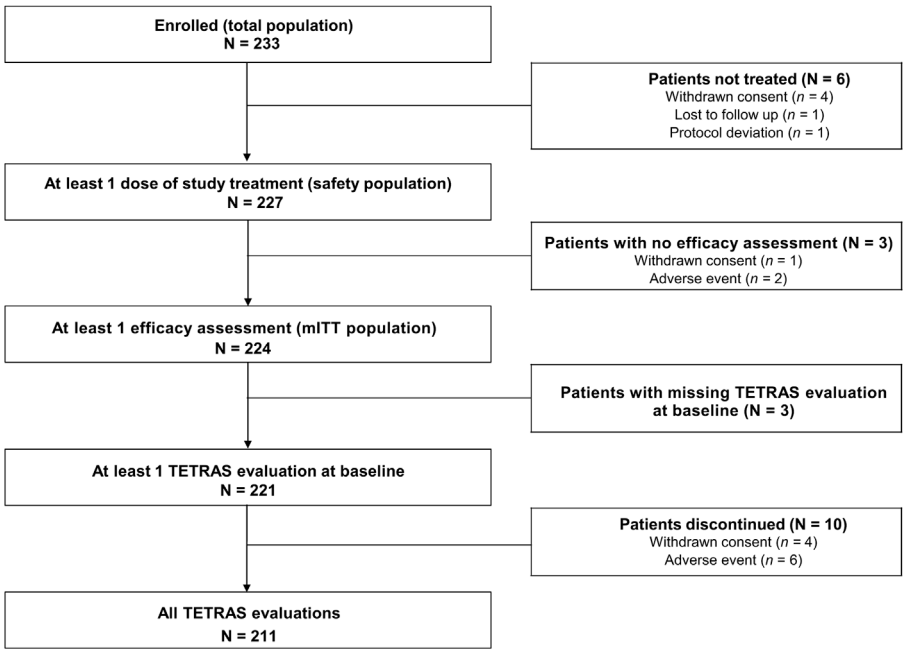


FIGURE 1 | Population flow chart. *Three patients were excluded from the primary endpoint analyses (one patient had no evaluable TETRAS data on Day 0, and two patients had no post-baseline TETRAS data); therefore, the primary endpoint analysis was performed using data from 221 patients. mITT, modified intent-to-treat; TETRAS, The Essential Tremor Rating Assessment Scale.

Total TETRAS scores were calculated if at least half of the 12 items were completed, and missing items were replaced with the average of the items completed. The mean change from baseline was presented with 95% confidence intervals (CIs) at each available visit. The primary endpoint was the change from baseline to the last follow-up visit (M3, or M1 if M3 not available). The overall change over time in TETRAS scores was evaluated using a repeated measures analysis of variance, with time as the fixed effect and patient as the random effect; TETRAS scores at M1, M3, or the last follow-up visit were compared with the baseline score using Dunnett's test. Subgroups were compared by an analysis of covariance for change in TETRAS scores at M1 and M3 versus baseline. The same analyses (mean change from baseline, overall change over time, and comparison of values at M1 or M3 vs. baseline) were performed for tacrolimus trough blood concentrations and C_0/D ratio. The mean change in C_0/D ratio from baseline to M1 and M3 was compared in subgroups using the Wilcoxon test. The association between TETRAS scores and tacrolimus trough blood concentrations was assessed using Spearman's rank correlation.

The mean change in SF-12 scores from baseline to M3 was presented for patients with evaluable data; SF-12 scores at M3 were compared with baseline using the Wilcoxon test for SF-12 individual scores and the Student t-test for SF-12 composite scores.

Laboratory parameters were summarized with descriptive statistics. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [27].

Statistical analyses were performed using SAS® software (version 9.4).

RESULTS

Participants

Over an 18-month period (15 June 2017 to 31 December 2018), 233 patients were recruited. Among these, 227 were included in the safety population, and 224 in the mITT efficacy population. Three patients had missing TETRAS evaluation at D0, and 10 patients had missing TETRAS evaluation at M1 or M3. Thus, TETRAS score analyses have been made on 221 patients at baseline and 211 patients at M1 and M3 (**Figure 1**).

In the mITT population, 57.6% of patients were male, and the median (IQR) age was 58 (46.0–67.5) years. The median (IQR) time from kidney transplantation to the switch to LCPT was 11.02 (4.75–28.77) months. The baseline demographic and disease-related characteristics of the mITT population are shown in **Table 1**.

Before switching to LCPT, 117 (52.2%) patients were receiving PR-TAC and 107 (47.8%) patients were receiving IR-TAC. Of the PR-TAC pretreated patients, 58.1% were male versus 57.0% of IR-TAC pretreated patients. The median (IQR) age was 56 (45.0–66.0) years in PR-TAC pretreated patients versus 61.0 (48.0–70.0) years in IR-TAC pretreated patients. The median (IQR) time from kidney transplantation to the switch to LCPT was 17.25 (6.10–31.74) months in PR-TAC pretreated patients versus 6.66 (4.03–15.34) months in IR-TAC pretreated patients.

Based on the C_0/D ratio cut-off value of 1.05 ng/mL/mg, 73 (33.8%) patients were characterized as fast metabolizers and 143 (66.2%) as slow metabolizers. Of the fast metabolizer patients, 56.2% were male versus 59.4% of the slow metabolizer patients. The median (IQR) age was 53 (42.0–60.0) years in fast metabolizer patients versus 61.0 (49.0–70.0) years in slow metabolizer patients. The

TABLE 1 | Patient demographics and disease-related characteristics at baseline (study population and modified intent-to-treat population).

Characteristic/demographic	Total mITT population (*N* = 224)
Male sex, *n* (%)	129 (57.6)
Median (IQR) age at enrolment, years	58.0 (46.0–67.5)
Initial cause of nephropathy, *n* (%)	
Polycystic kidney disease	44 (22.4)
Glomerulopathy	34 (17.3)
Diabetic nephropathy	17 (8.7)
Immunoglobulin A nephropathy	16 (8.2)
Hypertensive nephropathy	14 (7.1)
Vascular nephropathy	14 (7.1)
Interstitial nephropathy	12 (6.1)
Congenital nephropathy	11 (5.6)
Other	34 (17.3)
Dialysis received before transplant, *n* (%)	188 (83.9)
History of diabetes, *n* (%)	58 (25.9)
Median (IQR) time from transplant to LCPT switch, months	11.02 (4.8–28.8)
Post-transplantation treatment other than tacrolimus, *n* (%)	*n* = 222
	219 (98.6)
Antibiotics	219 (98.6)
Corticoids received post-transplant	144 (64.9)
Immunosuppressor other than tacrolimus	157 (70.7)
Induction (ATG or immunoglobulin)	99 (44.6)
Deceased donor, *n* (%)	185 (82.6)
Tacrolimus formulation at baseline (before the switch), *n* (%)	224 (100)
Prolonged-release tacrolimus (PR-TAC)	117 (52.2)
Advagraf® (PR-TAC)	117 (52.2)
Immediate-release tacrolimus (IR-TAC)	107 (47.8)
Prograf® (IR-TAC)	98 (43.8)
Adoport® (IR-TAC)	7 (3.1)
Modigraf® (IR-TAC)	2 (0.9)
Median (IQR) time since tremor onset, months	*n* = 172
	5.9 (2.3–17.9)
At least one other neurological symptom, *n* (%)	122 (54.5)
Serum creatinine, µmol/L	
Mean (SD)	139.6 (44.1)
Min; max	45.0; 321.0
eGFR, mL/min/1.73m^2	
Mean (SD)	48.6 (18.5)
Min; Max	16.8; 113.9

eGFR, estimated glomerular filtration rate; LCPT, extended-release tacrolimus; IQR, interquartile range; IR-TAC, immediate-release tacrolimus; PR-TAC, prolonged-release tacrolimus; SD, standard deviation.

median (IQR) time from kidney transplantation to the switch to LCPT was 9.18 (4.62–26.82) months in the fast metabolizers versus 11.54 (4.82–29.93) months in the slow metabolizers.

Primary Endpoint (Tremor)

The primary endpoint analysis included data from the 221 patients. The mean (95% CI) total TETRAS scores obtained at D0, M1, and M3 were 10.60 (9.61, 11.58), 6.81 (5.96, 7.67), and 5.94 (5.79, 6.79), respectively for the mITT population (**Figure 2A**). The overall decrease in TETRAS score over time for the mITT population was statistically significant ($p < 0.0001$), as were the decreases from baseline to either M1 or M3 (both $p < 0.0001$). The mean (95% CI) change in TETRAS score from baseline was −28.30% (−39.00%, −17.60%) at M1 and −38.68% (−49.77%, −27.60%) at M3. These results were confirmed by the primary endpoint analysis, with a mean (95% CI) change in TETRAS score from baseline to last follow-up visit of −37.63% (−48.32%, −26.95%; $p < 0.0001$). When categorized by change in TETRAS score, 151 patients (71.6%) at M1 and 163 (77.3%) at M3 were classified as "improved," 23 (10.9%) at M1 and 12 (5.7%) at M3 had "no change," and 37 (17.5%) at M1 and 36 (17.1%) at M3 were classified as "worsened."

Regarding the subgroup analysis by pretreatment (IR-TAC pretreated vs. PR-TAC pretreated), the mean (95% CI) total TETRAS scores obtained at D0, M1, and M3 were 10.52 (9.17, 11.87), 7.35 (6.10, 8.60), and 6.52 (5.31, 7.73), respectively, for PR-TAC pretreated patients and 10.68 (9.21, 12.14), 6.24 (5.07, 7.42), and 5.32 (4.13, 6.52), respectively, for IR-TAC pretreated patients. The overall decrease in TETRAS score after the switch was statistically significant ($p < 0.0001$) in the two groups, as were the decreases from baseline to either M1 or M3 (both $p < 0.0001$). There was no statistically significant difference between the two groups (IR-TAC pretreated patients and PR-TAC pretreated patients) in terms of change in TETRAS score from baseline to either M1 or M3 (**Figure 2A**).

Regarding the subgroup analysis by tacrolimus metabolizer status (fast metabolizers vs. slow metabolizers), the mean (95% CI) total

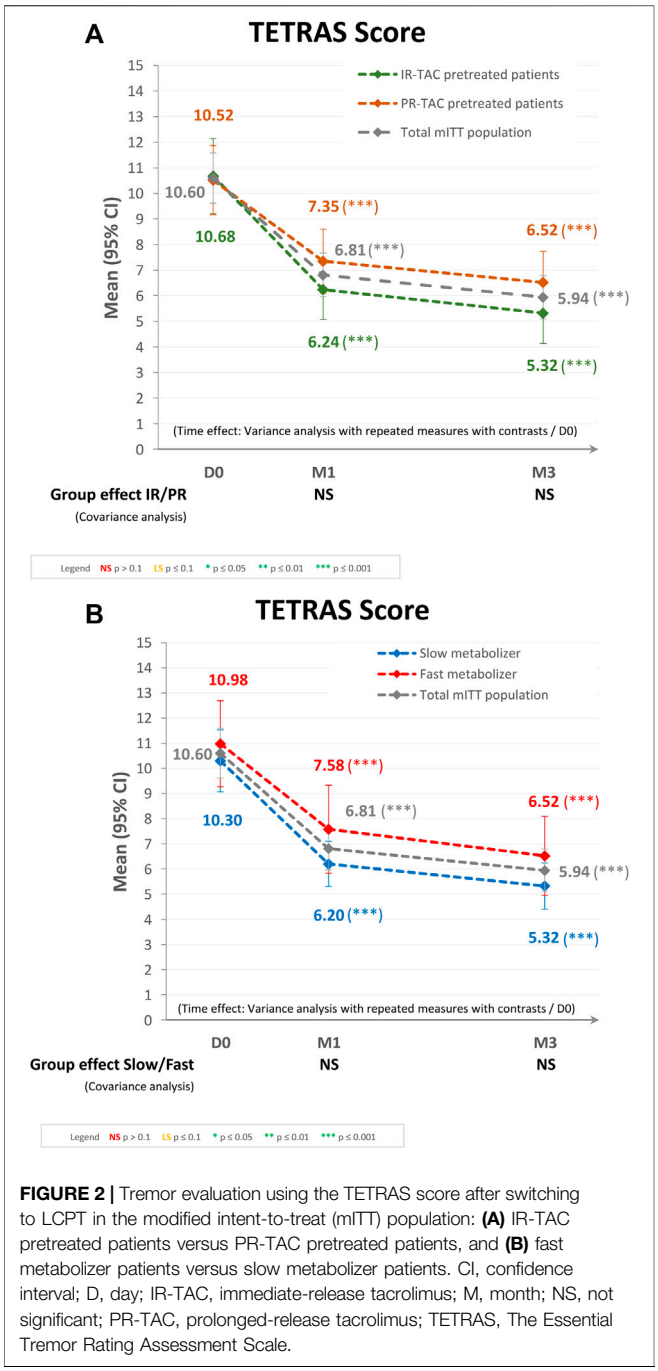


FIGURE 2 | Tremor evaluation using the TETRAS score after switching to LCPT in the modified intent-to-treat (mITT) population: **(A)** IR-TAC pretreated patients versus PR-TAC pretreated patients, and **(B)** fast metabolizer patients versus slow metabolizer patients. CI, confidence interval; D, day; IR-TAC, immediate-release tacrolimus; M, month; NS, not significant; PR-TAC, prolonged-release tacrolimus; TETRAS, The Essential Tremor Rating Assessment Scale.

TETRAS scores observed at D0, M1, and M3 were 10.98 (9.27, 12.68), 7.58 (5.83, 9.34), and 6.52 (4.95, 8.09), respectively, for the fast metabolizer group and 10.30 (9.07, 11.54), 6.20 (5.30, 7.09), and 5.32 (4.40, 6.23), respectively, for the slow metabolizer group. The overall decrease in TETRAS score after the switch was statistically significant ($p < 0.0001$) in the two groups, as were the decreases from baseline to either M1 or M3 (both $p < 0.0001$). There was no statistically significant difference between the two groups (fast metabolizers and slow metabolizers) in terms of change in TETRAS score from baseline to either M1 or M3 (**Figure 2B**).

Secondary Endpoints

Tacrolimus Dose and Trough Concentration

At baseline, the mean dose of tacrolimus (irrespective of the formulation) was 0.113 mg/kg/day. After switching to LCPT, the mean dose of tacrolimus was 0.071 mg/kg/day (4.89 mg/day); it was 0.067 mg/kg/day (4.60 mg/day) at M1 and 0.062 mg/kg/day (4.29 mg/day) at M3. While the mean tacrolimus dose decreased over time, the mean (95% CI) trough blood concentration increased from 7.04 (6.79, 7.29) ng/mL at D0 to 7.81 (7.45, 8.16) ng/mL at M1 and 7.59 (7.27, 7.92) ng/mL at M3. The mean (SD) change in trough blood concentration from baseline was +0.73 (3.09) ng/mL at M1 ($p = 0.0005$) and +0.55 (2.65) ng/mL at M3 ($p = 0.0103$). The overall increase in trough blood concentration over time was statistically significant ($p = 0.0006$).

There was no correlation between the change in tacrolimus trough blood concentration from D0 to M1 and the change in TETRAS score (Spearman's $\rho = -0.02$).

Trough Concentration/Dose (C_0/D) Ratio

Regarding the subgroups analysis by pretreatment (IR-TAC pretreated vs. PR-TAC pretreated), the mean (95% CI) C_0/D ratios observed at D0, M1, and M3 were 1.47 (1.27, 1.67), 2.59 (2.18, 3.00), and 2.66 (2.27, 3.04) ng/mL/mg, respectively, for the PR-TAC pretreated group and 1.68 (1.50, 1.86), 2.54 (2.14, 2.95), and 2.41 (2.13, 2.68) ng/mL/mg, respectively, for the IR-TAC pretreated group. The overall increase in C_0/D ratio post-switch to LCPT was statistically significant ($p < 0.0001$) in the two groups, and from baseline to either M1 or M3 (both $p < 0.0001$). However, there was no statistically significant difference in terms of C_0/D ratio between the two groups (**Figure 3A**).

Regarding the subgroups analysis by tacrolimus metabolizer status (fast metabolizers vs. slow metabolizers), the mean (95% CI) C_0/D ratios observed at D0, M1, and M3 were 0.69 (0.63, 0.74), 1.33 (1.14, 1.51), and 1.39 (1.21, 1.57) ng/mL/mg, respectively, for the fast metabolizer group and 2.01 (1.86, 2.16), 3.17 (2.79, 3.55), and 3.10 (2.80, 3.40) ng/mL/mg, respectively, for the slow metabolizer group. The overall increase in C_0/D ratio post-switch to LCPT was statistically significant ($p < 0.0001$) in the two groups, and from baseline to either M1 or M3 (both $p < 0.0001$). In the fast metabolizer group, the C_0/D ratio crossed over the threshold of 1.05 ng/mL/mg after the switch to LCPT. Furthermore, the difference between the two groups in terms of C_0/D ratio at M1 and M3 was statistically significant ($p < 0.0001$; **Figure 3B**).

Quality of Life

There was a statistically significant improvement from baseline in the individual SF-12 component scores of role-physical ($p = 0.0001$), bodily pain ($p = 0.0019$), role-emotional ($p < 0.0001$), social functioning ($p = 0.0069$), and mental health ($p = 0.0197$), as well as in the mental component summary scores ($p = 0.0002$). The improvement in the physical component summary score approached statistical significance ($p = 0.0707$; **Table 2**; **Figure 4**).

Other Neurologic Symptoms

The overall number of patients with at least one post-baseline evaluation and one other neurologic symptom decreased from

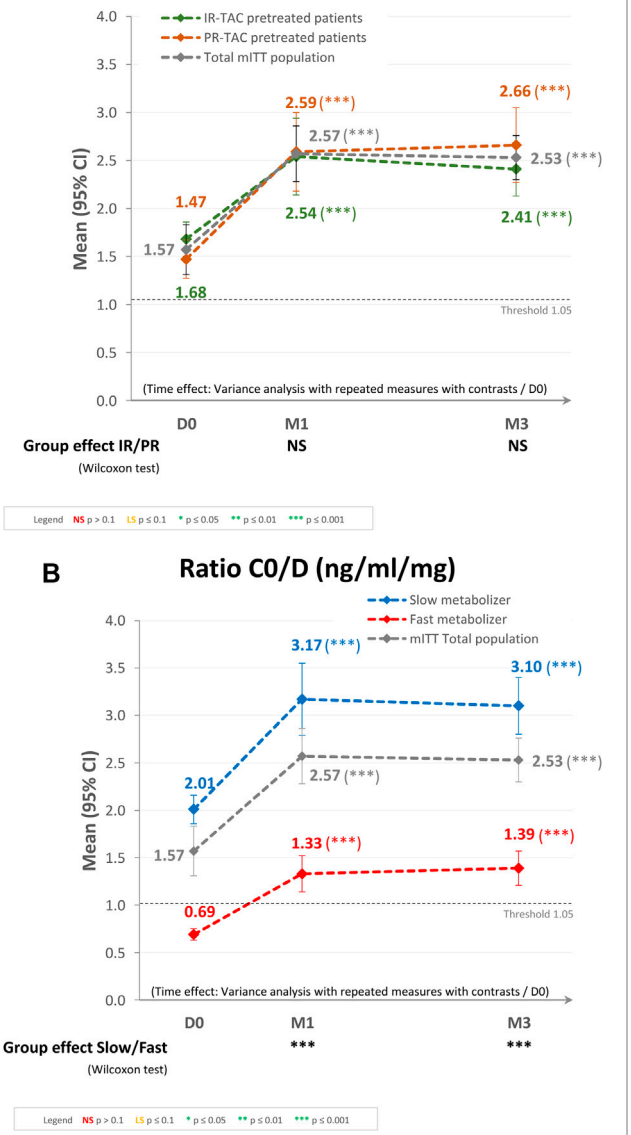


FIGURE 3 | Trough concentration/dose (C_0/D) ratio after switching to LCPT in the modified intent-to-treat (mITT) population: **(A)** IR-TAC pretreated patients versus PR-TAC pretreated patients; and **(B)** fast metabolizer patients versus slow metabolizer patients. CI, confidence interval; D, day; IR-TAC, immediate-release tacrolimus; M, month; NS, not significant; PR-TAC, prolonged-release tacrolimus.

121 (54.8%) at D0 to 103 (48.1%) at M1 and 83 (39.2%) at M3. All assessed neurologic symptoms reported at baseline had decreased in frequency by M1; subsequently, all but nightmares and photophobia decreased in frequency between M1 and M3. Although all neurologic symptoms decreased in frequency from D0 to M3, those symptoms reported in >15% of patients at D0 (i.e., headaches, insomnia, paresthesia/dysesthesia, and blurred vision) were still present in >10% of patients at M3.

Kidney Function and Other Laboratory Parameters

Kidney function was unchanged during the study: mean (SD) serum creatinine levels were 140.01 (44.71), 144.70 (49.54), and 143.30 (46.98) μmol/L at D0, M1, and M3, respectively. Mean (SD) eGFR values were 48.54 (18.59), 47.49 (18.94), and 47.54 (18.58) mL/min/1.73 m^2 at D0, M1, and M3, respectively. Other renal function parameters (creatinine clearance) were numerically similar between study time points (data not shown).

There were no notable differences in lipid profiles during the study, including total cholesterol, high-density lipoprotein cholesterol, and triglyceride levels. There were also no notable changes over time in other laboratory parameters (blood cell count, blood glucose, liver enzyme, proteinemia/proteinuria).

Adverse Events

During the 3-month follow-up, 117 patients (51.5%) presented with at least one AE and 43 (18.9%) with at least one treatment-related AE; 14 patients (6.2%) discontinued treatment due to AE(s), of whom eight discontinued due to a treatment-related AE. Serious AEs (SAEs) were reported in 39 patients (17.2%). Seven SAEs in six patients were considered to be related to LCPT (pneumocystis, hypertension, thrombotic microangiopathy, BK virus replication, basal cell carcinoma, epidermoid carcinoma, and cytomegalovirus infection). Three patients experienced a SAE considered unrelated to LCPT treatment that was fatal [pneumonia, head trauma (fall), and suicide].

Graft Rejection

Two humoral graft rejections were reported (humoral rejection and chronic active humoral rejection): one case of humoral rejection for which biopsy confirmed the rejection but it was considered not related as the patient already presented with donor-specific antibodies on the day of graft (at a mean fluorescence intensity of 1470); and one case of chronic active humoral rejection (biopsy performed BANFF 2015 category 2). There was another case of acute renal failure that was also considered as suspicion of graft rejection; a biopsy was planned following an increase in creatinine but was cancelled as the levels returned to normal.

DISCUSSION

In the ELIT study, statistically significant decreases in mean total TETRAS scores were observed in patients switching from IR-TAC or PR-TAC to once-daily LCPT (−37.63% from switch to last follow-up visit; $p < 0.0001$), irrespective of the previous tacrolimus formulation administered and metabolism status (fast vs. slow metabolizers), suggesting tremor improvement in kidney transplant patients. These results—in a larger population—are in line with those of the STRATO study [24].

No correlation between tacrolimus trough blood concentrations and TETRAS scores was shown; however, the improvements in TETRAS scores were observed despite an increase in tacrolimus trough blood concentrations, suggesting that other pharmacokinetic parameters, such as tacrolimus peak

TABLE 2 | Mean 12-item Short Form Survey (SF-12) scores over time (modified intent-to-treat population).

SF-12 component	*n*	Mean (SD) SF-12 score			*p*-value[a]
		Day 0	Month 3	Change from baseline	
Physical functioning	199	46.2 (9.9)	46.6 (10.4)	3.7 (26.8)	0.6604
Role-physical	200	49.9 (12.4)	53.1 (11.6)	3.2 (12.4)	**0.0001**
Bodily pain	199	42.8 (11.8)	45.5 (11.5)	2.7 (11.2)	**0.0019**
General health perceptions	198	41.7 (11.1)	42.2 (10.9)	0.5 (9.9)	0.6252
Physical component summary	194	44.4 (9.5)	45.5 (9.6)	1.2 (8.9)	0.0707
Vitality	200	37.7 (12.4)	38.4 (11.9)	0.7 (11.0)	0.2907
Role-emotional	198	50.0 (14.2)	53.7 (12.7)	3.7 (12.3)	**<0.0001**
Social functioning	200	44.8 (11.1)	47.2 (10.7)	2.4 (12.1)	**0.0069**
Mental health	200	47.5 (13.0)	49.2 (12.6)	1.7 (11.3)	**0.0197**
Mental component summary	194	46.1 (13.0)	48.8 (11.3)	2.7 (10.0)	**0.0002**

[a]*Significant* p-*values are shown in bold. Mean difference from baseline was evaluated statistically using the Wilcoxon test, except for the physical component summary and the mental component summary, for which the Student's t-test was used.*
SD, standard deviation.

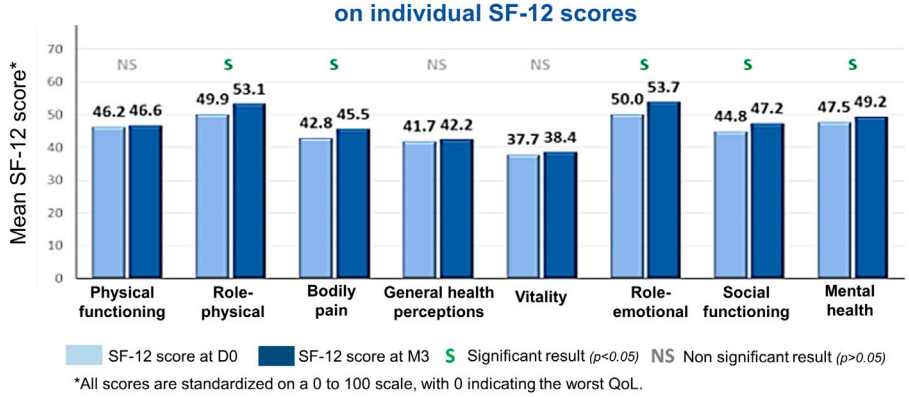


FIGURE 4 | Mean 12-item Short Form Survey (SF-12) scores from baseline to Month 3 (M3) in the modified intent-to-treat population. D0, Day 0 (baseline); QoL, quality of life.

blood concentrations (which were not evaluated in the current real-world study) or the C_0/D ratio improvement (as shown by the results of this study), may play a role in reducing the incidence of tacrolimus-induced tremor. Moreover, we had to enlarge the predefined trough concentration range from 4–8 to 4–15 ng/mL to facilitate inclusion, and we had to extend the study enrollment period (from 12 to 18 months; protocol amended). Nevertheless, 73.2% of study participants (164 of 224 patients) had a tacrolimus trough concentration between 4 and 8 ng/mL at baseline (last dosage before inclusion): four patients had a trough concentration <4 ng/mL and 55 patients had a trough concentration between 8 and 13.90 ng/mL.

Previous studies examining a possible correlation between the pharmacokinetic characteristics of tacrolimus and the development of neurotoxicity have shown inconsistent results. More severe CNI-related toxicities have been reported with a higher CNI C_{max} [24, 28]; this may explain why the TETRAS scores in this study improved following the switch to LCPT, which has a consistently lower C_{max} than all other tacrolimus formulations [14]. Although neurologic symptom reduction was not correlated with tacrolimus trough blood concentrations [24], neurologic symptom reduction has been observed after discontinuation of tacrolimus or a decrease in dose [29]. Our study suggests that LCPT is associated with a different profile of neurologic effects compared with IR-TAC or PR-TAC and highlights the need for mechanistic studies to improve understanding of the pathophysiology of neurologic adverse effects that consider differences in the pharmacokinetic characteristics (including peak and trough blood concentrations) of different tacrolimus formulations.

In the ELIT study, the initial dose of LCPT was 37.1% lower than the dose of IR-TAC or PR-TAC administered prior to the switch, and the LCPT dose was reduced at each study visit; however, the tacrolimus trough blood concentration increased significantly over time. The tacrolimus C_0/D ratio significantly improved post-switch to LCPT for all patients, irrespective of the previous tacrolimus formulation administered (IR-TAC or PR-TAC) and irrespective of the patients' metabolism status (fast or slow metabolizers of tacrolimus). We can make the hypothesis that the improvement of tremors and neurologic symptoms after switch to LCPT can be explained by the C_0/D ratio improvement. Previous studies have already shown that switching to LCPT increased tacrolimus

bioavailability, C/D ratio, and was associated with a noticeable recovery of renal function in fast metabolizers [22].

These results are consistent with previous reports using the MeltDose® technology, which demonstrated an increased bioavailability of LCPT compared with twice-daily formulations of tacrolimus [12, 14, 16] and with PR-TAC [30]. A comparative pharmacokinetic study of IR-TAC, PR-TAC, and LCPT formulations in stable renal transplant recipients demonstrated that there were significant differences between LCPT and both IR-TAC and PR-TAC, and that the formulations are not interchangeable with LCPT [12]. Based on the results of the ELIT study and exposure normalization analysis, a 36% total daily dose reduction is observed when converting from PR-TAC to LCPT and a 30% total daily dose reduction when converting from IR-TAC to LCPT. It is noteworthy that after the switch to LCPT, patients still had therapeutic drug exposure, despite the decreased dose. Further, the doses at each time point (D0, M1, and M3) were 59.7%–64.4% lower than that of the doses administered prior to the switch to LCPT. Interestingly, this is considerably less than the dosing conversion (1:0.7 on a mg:mg basis) outlined in the LCPT prescribing information [31], although it should be noted that the patients included in the current study received high tacrolimus doses at baseline and were experiencing tremors at baseline.

The switch to LCPT appeared to be associated with improvements in patient health-related QoL. We found statistically significant improvements from D0 to M3 in five of the eight individual components of the SF-12, as well as in the mental component summary. There was also an improvement, albeit not statistically significant, in the physical component summary. The observed clinical improvement in other neurologic symptoms is also likely to have been associated with this effect on QoL. Further, LCPT has been shown to improve psychomotor speed compared with cyclosporine [28], an effect that may also positively impact QoL.

No new efficacy or safety concerns were observed, including no clinically significant change in kidney function. This is consistent with evidence from liver or kidney transplant patients, which indicates that LCPT had less adverse impact on kidney function than the twice-daily tacrolimus formulation [13]. Switching to LCPT increased the bioavailability of tacrolimus and concentration-to-dose ratio, and was associated with a noticeable recovery of renal function in fast metabolizers [22]. It has been suggested that this reduced kidney toxicity may be due to a reduced peak tacrolimus concentration, in addition to improved bioavailability and reduced trough blood concentrations, after conversion to LCPT [13].

In the current study, the incidence of AEs (51.1% of patients had ≥1 AE; 18.9% had ≥1 treatment-related AE) and SAEs (17.2%) was higher than in the previous STRATO trial, in which 19.5% of patients experienced an AE, 2.4% a treatment-related AE, and no SAEs were reported [24]. This may be related to differences in study design (including duration), patient population, and sample size. STRATO was an open-label, multicenter, prospective, phase IIIb study, in which 38 stable kidney transplant patients with tremor were converted from twice-daily tacrolimus to once-daily LCPT and followed during the 7 days post-switch. In addition, the incidence of AEs in the ELIT study was lower than that reported by Budde et al. from a phase IV, randomized, open-label, parallel group study conducted in 10 European countries [19]. In that study of 200 patients over a 6-month period, 97.5% of patients had any AE, 36.5% had treatment-emergent adverse drug reactions, and 49.5% had an SAEs [19]. Further, in the LCPT international phase III study (double blind, randomized trial, 1-year follow-up; n = 268), 98.1% of study participants reported ≥1 AE and 61.9% reported ≥1 SAE [18], while in the LCPT phase III MELT study (two-armed, parallel group, prospective, randomized, open-label, multicenter, controlled, noninferiority trial; n = 162), 83.3% of patients had treatment-emergent AEs and 22.2% had a SAE [16]. The differences in the incidence of AEs in the ELIT study compared with these studies can be explained by the observational design of the ELIT study (generally less AEs reported). The incidence of AEs in the ELIT study was similar to that reported in the Spanish Better study (61.7% of patients experienced an AE and 27.1% experienced a SAE) [32], which had a similar study design (multicenter, prospective, observational; n = 133) to the ELIT study.

LCPT may offer a therapeutic alternative to other tacrolimus formulations, such as IR-TAC and PR-TAC, and allow for adequate balance between immunosuppression and adverse effects, given the large interpatient variability in tacrolimus bioavailability and absorption rates. This could be particularly relevant for patients who experience lower tacrolimus bioavailability due to intrinsic factors, such as age [33], race [34], sex [35], and/or genetic variations in cytochrome P450 3A and P-glycoprotein expression [36–38].

To our knowledge, the ELIT study is the first large, prospective, multicenter trial to investigate the impact of switching from IR-TAC or PR-TAC to LCPT on tremor in kidney transplant patients. The non-interventional design of the study is a strength, as the results reflect outcomes in standard clinical practice and therefore are generalizable to other clinical sites in France. However, the study does have a few limitations. Firstly, due to the observational nature of the study and the associated less stringent inclusion criteria, the study population was heterogeneous (e.g., the reasons for switching to LCPT and the TETRAS score at baseline were not set as inclusion criteria) and missing data may have limited the internal consistency of the results. Secondly, 65% of the population of the study was receiving corticosteroids as well as tacrolimus, which could have influenced tremor. Another limitation is that a subjective tremor assessment scale (TETRAS) was used rather than a more objective tremor assessment (such as accelerometers). However, in the study of patients in real-life conditions, using devices such as accelerometers is not practical, whereas TETRAS scores have been validated for use in this setting. The absence of a control group means that caution is required in the interpretation of the effect of LCPT treatment on tremor and health-related QoL. Furthermore, care is needed in the interpretation of the C_0/D ratio improvement and its potential link with clinical outcomes. Therefore, the study results need to be confirmed in a randomized, controlled, international trial.

In conclusion, the results of the ELIT study suggest that LCPT could be beneficial to renal transplant patients. We observed an improvement in tacrolimus-induced tremor, as assessed with the TETRAS scale. Treatment with LCPT was also associated with a reduction in the daily dose of tacrolimus, while allowing a therapeutic trough blood concentration to be maintained. There was a trend towards improvement in other neurological symptoms, as well as significant improvements in patient health-

related QoL. Further exploration of the pathophysiology of CNI-related toxicities and robust clinical investigations to fully discern the improved tolerability with LCPT are warranted.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the French Authority for computerized research data (Comité Consultatif sur le Traitement de l'Information en Matière de Recherche dans le domaine de la Santé, C.C.T.I.R.S.) and all subjects provided written consent for the use of their data for the purpose of this study. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

PG has received fees from BMS and Chiesi. YL has received speaker fees and consultant fees from AstraZeneca, Chiesi, and Hemarina. NK has received speaker fees and advisory board fees from Astellas, AstraZeneca, Biotest, CSL Behring, Chiesi, ExeViR, Hansa, Merck Sharp and Dohme, GlaxoSmithKline, Novartis Pharma, Sanofi, Sandoz, and Takeda. BM and YA work for Chiesi SAS.

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.

Chiesi SAS France provided funding for this manuscript, and contributed to the study design; collection, analysis, and interpretation of the data; and the writing of the report. The decision to submit the report for publication was made by the authors.

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REFERENCES

1. Scalea JR, Levi ST, Ally W, Brayman KL. Tacrolimus for the Prevention and Treatment of Rejection of Solid Organ Transplants. *Expert Rev Clin Immunol* (2016) 12:333–42. doi:10.1586/1744666X.2016.1123093
2. Hart A, Smith JM, Skeans MA, Gustafson SK, Wilk AR, Robinson A, et al. OPTN/SRTR 2016 Annual Data Report: Kidney. *Am J Transpl* (2018) 18(1): 18–113. doi:10.1111/ajt.14557
3. Agence de la Biomédecine. *The Medical and Scientific Report 2021: Kidney Transplant* (2021).
4. Malvezzi P, Rostaing L. The Safety of Calcineurin Inhibitors for Kidney-Transplant Patients. *Expert Opin Drug Saf* (2015) 14:1531–46. doi:10.1517/14740338.2015.1083974
5. Peters DH, Fitton A, Plosker GL, Faulds D. Tacrolimus. A Review of its Pharmacology, and Therapeutic Potential in Hepatic and Renal Transplantation. *Drugs* (1993) 46:746–94. doi:10.2165/00003495-199346040-00009
6. Winkler M, Christians U. A Risk-Benefit Assessment of Tacrolimus in Transplantation. *Drug Saf* (1995) 12:348–57. doi:10.2165/00002018-199512050-00006
7. Pirsch JD, Miller J, Deierhoi MH, Vincenti F, Filo RS. A Comparison of Tacrolimus (FK506) and Cyclosporine for Immunosuppression After Cadaveric Renal Transplantation. FK506 Kidney Transplant Study Group. *Transplantation* (1997) 63:977–83. doi:10.1097/00007890-199704150-00013
8. Bulatova N, Yousef AM, Al-Khayyat G, Qosa H. Adverse Effects of Tacrolimus in Renal Transplant Patients From Living Donors. *Curr Drug Saf* (2011) 6: 3–11. doi:10.2174/157488611794480043
9. Bechstein WO. Neurotoxicity of Calcineurin Inhibitors: Impact and Clinical Management. *Transpl Int* (2000) 13:313–26. doi:10.1007/s001470050708
10. Eidelman BH, Abu-Elmagd K, Wilson J, Fung JJ, Alessiani M, Jain A, et al. Neurologic Complications of FK 506. *Transpl Proc* (1991) 23:3175–8.
11. Abouljoud MS, Kumar MS, Brayman KL, Emre S, Bynon JS, OLN-452 Study Group. Neoral Rescue Therapy in Transplant Patients With Intolerance to Tacrolimus. *Clin Transpl* (2002) 16:168–72. doi:10.1034/j.1399-0012.2002.01054.x
12. Tremblay S, Nigro V, Weinberg J, Woodle ES, Alloway RR. A Steady-State Head-to-Head Pharmacokinetic Comparison of All FK-506 (Tacrolimus) Formulations (ASTCOFF): An Open-Label, Prospective, Randomized, Two-Arm, Three-Period Crossover Study. *Am J Transpl* (2017) 17:432–42. doi:10.1111/ajt.13935
13. von Einsiedel J, Thölking G, Wilms C, Vorona E, Bokemeyer A, Schmidt HH, et al. Conversion From Standard-Release Tacrolimus to MeltDose® Tacrolimus (LCPT) Improves Renal Function After Liver Transplantation. *J Clin Med* (2020) 9:1654. doi:10.3390/jcm9061654
14. Gaber AO, Alloway RR, Bodziak K, Kaplan B, Bunnapradist S. Conversion From Twice-Daily Tacrolimus Capsules to Once-Daily Extended-Release Tacrolimus (LCPT): A Phase 2 Trial of Stable Renal Transplant Recipients. *Transplantation* (2013) 96:191–7. doi:10.1097/TP.0b013e3182962cc1
15. Garnock-Jones KP. Tacrolimus Prolonged Release (Envarsus®): A Review of its Use in Kidney and Liver Transplant Recipients. *Drugs* (2015) 75:309–20. doi:10.1007/s40265-015-0349-2
16. Bunnapradist S, Ciechanowski K, West-Thielke P, Mulgaonkar S, Rostaing L, Vasudev B, et al. Conversion From Twice-Daily Tacrolimus to Once-Daily Extended Release Tacrolimus (LCPT): The Phase III Randomized MELT Trial. *Am J Transpl* (2013) 13:760–9. doi:10.1111/ajt.12035
17. Budde K, Bunnapradist S, Grinyo JM, Ciechanowski K, Denny JE, Silva HT, et al. Novel Once-Daily Extended-Release Tacrolimus (LCPT) Versus Twice-Daily Tacrolimus in De Novo Kidney Transplants: One-Year Results of Phase III, Double-Blind, Randomized Trial. *Am J Transpl* (2014) 14:2796–806. doi:10.1111/ajt.12955
18. Rostaing L, Bunnapradist S, Grinyo JM, Ciechanowski K, Denny JE, Silva HT, Jr., et al. Novel Once-Daily Extended-Release Tacrolimus Versus Twice-Daily Tacrolimus in De Novo Kidney Transplant Recipients: Two-Year Results of Phase 3, Double-Blind, Randomized Trial. *Am J Kidney Dis* (2016) 67:648–59. doi:10.1053/j.ajkd.2015.10.024
19. Budde K, Rostaing L, Maggiore U, Piotti G, Surace D, Geraci S, et al. Prolonged-Release Once-Daily Formulation of Tacrolimus Versus Standard-Of-Care Tacrolimus in De Novo Kidney Transplant Patients Across Europe. *Transpl Int* (2022) 35:10225. doi:10.3389/ti.2021.10225
20. van Gelder T, Meziyerh S, Swen JJ, de Vries APJ, Moes D. The Clinical Impact of the C_0/D Ratio and the CYP3A5 Genotype on Outcome in Tacrolimus Treated Kidney Transplant Recipients. *Front Pharmacol* (2020) 11:1142. doi:10.3389/fphar.2020.01142

21. Thölking G, Fortmann C, Koch R, Gerth HU, Pabst D, Pavenstädt H, et al. The Tacrolimus Metabolism Rate Influences Renal Function After Kidney Transplantation. *PLoS One* (2014) 9:e111128. doi:10.1371/journal.pone.0111128
22. Thölking G, Tosun-Koç F, Jehn U, Koch R, Pavenstädt H, Suwelack B, et al. Improved Kidney Allograft Function After Early Conversion of Fast IR-Tac Metabolizers to LCP-Tac. *J Clin Med* (2022) 11:1290. doi:10.3390/jcm11051290
23. Thölking G, Schütte-Nütgen K, Schmitz J, Rovas A, Dahmen M, Bautz J, et al. A Low Tacrolimus Concentration/Dose Ratio Increases the Risk for the Development of Acute Calcineurin Inhibitor-Induced Nephrotoxicity. *J Clin Med* (2019) 8:1586. doi:10.3390/jcm8101586
24. Langone A, Steinberg SM, Gedaly R, Chan LK, Shah T, Sethi KD, et al. Switching STudy of Kidney TRansplant PAtients with Tremor to LCP-TacrO. *Clin Transpl* (2015) 29:796–805. doi:10.1111/ctr.12581
25. Elble R, Comella C, Fahn S, Hallett M, Jankovic J, Juncos JL, et al. Reliability of a New Scale for Essential Tremor. *Mov Disord* (2012) 27:1567–9. doi:10.1002/mds.25162
26. Ware J, Jr., Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: Construction of Scales and Preliminary Tests of Reliability and Validity. *Med Care* (1996) 34:220–33. doi:10.1097/00005650-199603000-00003
27. 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) 150:604–12. doi:10.7326/0003-4819-150-9-200905050-00006
28. Heits N, Keserovic D, Mund N, Ehmke N, Bernsmeier A, Hendricks A, et al. Cognitive Evaluation in Liver Transplant Patients Under Calcineurin Inhibitor Maintenance Therapy. *Transpl Direct* (2017) 3:e146. doi:10.1097/TXD.0000000000000658
29. Wijdicks EF, Wiesner RH, Dahlke LJ, Krom RA. FK506-Induced Neurotoxicity in Liver Transplantation. *Ann Neurol* (1994) 35:498–501. doi:10.1002/ana.410350422
30. Kamar N, Cassuto E, Piotti G, Govoni M, Ciurlia G, Geraci S, et al. Pharmacokinetics of Prolonged-Release Once-Daily Formulations of Tacrolimus in De Novo Kidney Transplant Recipients: A Randomized, Parallel-Group, Open-Label, Multicenter Study. *Adv Ther* (2019) 36:462–77. doi:10.1007/s12325-018-0855-1
31. European Medicines Agency. *Envarsus (Tacrolimus): Summary of Product Characteristics* (2019). Available from: https://www.ema.europa.eu/en/documents/product-information/envarsus-epar-product-information_en.pdf.
32. Fernandez Rivera C, Calvo Rodríguez M, Poveda JL, Pascual J, Crespo M, Gomez G, et al. Bioavailability of Once-Daily Tacrolimus Formulations Used in Clinical Practice in the Management of De Novo Kidney Transplant Recipients: The BETTER Study. *Clin Transpl* (2022) 36:e14550. doi:10.1111/ctr.14550
33. Heldal K, Hartmann A, Leivestad T, Svendsen MV, Foss A, Lien B, et al. Clinical Outcomes in Elderly Kidney Transplant Recipients Are Related to Acute Rejection Episodes Rather Than Pretransplant Comorbidity. *Transplantation* (2009) 87:1045–51. doi:10.1097/TP.0b013e31819cdddd
34. Fitzsimmons WE, Bekersky I, Dressler D, Raye K, Hodosh E, Mekki Q. Demographic Considerations in Tacrolimus Pharmacokinetics. *Transpl Proc* (1998) 30:1359–64. doi:10.1016/s0041-1345(98)00275-9
35. Bloom RD, Trofe-Clark J, Wiland A, Alloway RR. A Randomized, Crossover Pharmacokinetic Study Comparing Generic Tacrolimus vs the Reference Formulation in Subpopulations of Kidney Transplant Patients. *Clin Transpl* (2013) 27:E685–93. doi:10.1111/ctr.12256
36. Zhang Y, Benet LZ. The Gut as a Barrier to Drug Absorption: Combined Role of Cytochrome P450 3A and P-Glycoprotein. *Clin Pharmacokinet* (2001) 40:159–68. doi:10.2165/00003088-200140030-00002
37. Macphee IA, Fredericks S, Tai T, Syrris P, Carter ND, Johnston A, et al. Tacrolimus Pharmacogenetics: Polymorphisms Associated With Expression of Cytochrome P4503A5 and P-Glycoprotein Correlate With Dose Requirement. *Transplantation* (2002) 74:1486–9. doi:10.1097/00007890-200212150-00002
38. Thangavel C, Boopathi E, Shapiro BH. Inherent Sex-Dependent Regulation of Human Hepatic CYP3A5. *Br J Pharmacol* (2013) 168:988–1000. doi:10.1111/j.1476-5381.2012.02222.x

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CONSENSUS REPORT
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European Consensus on the Management of Sensitized Kidney Transplant Recipients: A Delphi Study

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An increasing number of sensitized patients awaiting transplantation face limited options, leading to fatalities during dialysis and higher costs. The absence of established evidence highlights the need for collaborative consensus. Donor-specific antibodies (DSA)-triggered antibody-mediated rejection (AMR) significantly contributes to kidney graft failure, especially in sensitized patients. The European Society for Organ Transplantation (ESOT) launched the ENGAGE initiative, categorizing sensitized candidates by AMR risk to improve patient care. A systematic review assessed induction and maintenance regimens as well as antibody removal strategies, with statements subjected to the Delphi methodology. A Likert-scale survey was distributed to 53 European experts (Nephrologists, Transplant surgeons and Immunologists) with experience in kidney transplant recipient care. A rate ≥75% with the same answer was considered consensus. Consensus was achieved in 95.3% of statements. While most recommendations aligned, two statements related to complement inhibitors for AMR prophylaxis lacked consensus. The ENGAGE consensus presents contemporary recommendations for desensitization and immunomodulation strategies, grounded in predefined risk categories. The adoption of tailored, patient-specific measures is anticipated to streamline the care of sensitized recipients undergoing renal allografts. While this approach holds the promise of enhancing transplant accessibility and fostering long-term success in transplantation outcomes, its efficacy will need to be assessed through dedicated studies.

Keywords: kidney transplantation, desensitization, immunomodulation, systematic review, Delphi

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INTRODUCTION

The incidence of chronic kidney disease is rocketing worldwide and it is widely acknowledged that kidney transplantation represents the best therapeutic option for patients reaching end-stage kidney failure [1]. However, there is a rapid increasing number of highly sensitized patients waitlisted worldwide, who have limited access to transplantation. 2024 OPTN data from US [2] show that 11% of waiting list kidney transplant candidates can be defined as highly sensitized (HS), displaying a cPRA>80% (5% of listed patients display >98% cPRA), and 45% of candidates have some degree of sensitization with a cPRA>1%. The Eurotransplant data report that 35% of candidates display a virtual PRA>0% in 2023 [3] and the percentage of >85% PRA listed patients increased from 3.4% in 2014 to 6% in 2019, whereas the percentage of sensitized patients at any degree (PRA between 6% and 84%) remained stable (14%). Country-specific reports show a percentage of HS candidates varying from 20% to 30% depending on the assay utilized (20% with cPRA>98% in Spain, 25% with cPRA >85% in France, 28% with cRF >85% in United Kingdom). In Australia the proportion appears similar, with 30% patients having cPRA >80% [4–9]. In the absence of consensual evidence-based data regarding the way these high immunological risk patients should be managed, a large proportion of them remain on chronic dialysis, which detrimentally impact both on their quantity and quality of life and represents a higher financial burden for the society [10–13].

In 2021, the European Society for Organ Transplantation (ESOT) initiated the EuropeaN Guidelines for the mAnagement of Graft rEcipients (ENGAGE) program. That same year the ENGAGE working group proposed a stratification of the humoral risk for candidate to a solid organ transplantation [1]. Based on patient's "immunological" history and the results of single-antigen bead assay, cytotoxic (CDC) and flow cytometry crossmatches, sensitized candidates can be distributed into five categories (**Figure 1**) with decreasing risk for AMR from Category 1 (Patients with day 0 DSA with positive CDC crossmatch) to 5 (patients with no DSA and no cellular memory).

Following the publication of this stratification, the ENGAGE II working group was established to discuss how patient management should be adapted in the five ENGAGE categories. The ENGAGE II working group includes members from across Europe, selected among ESOT/EKITA recognised experts in the field of transplant immunology, kidney transplantation, and the management of high-risk kidney transplant candidates or recipients. The approach was based on two consecutive steps. In the first step, a systematic review of the literature was conducted leading to the generation of a list of evidence-based proposals on induction therapies, antibody removal strategies and new biological drugs and maintenance immunosuppression. Consensus about these proposals was then established in each ENGAGE category using the Delphi methodology.

METHODS

The Steering Committee of ENGAGE II working group included members of the ENGAGE I and the TLJ WS06, all the previous experts accepted either as panellists or scientific committee. The requisites to be involved were to be representative of different European Countries with experience in desensitization based on scientific publications or participation to multicentre clinical

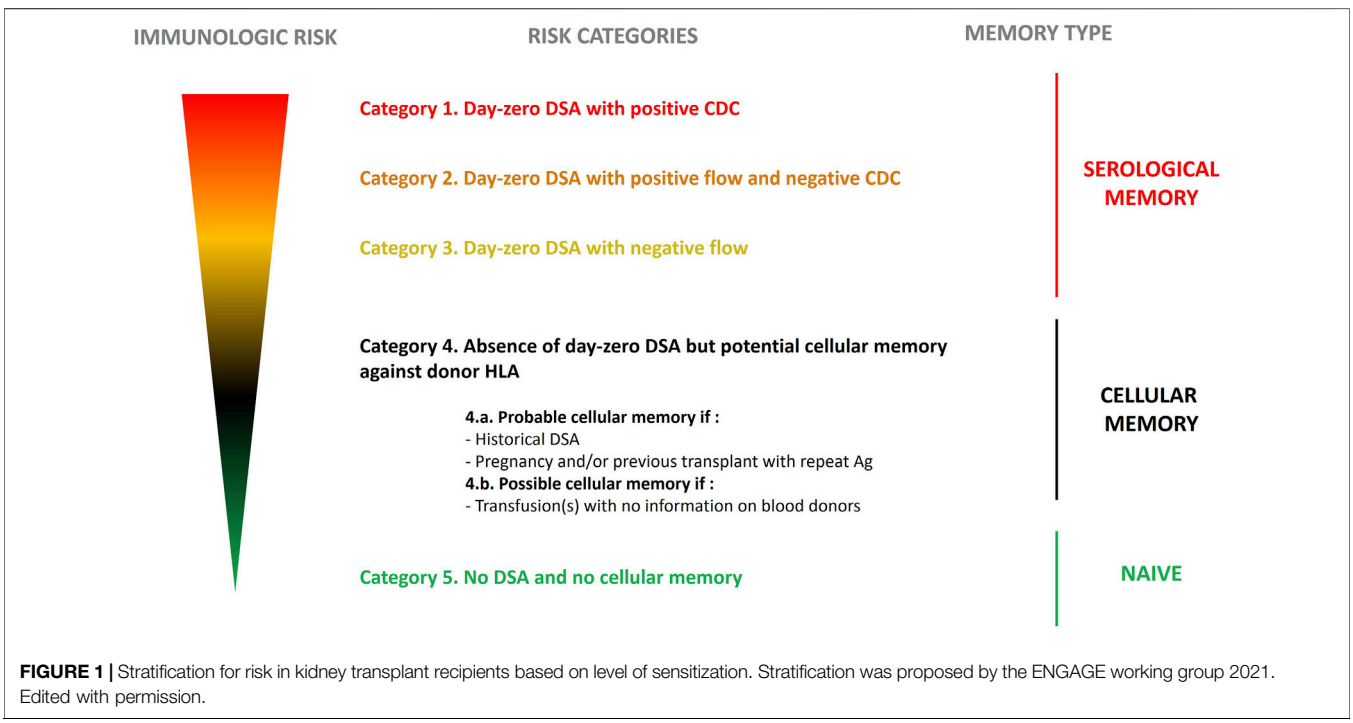

FIGURE 1 | Stratification for risk in kidney transplant recipients based on level of sensitization. Stratification was proposed by the ENGAGE working group 2021. Edited with permission.

studies on HS patients. None of the contacted centres or experts declined to participate, witnessing the high interest of this topic in selected transplant centres. The Scientific Committee for the evidence-based evaluation and consensus generation consisted of ten members, Lucrezia Furian (Italy; co-Chair), Olivier Thaunat (France; co-Chair), Nizam Mamode (United Kingdom), Oriol Bestard (Spain), Maarten Naesens (Belgium), Klemens Budde (Germany), Fabio Vistoli (Italy), Emanuele Cozzi (Italy), Soren Schwartz Sorensen (Denmark) and Fritz Diekmann (Spain), all academic kidney transplant experts.

Systematic Literature Review

A systematic search of the published literature was conducted to identify studies reporting on induction regimens in sensitised kidney transplant recipients and studies reporting antibody removal strategies and new biological agents in low to very high-risk kidney transplant recipients (**Supplementary Figure S1**).

Two clinical questions were formulated according to the PICO (Population, Intervention, Comparison, Outcome) structure to define the search strategy as well as the inclusion and exclusion criteria for selection of publications. Scientific committee met several times online to define the scope, the PICOs, and discuss the results.

The first clinical question was "*What is the efficacy of different induction agents or protocols on transplant outcomes in low to very high-risk kidney transplant recipients*?". The population (P) was defined as low to very high-risk kidney transplant recipients (all ages), intervention (I) as induction agents or protocols, no comparators (C) were considered and the outcome (O) was defined as 1-year patient and graft survival, acute rejection rates type of rejection according to the Banff Classification, 5- and 10-year graft and patient survival, and development of DSAs. Systematic reviews, randomised controlled trials, registry analyses, case series were considered relevant. Publications were excluded if they were published before 2000 or in any language other than English.

The second clinical question was "*What are the antibody removal strategies and maintenance immunosuppression available to facilitate the access to kidney transplantation and to obtain acceptable outcomes in sensitized recipients*?". For this question the P was defined as adult sensitized patients, the I as antibody removal strategies and maintenance immunosuppression, no C was considered and the O was defined as AMR, infections, graft function, graft survival, patient survival. Systematic reviews randomised controlled trials, registry analyses, case series were considered relevant. Publications were excluded if they were published before 1995 or in any language other than English. The decision to exclude publications prior to 2000 and 1995, respectively, was taken because more recent publications generally included advancements in antibody detection technologies, diagnostic criteria for rejection and immunosuppressive treatment changes, among others. However, older papers that included high-quality research could be included as supporting evidence, with expert group agreement.

Literature Search Strategy, Study Selection and Data Collection

Literature searches for both clinical questions were developed by the Centre for Evidence in Transplantation, University of Oxford, United Kingdom. The search strategy including the list of search queries used per each bibliographic source is provided in **Supplementary Table S1**. The literature searches were conducted in the Transplant Library (www.transplantlibrary.com), Medline® and Embase® databases, and included free text and controlled vocabulary terms. The titles and abstracts were screened by one reviewer and a list of potentially eligible reports was identified. The review of the literature was refined by a subgroup for each PICO, consisting of two members of the scientific committee who independently evaluated the evidences in the literature. **Figure 2** describes both PRISMA flow diagram for the study selection process.

Consensus Statement Development

Based on the evidence generated through the systematic literature search, the clinical members of the induction therapies, antibody removal strategies and maintenance immunosuppression subgroups drafted statements on induction, desensitization and immunomodulation. Statements were developed for ENGAGE risk categories 1–4b. No specific statements were developed for risk category 5 as these transplant candidates, who present with no DSA and no cellular memory that indicates heightened risk of rejection, were not the focus of the current work. Draft statements for the Delphi process were discussed, revised and approved by the full working group. Statements were then presented to a larger number of experts who qualified as voting members of the Delphi panel (**Supplementary Figure S1**).

Delphi Methodology

We employed the Delphi methodology to achieve a global view of current desensitization and immunomodulation strategies during kidney transplantation from a clinical immunology perspective. The process was undertaken between May and September 2022. The Delphi Review Group members were selected by the Scientific Committee based on their specialty (nephrologists, transplant surgeons, immunologists) and their experience in the care of kidney transplantation recipients (minimum of 5 years). An online questionnaire was sent in two waves to nephrologists, transplant surgeons and immunologists of the selected countries. For the first wave, panel members were invited to vote individually on whether they agree, partially agree or disagree with each statement. In case of disagreement or partial disagreement, panel members were asked to briefly explain the reason for their disagreement/partial disagreement with the statement and were invited to re-write the statement as they considered more appropriate. Data were analysed globally. The level of agreement or disagreement was defined by the Scientific Committee when 75% of more of the experts agree on the assessment. Following completion of the first wave, those statements for which consensus had not been achieved were re-written and clarified with some definitions by the Scientific Committee according to the insights provided by the panel

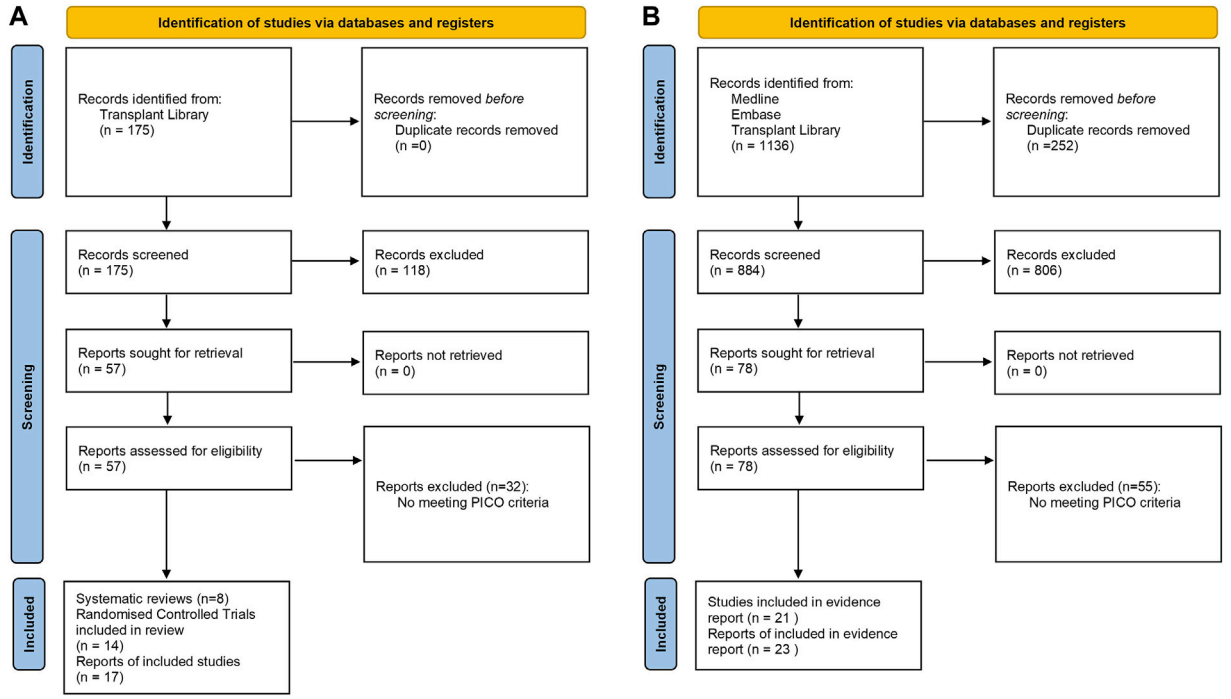

FIGURE 2 | PRISMA flow diagrams for **(A)** efficacy of induction agent and **(B)** antibody removal strategy.

members for disagreed/partially disagreed. The second wave consisted of the rewritten statements that had not achieved consensus during the first wave.

RESULTS

For the systematic literature search regarding the first clinical question, a total of 175 publications were identified from Transplant Library. For the second clinical question, 1,136 publications were identified from Medline®, Embase® and Transplant Library databases. A total of 43 statements were developed by the Scientific Committee based on the systematic literature review (**Supplementary Table S2**).

Delphi Review Group

Considering the highly specific topic addressed by the questionnaire, different strategies of recruitment were simultaneously taken (**Supplementary Table S3**). The Delphi Review Group consisted of 53 experts from across Europe (**Supplementary Table S4**).

Category 1 Patients (DSA Present With Positive CDC Crossmatch at Day 0)

All statements for this group reached consensus except for Statement 7 referring to the use of complement inhibitors as an adjunction to a desensitization strategy (**Figure 3**). In all, 98% of the Delphi Review Group agreed that kidney transplantation should be avoided unless no other options is available. Most members agreed that if kidney transplantation is considered, a CDC negative crossmatch must be obtained through desensitization before transplantation, and strategies to prevent and treat antibody rebound must be carefully planned (agreement rate 96%). Useful tools beyond careful clinical surveillance are monitoring with DSA screening and surveillance biopsy (agreement rate 96%). There was good agreement that plasma exchange (PEX) and intravenous immunoglobulin (IVIg) should be part of the first line desensitization strategy to provide a negative CDC crossmatch prior to transplantation (agreement rate 75%). Also, imlifidase might be considered as a desensitization strategy for deceased kidney transplantation in very selected patients for whom there are no other treatment options (agreement rate 92%). Regarding induction therapies, experts agreed that T-lymphocyte-depleting agents should be used in these patients rather than IL-2RA (agreement rate 94%). T-cell depleting therapy such as alemtuzumab or antithymocyte globulins (ATG) can be used (agreement rate 94%). The B-cell depleting agent rituximab might be considered as an adjunct to prevent antibody rebound (agreement rate 89%). It was agreed by 91% of the Delphi Review Group that patients in Category 1 should receive maintenance immunosuppression with tacrolimus, mycophenolate and steroids. Also, mTOR inhibitors can be considered in combination with tacrolimus instead of mycophenolate, especially when it cannot be tolerated or when infectious complications due to mycophenolate occur (agreement rate 81%). A total of 92% of the Delphi Review Group agreed that a planned minimization or withdrawal of immunosuppression should be avoided in these patients.

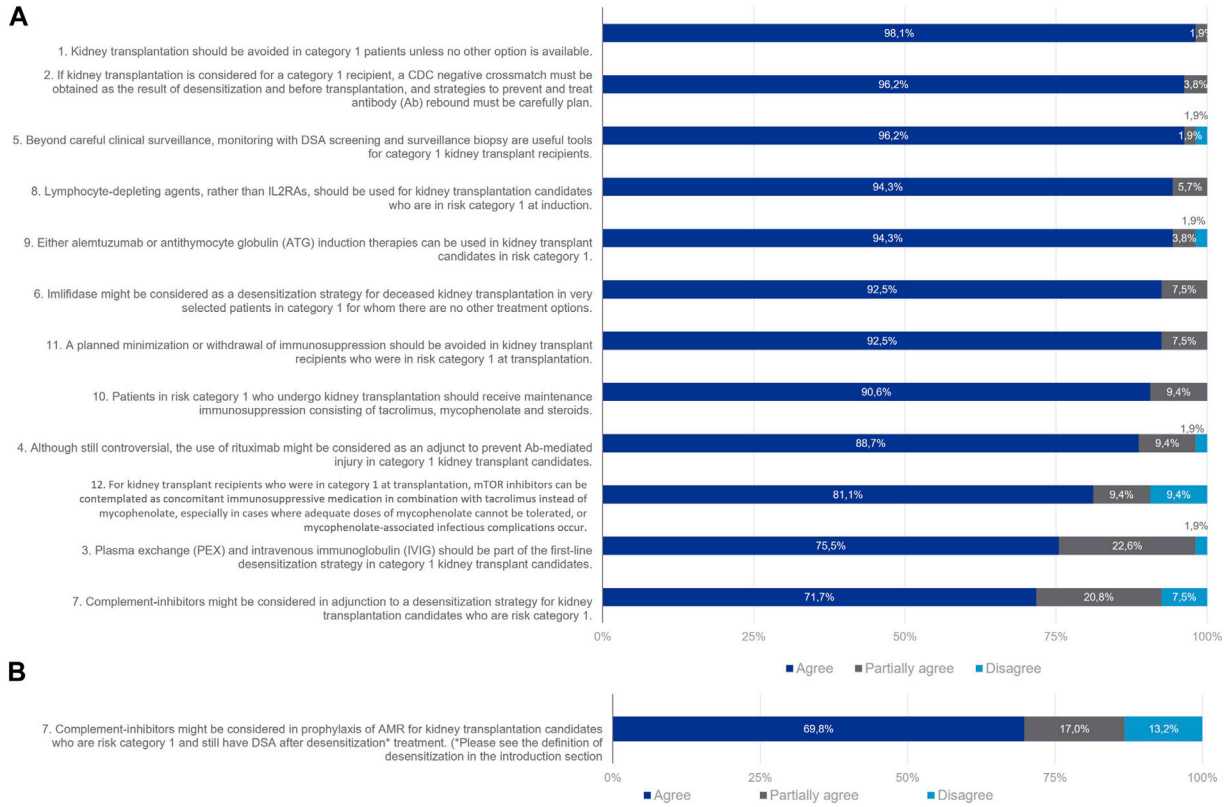

FIGURE 3 | Panellist responses for Category 1 (patients with DSA present and positive CDC crossmatch at D0 and Day 0) after **(A)** Wave 1 and **(B)** Wave 2.

Consensus remained elusive regarding the utilization of complement inhibitors as a prophylactic measure against antibody-mediated rejection (AMR) for patients in this particular cohort who retained donor-specific antibodies (DSA) post-desensitization treatment. In the initial assessment, 72% of the Delphi Review Group supported the notion that complement inhibitors could be considered as an adjunct to desensitization strategies, while 21% expressed partial agreement and 8% dissented. Given the absence of consensus after the first round, the statement underwent refinement for the second round, incorporating a specific definition of desensitization (herein strictly referring to drugs or procedures designed to diminish the titre of anti-donor antibodies, either directly or by targeting antibody-producing cells or their precursors). Nevertheless, consensus remained unattainable in the second wave, with 70% of the Delphi Review Group endorsing the revised statement, 17% offering partial agreement, and 13% dissenting from the statement.

Category 2 Patients (DSA Present With Positive Flow and Negative CDC Crossmatch at Day 0)

For risk Category 2 patients, all statements reached consensus except statement 7 referring to the use of complement inhibitors in prophylaxis of AMR (**Figure 4**). 83% of the Delphi Review Group agreed that, preferably, kidney transplantation should be avoided, but if there are no other options, it could be considered on a case-by-case basis. In that case, strategies to prevent and treat antibody rebound must be cautiously planned (agreement rate 87%). Useful tools beyond careful clinical surveillance are monitoring with DSA screening and surveillance biopsy (agreement rate 96%). As agreed by 77% of the Delphi Review Group, PEX and IVIg should be part of the first line desensitization strategy, to provide a negative crossmatch prior to transplantation. Also, imlifidase might be considered for deceased kidney transplantation in selected patients for whom there are no other treatment options (agreement rate 91%). The Group agreed that T-lymphocyte-depleting agents should be used as induction therapy in these patients, rather than interleukin 2 receptor antagonists (IL-2RAs; agreement rate 93%). Alemtuzumab or antithymocyte globulins (ATG) can be used (agreement rate 91%). The B-cell depleting agent rituximab might be considered as an adjunct to antibody-mediated injury (agreement rate 91%). Immunosuppression should be maintained for this group, as agreed by 93% of the Delphi Review Group with tacrolimus, mycophenolate and steroids. Also, mTOR inhibitors can be contemplated in combination with tacrolimus instead of mycophenolate, especially when the latter cannot be tolerated or when infectious complications due to mycophenolate occur

FIGURE 4 | Panellist responses for Category 2 (patients with DSA, negative CDC crossmatch and positive now cytometry crossmatch on Day 0) after **(A)** Wave 1 and **(B)** Wave 2.

(agreement rate 83%). Moreover, planned minimizations or withdrawal of immunosuppression should be avoided in these patients (agreement rate 91%).

As for patients of Category 1, consensus proved elusive on the statement concerning the use of complement inhibitors as an adjunct to desensitization strategies for the prophylaxis of AMR in patients retaining donor-specific antibodies (DSA) post-desensitization treatment. In the initial wave, 68% of the Delphi Review Group concurred that complement inhibitors could be considered in tandem with desensitization strategies, 21% partially agreed, and 11% disagreed. In the second wave, the statement was refined to specifically address AMR prophylaxis in patients with persisting DSA. Despite this focus, consensus further diminished for the rephrased statement, with 60% in agreement, 19% partially in agreement, and 21% in disagreement. Overall, while the Delphi results may not endorse the use of complement inhibitors as AMR prophylaxis, there remains interest in exploring this therapeutic class for treating confirmed episodes of AMR.

Category 3 Patients (DSA Present and Negative Flow and CDC Cross Match at Day 0)

For Category 3 patients, consensus was reached for all proposed statements at Wave one (**Figure 5**). 83% of the Delphi Review Group agreed that other options for transplantation (such as compatible living donor transplant or kidney paired donation) should be objectively considered for kidney transplant candidates in Category 3, since these patients are at higher immunological risk than those in categories 4 and 5. Moreover, 96% of the Group agreed that these patients require a thorough risk/benefit analysis, and strategies to prevent and treat antibody rebound need to be carefully planned. Useful tools beyond clinical surveillance are monitoring with DSA screening and surveillance biopsy (agreement rate 94%). For desensitization, PEX and IVIg might be considered (agreement rate 77%). Additionally, rituximab might be considered as an adjunct to prevent antibody-mediated injury (agreement rate 79%). For induction therapy, T-lymphocyte-depleting agents should be used, rather than IL-2RAs (agreement rate 85%). Alemtuzumab or ATG can be used (agreement rate 89%). According to 93% of the Delphi Review Group, immunosuppression should be maintained, with tacrolimus, mycophenolate and steroids. Also, mTOR inhibitors can be contemplated in combination with tacrolimus instead of mycophenolate, especially when the latter cannot be tolerated or when infectious complications due to mycophenolate occur (agreement rate 89%). Further, planned minimizations or withdrawal of immunosuppression should be avoided in these patients (agreement rate 81%).

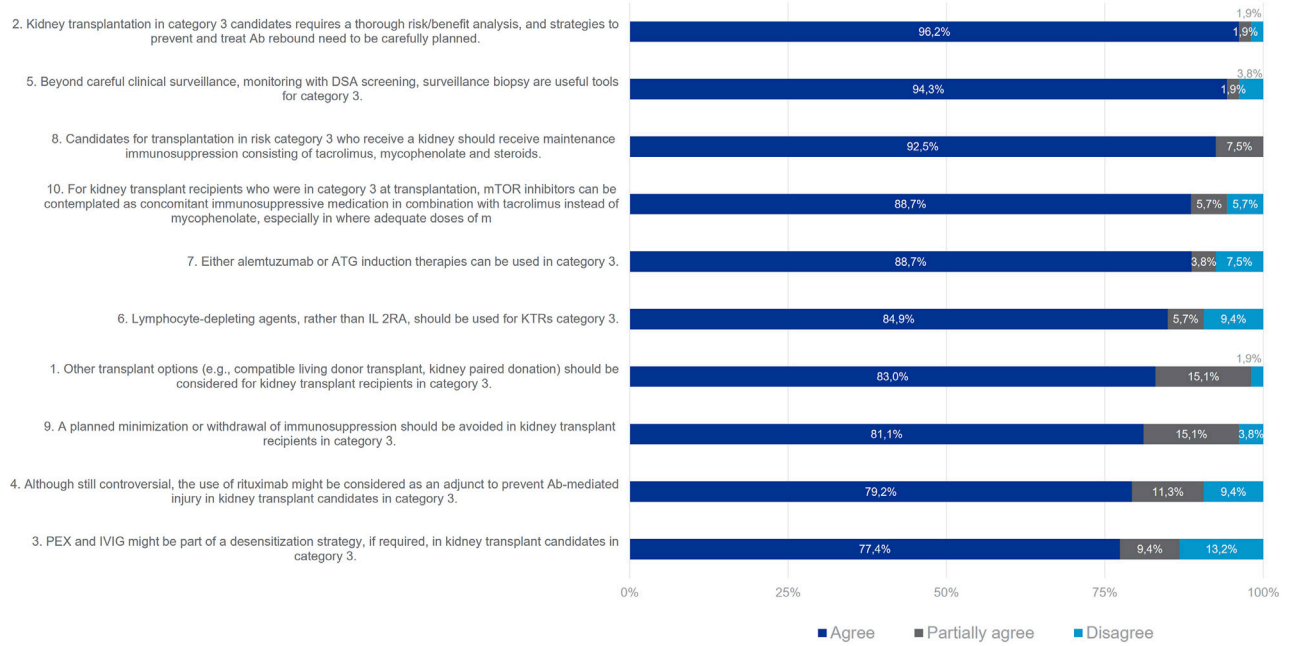

FIGURE 5 | Panellist responses for Category 3 (patients with DSA and negative flow cytometry crossmatch on Day 0).

Category 4 Patients (Without DSA on Day 0 But With Potential Cellular Memory Against Donor HLA)

In ENGAGE stratification [1], category 4 was further divided into category 4a, with "probable" cellular memory, in case of positive history of DSA, pregnancy and/or previous transplant with repeated antigens, and category 4b with "possible" cellular memory if they have a history of transfusions and/or pregnancies with no information on the HLA type patient was exposed to.

Most members of the Delphi Review Group (89%) agreed that for Category 4a patients post-transplant monitoring and strategies to control antibody-mediated injury need to be considered (**Figure 6**). Useful tools beyond careful clinical surveillance are monitoring with DSA screening and surveillance biopsy (agreement rate 87%). Lymphocyte-depleting agents should be considered for patients in Category 4a, rather than IL-2Ras alone (agreement rate 76%). Alemtuzumab or ATG (i.e., T-cell depleting agents) can be used as induction therapies for this group (agreement rate 81%) since as naïve alloantibody response, recall responses also require T cell help [14]. In all, 87% of the Delphi Review Group agreed that patients in Category 4a should receive maintenance immunosuppression with tacrolimus, mycophenolate and steroids. Also, mTOR inhibitors can be contemplated in combination with tacrolimus instead of mycophenolate, especially when the latter cannot be tolerated or when infectious complications due to mycophenolate occur (agreement rate 94%). A total of 81% of the Group agreed that a planned strategy of minimization of maintenance immunosuppression should be avoided in these patients in Category 4a. Initially, no consensus was reached during Wave one, as 66% of the Group agreed, while 25% partially agreed and 9% disagreed. For Wave two, a clear definition of minimization (a planned strategy of reduction of maintenance immunosuppression consisting in reducing calcineurin inhibitor (CNI) trough levels and/or antimetabolites doses below the standard values and/or withdrawing corticosteroids) was included and consensus was achieved. However, withdrawal of steroids or lower than usual doses of tacrolimus/MMF in these patients was also considered appropriate by the Delphi Review Group, depending on time after transplantation, occurrence of acute rejection and side effects of immunosuppression.

Given the current lack of routinely accessible tests to evaluate the humoral cellular memory of kidney transplant candidates, the Delphi Review Group reached a consensus that patients in Category 4b do not necessitate additional treatment beyond the standard of care, with an agreement rate of 81%. While this finding might suggest the potential exclusion of Category 4b from the classification, the ENGAGE working group recommends retaining this category. Doing so emphasizes the critical unmet medical need and encourages research on alloreactive memory B cells. The simplification of the classification awaits robust evidence on the role of these subsets and the development of reliable assays to screen for their presence.

Category 5 Patients (With No DSA and No Cellular Memory)

No specific statements were developed for this category as these transplant candidates (who present with no DSA, and no cellular memory indicating heightened risk of rejection) were not the focus of the review. It was agreed that these patients do not

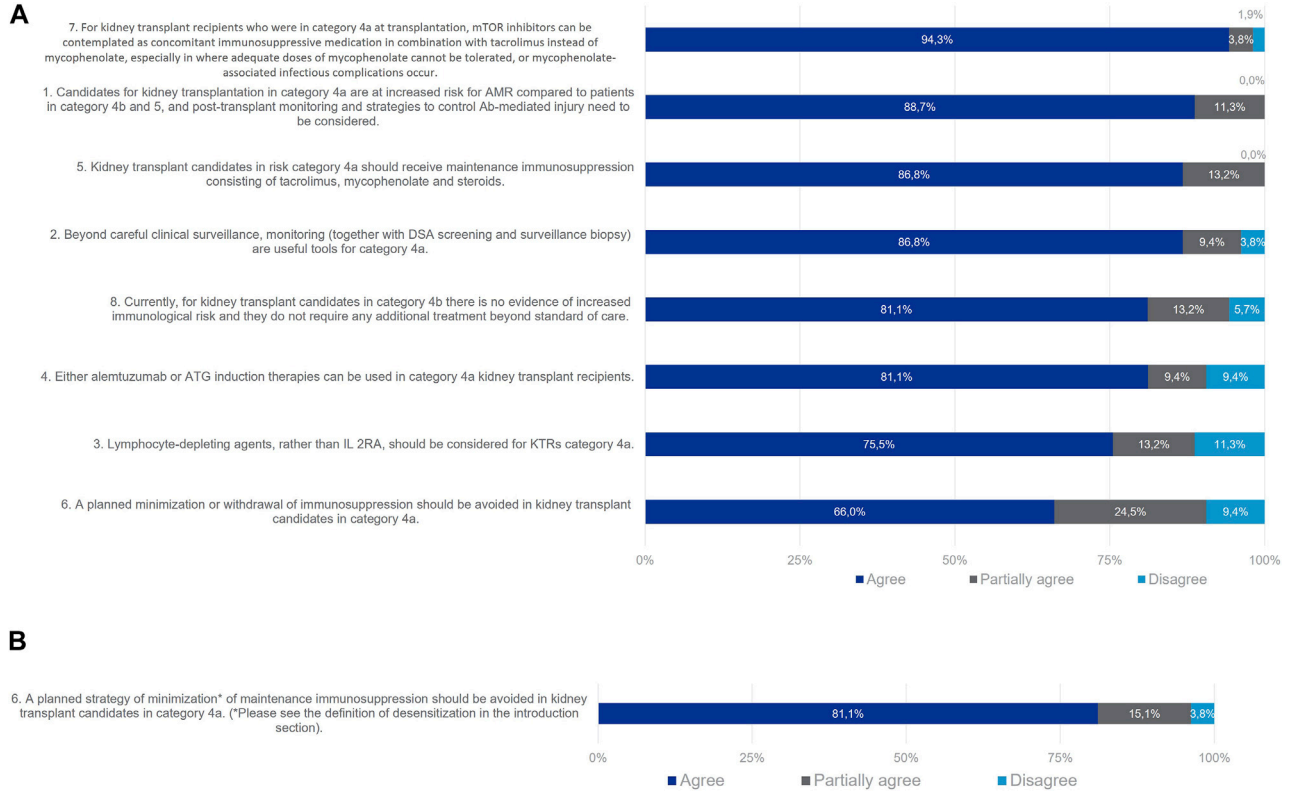

FIGURE 6 | Panellist responses for Category 3 (patients with absence of DSA with but potential cellular memory against donor HLA antigens on day 0) after **(A)** Wave 1 and **(B)** wave 2.

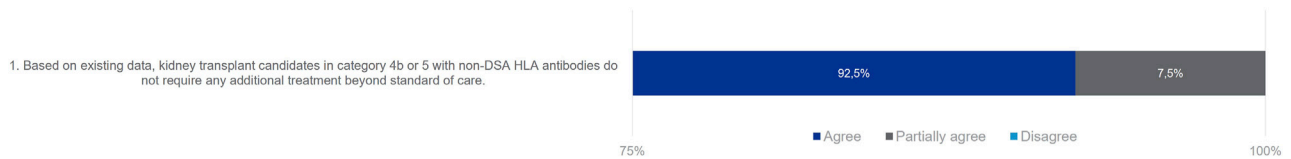

FIGURE 7 | Panellist responses for category 5 (Patients with no DSA and no cellular memory on Day 0).

require any additional treatment beyond standard of care (agreement rate 93%; **Figure 7**).

DISCUSSION

Through a process of systematic literature searching, statement development and Delphi-based consensus achievement a group of European experts in the field of kidney transplantation agreed a series of recommendations for desensitization and immunomodulation strategies based on previously defined risk categories [1]. For patients in Categories 1 and 2 the recommendation is that kidney transplantation should be avoided unless no other option is available. In this situation, for patients in category 1, a CDC negative crossmatch must be obtained through desensitization before transplantation and in both categories 1 and 2, strategies to prevent and treat antibody rebound must be carefully planned. Importantly, in the survey used to establish the consensus, the focus was primarily on the combination of plasma exchange (PEX) and intravenous immunoglobulin (IVIG) for desensitization. It's crucial to note that this choice was made for the sake of simplicity, and while historically the first therapeutic approach, it is no longer the sole option available to clinicians. Among the alternative extracorporeal therapies capable of removing circulating HLA antibodies, both double-filtration plasmapheresis (DFPP) and immunoadsorption (IA) have demonstrated efficacy for desensitization, as supported by studies [15, 16]. Currently, there is no conclusive evidence favouring one technique over another, and studies comparing different apheresis techniques for

HLA desensitization are limited. For instance, a study by Maillard et al. revealed a higher relative reduction of MFI with IA compared to three consecutives daily PLEX sessions (−69% vs. −58%, respectively, $p = 0.003$), despite IA treating a lower total volume of plasma (105 ± 6 vs. 160 ± 16 mL/kg after IA and PEX, respectively) [17]. However, a significant drawback of this study was its departure from routine clinical practice, where more than one IA or three PEX sessions are typically performed. Another recent monocentric study analysing 881 sessions (107 DFPP, 54 PEX, 720 IA) in 45 patients reported successful procedures leading to HLA incompatible kidney transplantation in 39 patients (87%) after 29 (15–51) days. IA, PE, and a lower maximal DSA MFI were associated with a greater decrease in intra-session class II DSA [15]. Apart from efficacy, the choice of the apheresis technique also considers safety. Compared to PEX, IA offers semi-specific plasma treatment, eliminating the need for albumin or plasma substitution [18]. However, the rational use of fresh-frozen plasma effectively mitigates hypofibrinogenemia-induced haemorrhagic risk associated with PEX. Therefore, all three techniques exhibited good tolerance in the study by Noble et al, with severe adverse events occurring in only 1.9% of the 881 (DFPP had a slightly higher occurrence of adverse events: 6.5%; $p < 0.01$). Lastly, it's essential to also consider the financial and practical aspects, unfortunately IA columns comes at a higher cost are not universally available across all countries [19].

For patients of category 1 to 3 other transplant options should be preferred each time possible, such as compatible living donor transplant or kidney paired donation, or awaiting on a national prioritization program if acceptable waiting times are expected according to transplant calculators that address the likelihood of a compatible deceased donor transplant for sensitized patients [20]. They require a thorough risk/benefit analysis and strategies to control antibody-mediated injury. Patients in Category 4a require post-transplant monitoring and strategies to control antibody-mediated injury as they are at increased risk for AMR compared with patients in Category 5, who do not require any additional treatment beyond standard of care [21–25].

For patients in Categories 1, 2, 3 and 4a, careful clinical surveillance and monitoring with DSA screening and surveillance biopsy is recommended [26, 27]. With regard to desensitization strategies for patients in Categories 1, 2 and 3, it is suggested that PEX and IVIg should be part of the first line treatment. Moreover, Imlifidase could be considered for deceased kidney transplantation in selected patients in Categories 1 and 2 for whom no other treatment options are available [21, 28–31]. As far as induction therapy for patients in Categories 1, 2, 3 and 4a, lymphocyte-depleting agents rather than IL-2RAs alone are recommended and either alemtuzumab or ATG can be considered [4, 21, 32, 33]. The experts also agreed that rituximab might be considered as an adjunct to prevent antibody rebound and therefore could be included at the time of transplantation as an induction agent for patients in Categories 1, 2, 3 [21, 33–36]. This approach, although not directly evaluated in the present questionnaire, is even making better sense for patients from category 4a, who have by definition lost their serological memory (disappearance of DSA from the circulation) but remain at high risk of having persisting alloreactive memory B cells [37]. In the latter, the use of rituximab (a B-cell depleting agent with a safe tolerance profile) has been suggested as an alternative to T-cell depleting agents to prevent DSA rebound without increasing the risk of infectious and cancerous complications [38].

For maintenance immunosuppression for patients in Categories 1, 2, 3 and 4a, it is recommended that they receive treatment with tacrolimus, mycophenolate and steroids. Also, in some cases mTOR inhibitors in combination with tacrolimus instead of mycophenolate can be contemplated. Planned strategies of minimization of this maintenance immunosuppression should be avoided in these patients. Immunosuppression should be adapted and maintained in these transplant recipients unless there are treatment-related adverse events that are severe enough to alter the regimen [39].

In Category 4a patients, consensus remained elusive during Wave one concerning the statement on maintenance immunosuppression. Disagreement primarily stemmed from the belief that minimizing immunosuppression should be approached on a case-by-case basis, contingent upon DSA monitoring and surveillance biopsies. For Wave two, the statement underwent a revision, incorporating a clear definition of minimization as a planned strategy involving the reduction of maintenance immunosuppression, entailing lowering CNI trough levels and/or antimetabolite doses below standard values. However, specific target levels and doses were not delineated, and/or the withdrawal of corticosteroids was suggested. Consensus was attained in Wave two, with agreement that planned minimization strategies should be avoided for these patients. Nevertheless, experts acknowledged that, based on time post-transplantation, absence of prior acute rejection history, and immunosuppression-related side effects, the consideration of steroid withdrawal or reduced tacrolimus/MMF doses could be appropriate for select patients in this category.

In regard to kidney transplantation candidates in Categories 1 and 2, no consensus emerged regarding the use of complement inhibitors. Predominant reasons for disagreement centred around the current lack of evidence supporting this recommendation, given that complement inhibition does not reduce DSA titres. While the use of complement inhibitors may not be recommended for patients with high preformed DSA titres, these agents could still prove beneficial in addressing complement-mediated injury during an episode of AMR [6, 40–42].

According to the recent guideline from a European Society of Organ Transplantation (ESOT) working group, concerning the management of kidney transplant patients with HLA antibodies [6], highly sensitized patients should be prioritized in kidney allocation schemes and linking allocation schemes may increase opportunities. If strategies for finding a compatible kidney are very unlikely to yield a transplant, desensitization may be considered balancing the benefit/risk with staying on chronic dialysis therapy for long periods of time, if not for ever, and should be preferentially performed with PLEX or immunoadsorption (IA), supplemented with IVIg and/or anti-CD20 antibody treatment. Newer therapies such as imlifidase

may offer a unique opportunity, especially for deceased-donor transplant candidates, to significantly reduce, albeit only transiently, the risk for hyperacute and accelerated graft rejection and thus, may provide access to transplantation. To date, few studies have compared HLA incompatible transplantation with remaining on the waiting list, and comparisons of morbidity or quality of life do not exist. The use of Kidney-paired Exchange Programmes (KEP) is preferred to desensitization, but highly sensitized patients should not be left on a KEP list indefinitely if the option of a direct incompatible transplant exists.

To our knowledge, this is the first study undertaken as a cohesive effort to provide an international expert consensus on desensitization and immunomodulation in kidney transplant patients according to each patient recently determined humoral risk category. A high level of consensus was achieved among this group of European experts for the management of desensitization and immunomodulation strategies of kidney transplantation recipients according to defined pre-transplantation patients humoral risk profiles. The actions to be undertaken for each patient risk category may help to improve these patients' management, access to transplantation and long-term success.

AUTHOR CONTRIBUTIONS

LF and OT contributed to coordination, conception and design of the study, edit the first draft of the manuscript, review, read, and approved the submitted version. OB, KB, EC, FD, NM, MN, SSS, and FV contributed to conception and design of the study, revision, read, and approved the submitted version. LHMP performed the systematic review of the literature and contributed to manuscript revision, read, and approved the submitted version.

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CONFLICT OF INTEREST

Author LF has participated in advisory board and/or speakers bureau for Novartis Farmacéutica, S.A., Chiesi Farmaceutici S.p.A, Hansa Biopharma, Alexion Pharmaceuticals, Astellas Pharma Inc. Author OB has received consulting fees and research support by Hansa Biophama. Author KB declares honoraria, research grant, and or travel support from: Aicuris, Alexion, Astellas, AstraZeneca, Biohope, Bristol-Myers Squibb, CareDx, Carealytics Digital Health, Chiesi, CSL Behring, DTB GmbH, Eledon, Fresenius, Hansa, HiBio, MSD, Natera, Neovii, Oncocyte, OSKA, Otsuka, Paladin, Pfizer, Pirche, Sanofi, smart Care solutions, Stada, Takeda, Veloxis, Vifor and Xenothera. Author EC has participated in advisory boards or acted as a consultant for Novartis, Astellas, Hansa Biopharma and eGenesis. Author FD has received speaker honoraria from Hansa Biopharma. His institution is receiving research funds from Hansa Biopharma for an investigator-initiated clinical trial. Author NM has done consultancy work with Hansa Biophama. Author SSS has participated in advisory board for Hansa Biopharma. Author OT has participated in advisory board and/or speakers bureau for Adocia, Biotest and Chiesi Farmaceutici S.p.A.

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.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.12475/full#supplementary-material

REFERENCES

1. Bestard O, Couzi L, Crespo M, Kessaris N, Thaunat O. Stratifying the Humoral Risk of Candidates to a Solid Organ Transplantation: A Proposal of the ENGAGE Working Group. *Transpl Int* (2021) 34:1005–18. doi:10.1111/tri.13874
2. Health Resources and Services Administration USD of H& HS. *OPTN: Organ Procurement and Transplantation Network - OPTN*. Washington, DC: HHS Press Office.
3. Eurotransplant International Foundation. Eurotransplant Statistics Report Library (2024). Available from: https://statistics.eurotransplant.org (Accessed January 13, 2024).
4. Manook K, Koeser L, Ahmed Z, Robb M, Johnson R, Shaw O, et al. Post-listing survival for 395 highly sensitised patients on the UK kidney transplant waiting list: a matched cohort analysis *Lancet [Internet]* (2017) 389(10070):727–34. doi:10.1016/S0140-6736(16)31595-1
5. Heidt S, Claas FHJ. Transplantation in Highly Sensitized Patients: Challenges and Recommendations. *Expert Rev Clin Immunol* (2018) 14(8):673–9. doi:10.1080/1744666X.2018.1498335
6. 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 [Internet]* (2022) 35:1–16. doi:10.3389/ti.2022.10511
7. Stewart DE, Kucheryavaya AY, Klassen DK, Turgeon NA, Formica RN, Aeder MI. Changes in Deceased Donor Kidney Transplantation One Year After KAS Implementation. *Am J Transpl* (2016) 16(6):1834–47. doi:10.1111/ajt.13770
8. Divard G, Goutaudier V. Global Perspective on Kidney Transplantation: France. *Kidney360.* (2021) 2(10):1637–40. doi:10.34067/KID.0002402021
9. Sypek MP, Kausman JY, Watson N, Wyburn K, Holt SG, Hughes P, et al. The Introduction of cPRA and Its Impact on Access to Deceased Donor Kidney Transplantation for Highly Sensitized Patients in Australia. *Transplantation* (2021) 105(6):1317–25. doi:10.1097/TP.0000000000003410

10. Boenink R, Stel VS, Waldum-Grevbo BE, Collart F, Kerschbaum J, Heaf JG, et al. Data From the ERA-EDTA Registry Were Examined for Trends in Excess Mortality in European Adults on Kidney Replacement Therapy. *Kidney Int* (2020) 98(4):999–1008. doi:10.1016/j.kint.2020.05.039
11. Chen CC, Pouliquen E, Broisat A, Andreata F, Racapé M, Bruneval P, et al. Endothelial Chimerism and Vascular Sequestration Protect Pancreatic Islet Grafts From Antibody-Mediated Rejection. *J Clin Invest* (2018) 128(1):219–32. doi:10.1172/JCI93542
12. 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 Transpl* (2012) 12(2):388–99. doi:10.1111/j.1600-6143.2011.03840.x
13. 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 Transpl* (2012) 12(5):1157–67. doi:10.1111/j.1600-6143.2012.04013.x
14. Chen CC, Koenig A, Saison C, Dahdal S, Rigault G, Barba T, et al. CD4+ T Cell Help Is Mandatory for Naive and Memory Donor-Specific Antibody Responses: Impact of Therapeutic Immunosuppression. *Front Immunol* (2018) 9(FEB):275. doi:10.3389/fimmu.2018.00275
15. Noble J, Metzger A, Bennani HN, Daligault M, Masson D, Terrec F, et al. Apheresis Efficacy and Tolerance in the Setting of Hla-Incompatible Kidney Transplantation. *J Clin Med* (2021) 10(6):1316. doi:10.3390/jcm10061316
16. Bartel G, Wahrmann M, Regele H, Ž K, Fischer G, Druml W, et al. Peritransplant Immunoadsorption for Positive Crossmatch Deceased Donor Kidney Transplantation. *Am J Transpl* (2010) 10(9):2033–42. doi:10.1111/j.1600-6143.2010.03226.x
17. Maillard N, Absi L, Claisse G, Masson I, Alamartine E, Mariat C. Protein A-Based Immunoadsorption Is More Efficient Than Conventional Plasma Exchange to Remove Circulating Anti-HLA Antibodies. *Blood Purif* (2015) 40(2):167–72. doi:10.1159/000437041
18. Belàk M, Borberg H, Jimenez C, Oette K. Technical and Clinical Experience With Protein A Immunoadsorption Columns. *Transfus Sci* (1994) 15(4): 419–22. doi:10.1016/0955-3886(94)90174-0
19. Braun N, Bosch T. Immunoadsorption, Current Status and Future Developments. *Expert Opin Investig Drugs* (2000) 9(9):2017–38. doi:10.1517/13543784.9.9.2017
20. NHS Blood and Transplant. Tools, Policies, and Guidance/Calculators (2023). Available from: https://www.odt.nhs.uk/transplantation/tools-policies-and-guidance/calculators/ (Accessed October 13, 2023).
21. Huber L, Naik M, Budde K. Desensitization of HLA-Incompatible Kidney Recipients. *N Engl J Med* (2011) 365(17):1644–5. doi:10.1056/NEJMc1109936
22. Gosset C, Viglietti D, Rabant M, Verine J, Aubert O, Glotz D, et al. Circulating Donor-Specific Anti-HLA Antibodies Are a Major Factor in Premature and Accelerated Allograft Fibrosis. *Kidney Int* (2017) 92:729–42. doi:10.1016/j.kint.2017.03.033
23. Ziemann M, Alternamm W, Angert K, Arns W, Bachmann A, Bakchoul T, et al. Preformed Donor-Specific HLA Antibodies in Living and Deceased Donor Transplantation. *Clin J Am Soc Nephrol* (2019) 14:1056–66. doi:10.2215/CJN.13401118
24. Ziemann M, Suwelack B, Banas B, Budde K, Einecke G, Hauser I, et al. Determination of Unacceptable HLA Antigen Mismatches in Kidney Transplant Recipients. *HLA* (2022) 100(1):3–17. doi:10.1111/tan.14521
25. 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 Transplantion Society Working Group. *Transplantation* (2020) 104:911–22. doi:10.1097/TP.0000000000003095
26. Loupy A, Vernerey D, Tinel C, Aubert O, Duong van Huyen JP, Rabant M, et al. Subclinical Rejection Phenotypes at 1 Year Post-Transplant and Outcome of Kidney Allografts. *J Am Soc Nephrol* (2015) 26:1721–31. doi:10.1681/ASN.2014040399
27. Amrouche L, Aubert O, Suberbielle C, Rabant M, van Huyen JPD, Martinez F, et al. Long-Term Outcomes of Kidney Transplantation in Patients With High Levels of Preformed DSA: The Necker High-Risk Transplant Program. *Transplantation* (2017) 101:2440–8. doi:10.1097/TP.0000000000001650
28. Kjellman C, Maldonado AQ, Sjoholm K, Lonze BE, Montgomery RA, Runstrom A, et al. Outcomes at 3 Years Posttransplant in Imlifidase-Desensitized Kidney Transplant Patients. *Am J Transpl* (2021) 21:3907–18. doi:10.1111/ajt.16754
29. Orandi BJ, Luo X, Massie AB, Garonzik-Wang JM, Lonze BE, Ahmed R, et al. Survival Benefit With Kidney Transplants From HLA-Incompatible Live Donors. *N Engl J Med* (2016) 374(940–50):940–50. doi:10.1056/NEJMoa1508380
30. Jordan SC, Legendre C, Desai NM, Lorant T, Bengtsson M, Lonze B, et al. Imlifidase Desensitization in Crossmatch-Positive, Highly Sensitized Kidney Transplant Recipients: Results of an International Phase 2 Trial (Highdes). *Transplantation* (2021) 105:1808–17. doi:10.1097/TP.0000000000003496
31. Jordan S, Tyan D, Stablein D, McIntosh M, Rose S, Vo A, et al. Intravenous Immunoglobulin as an Agent to Lower Allosensitization and Improve Transplantation in Highly Sensitized Adult Patients With End-Stage Renal Disease: Report of the NIH IG02 Trial. *J Am Soc Nephrol* (2004) 15:3256–62. doi:10.1097/01.ASN.0000145878.92906.9F
32. Zheng J, Song W. Alemtuzumab Versus Antithymocyte Globulin Induction Therapies in Kidney Transplantation Patients: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Medicine (Baltimore)* (2017) 96: e7151. doi:10.1097/MD.0000000000007151
33. Clatworthy MR, Watson CJE, Plotnek G, Bardsley V, Chaudhry AN, Bradley J, et al. B-Cell-Depleting Induction Therapy and Acute Cellular Rejection. *N Engl J Med* (2009) 360:2683–5. doi:10.1056/NEJMc0808481
34. Sood P, Hariharan S. Rituximab Induction for Prevention of HLA-Antibody Rebound. *Nat Rev Nephrol* (2014) 10:682–3. doi:10.1056/NEJMc0808481
35. Vo AA, Lukovsky M, Toyoda M, Wang J, Reinsmoen NL, Lai CH, et al. Rituximab and Intravenous Immune Globulin for Desensitization During Renal Transplantation. *N Engl J Med* (2008) 359:242–51. doi:10.1056/NEJMoa0707894
36. van den Hoogen MWF, Kamburova EG, Baas MC, Steenbergen EJ, Florquin S, Koenen H, et al. Rituximab as Induction Therapy After Renal Transplantation: A Randomized, Double-Blind, Placebo-Controlled Study of Efficacy and Safety. *Am J Transpl* (2015) 15:407–16. doi:10.1111/ajt.13052
37. Luque S, Lúcia M, Bestard O. Refinement of Humoral Immune Monitoring in Kidney Transplantation: The Role of "Hidden" Alloreactive Memory B Cells. *Transpl Int* (2017) 30(10):955–68. doi:10.1111/tri.13014
38. Tomita Y, Iwadoh K, Ogawa Y, Miki K, Kato Y, Kai K, et al. Single Fixed Low-Dose Rituximab as Induction Therapy Suppresses De Novo Donor-Specific Anti-HLA Antibody Production in ABO Compatible Living Kidney Transplant Recipients. *PLoS One* (2019) 14(10):e0224203–15. doi:10.1371/journal.pone.0224203
39. KDIGO. KDIGO Clinical Practice Guideline for the Care of Kidney Transplant Recipients. *Am J Transpl* (2009) 9(Suppl. 3):S1–S155. doi:10.1111/j.1600-6143.2009.02834.x
40. Marks WH, Mamode N, Montgomery RA, Stegall MD, Ratner LE, Cornell L, et al. Safety and Efficacy of Eculizumab in the Prevention of Antibody-Mediated Rejection in Living-Donor Kidney Transplant Recipients Requiring Desensitization Therapy: A Randomized Trial. *Am J Transpl* (2019) 19:2876–88. doi:10.1111/ajt.15364
41. Glotz D, Russ G, Rostaing L, Legendre C, Tufveson G, Chadban S, et al. Safety and Efficacy of Eculizumab for the Prevention of Antibody-Mediated Rejection After Deceased-Donor Kidney Transplantation in Patients With Preformed Donor-Specific Antibodies. *Am J Transpl* (2019) 19:2865–75. doi:10.1111/ajt.15397
42. Lefaucheur C, Viglietti D, Hidalgo LG, Ratner LE, Bagnasco SM, Batal I, et al. Complement-Activating Anti-HLA Antibodies in Kidney Transplantation: Allograft Gene Expression Profiling and Response to Treatment. *J Am Soc Nephrol* (2018) 29(2):620–35. doi:10.1681/ASN.2017050589

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SYSTEMATIC REVIEW AND META-ANALYSIS
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Prevalence of Musculoskeletal and Metabolic Disorders in Kidney Transplant Recipients: A Systematic Review and Meta-Analysis

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Introduction: Musculoskeletal disorders could be associated with metabolic disorders that are common after kidney transplantation, which could reduce the quality of life of patients. The aim of this study was to assess the prevalence of both musculoskeletal and metabolic disorders in kidney transplant patients.

Methods: MEDLINE, CINAHL, Cochrane Library, EMBASE and Web of Science were searched from their inception up to June 2023. DerSimonian and Laird random-effects method was used to calculate pooled prevalence estimates and their 95% confidence intervals (CIs).

Results: 21,879 kidney transplant recipients from 38 studies were analysed. The overall proportion of kidney transplant patients with musculoskeletal disorders was 27.2% (95% CI: 18.4–36.0), with low muscle strength (64.5%; 95% CI: 43.1–81.3) being the most common disorder. Otherwise, the overall proportion of kidney transplant patients with metabolic disorders was 37.6% (95% CI: 21.9–53.2), with hypovitaminosis D (81.8%; 95% CI: 67.2–90.8) being the most prevalent disorder.

Conclusion: The most common musculoskeletal disorders were low muscle strength, femoral osteopenia, and low muscle mass. Hypovitaminosis D, hyperparathyroidism, and hyperuricemia were also the most common metabolic disorders. These disorders could be associated with poorer quality of life in kidney transplant recipients.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero/, identifier [CRD42023449171].

Keywords: renal transplant, musculoskeletal, metabolic, proportion, meta-analysis

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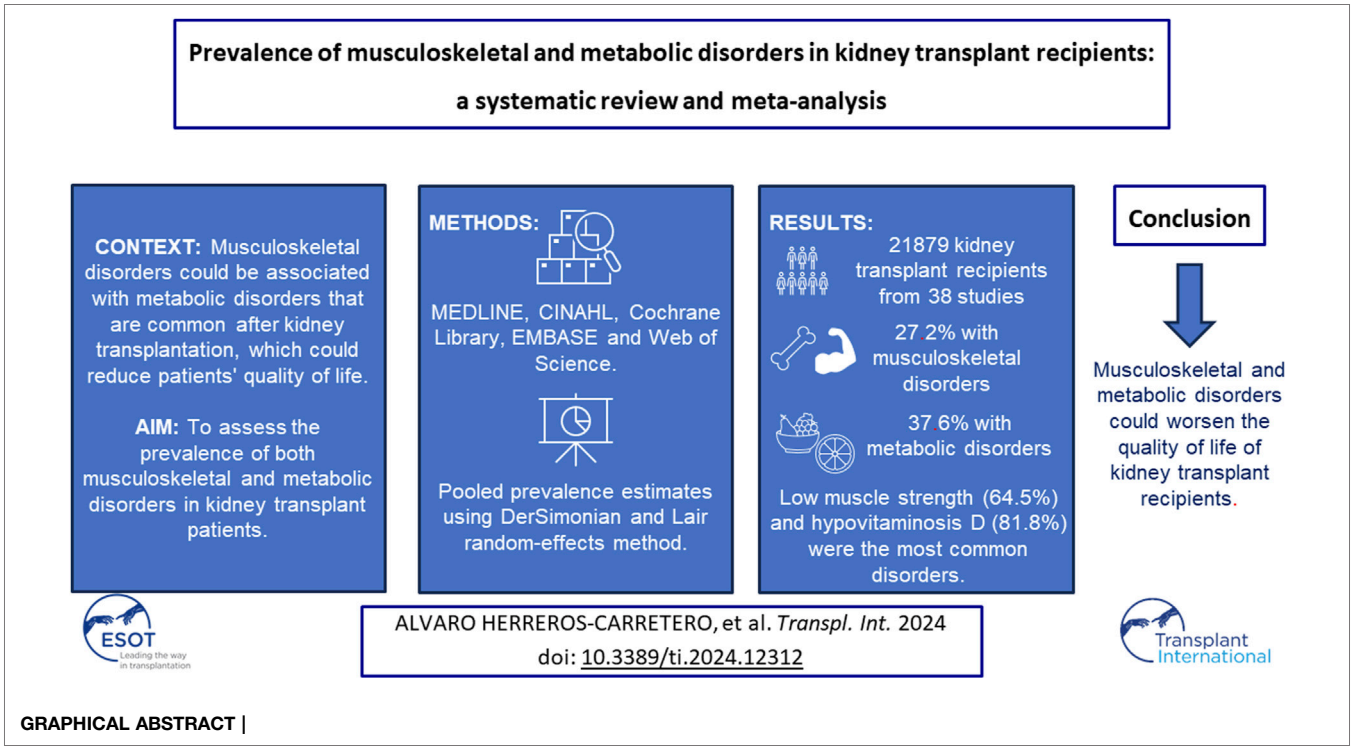
Abbreviations: BMD, Bone mineral density; CI, Confidence intervals; FGF-23, Fibroblast growth factor 23; GODT3, Global Observatory on Donation and Transplantation; JBI, Joanna Briggs Institute; MOOSE, Meta-analyses of Observational Studies in Epidemiology; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis; PROSPERO, International Prospective Register of Systematic Reviews; PTH, Parathyroid hormone; WOS, Web of Science.



GRAPHICAL ABSTRACT |

INTRODUCTION

Renal transplantation represents the best therapy for patients diagnosed with end-stage renal disease. Major advances in surgical techniques and immunosuppressive treatment have led to a substantial improvement in the survival of these patients over the last few decades, resulting in a higher quality of life and lower treatment-related costs compared to dialysis [1, 2]. This surgical procedure involves the replacement of a healthy kidney, either from a living or deceased donor, in a patient whose kidneys are not functioning properly [1]. According to the Global Observatory on Donation and Transplantation (GODT3), a total of 65,668 kidney transplants were performed worldwide in 2021, making the kidney commonly the most transplanted organ [3].

Despite the improvement in the patient's clinical status compared to the patient's previous disease status, this therapy does not imply a cure [4]. The evolution of kidney transplant recipients will depend fundamentally on the use of immunosuppressive drugs, the origin of the transplanted kidney, the characteristics of the patient and several events that may occur in the post-transplant period [1], which pose certain risks to the health and quality of life of the transplant recipient. These post-transplant events include renal, infectious, urological, surgical, cardiovascular, and cerebrovascular complications, side effects of the drugs used to prevent rejection, and metabolic disorders [1].

In relation to the above, there are several metabolic disorders, such as hypercalcemia, hypophosphatemia, hyperparathyroidism, and hypovitaminosis D, among others, which are common in these patients and have the potential to cause loss of bone mineral density (BMD), as occurs with the use of glucocorticoids, whose doses are higher immediately after transplantation [2, 4, 5]. This loss of BMD leads to several musculoskeletal disorders that can affect the quality of life of transplant patients and need to be controlled.

Musculoskeletal disorders include a group of pathologies suffered by many patients after surgery, the exact prevalence of which is not yet well known [6]. This group includes disorders such as osteopenia, osteoporosis, and sarcopenia, which involve both a reduction in bone density and a reduction in strength and muscle mass, respectively [7]. Although it is a common complication in these patients, involving the loss of bone and muscle mass, especially in the first months after transplantation, both diagnosis and treatment to prevent these pathologies are still inadequate [8, 9]. Furthermore, there is a lack of studies that accurately synthesize and estimate the proportion of musculoskeletal and metabolic disorders in renal transplant patients. Therefore, the aim of this study was to carry out a systematic review and meta-analysis to determine the prevalence of musculoskeletal disorders and their related metabolic disorders in kidney transplant patients.

METHODS

This systematic review adhered to the Cochrane Collaboration Handbook, the Meta-analyses of Observational Studies in Epidemiology (MOOSE) guidelines, and the "Preferred

TABLE 1 | Characteristics of the studies included (*n* = 38).

Author and year	Country	Study design	Sample characteristics					Outcome (prevalence)
			Transplant year	n	Age and gender (% women)	Time since transplant	Time on haemodialysis prior to transplant	
Alagoz S. et al. [11] 2019	Turkey	Retrospective longitudinal	2002–2012	176	32.9 ± 11.8 (38.1)	1 month	33.8 ± 33.1 months	Hypercalcemia (18.2%) Hypophosphatemia (33.3%) Hyperparathyroidism (45.3%)
						12 months		Hypercalcemia (17.2%) Hypophosphatemia (8.6%) Hyperparathyroidism (29.4%)
						60 months		Hypercalcemia (13.2%) Hypophosphatemia (11.4%) Hyperparathyroidism (9.2%)
Amin T. et al. [12] 2016	Australia	Cross-sectional	1971–2011	679	55 ± 13 (39)	≥3 months	28.8 ± 24 months	Hypercalcemia (15%)
Batteux B. et al. [13] 2020	France	Prospective longitudinal	2012–2018	310	51.1 ± 12.8 (37.4)	1 month	30 months	Osteopenia: lumbar area (34.5%); femoral area (53.5%) Osteoporosis: lumbar area (6.1%); femoral area (10%)
Berga JK. Et al [14]. 2010	Spain	Retrospective longitudinal	—	110	50.2 ± 11 (53)	—	—	Hypovitaminosis D (96.4%): insufficiency (43.6%), deficiency (52.7%)
Braga Jr JWR. et al. [15] 2006	Brazil	Cross-sectional	2000	191	44.8 ± 0.8 (50.8)	87 ± 3.7 months	46.48 ± 3.03 months	Osteopenia: lumbar area (32.5%); femoral area (33%) Osteoporosis: lumbar area (11.5%); femoral area (11%) Fractures (24.1%)
Chan W. et al. [16] 2019	United Kingdom	Prospective longitudinal	2010–2013	128	49 ± 15 (44)	60 (12–132) months	—	Sarcopenia (28.9%) Low muscle strength (64.1%) Low muscle mass (35.9%)
Conley E. et al. [17] 2008	United States	Retrospective longitudinal	1998–2006	554	46.3 ± 0.5 (42.2)	14 months	—	Fractures (13%)
Einollahi E. et al. [18] 2013	Iran	Cross-sectional	2008–2011	4,217	38 ± 15 (36)	60 months	—	Hyperuricemia (31.8%)
Evenepoel P. et al. [19] 2019	Belgium	Cross-sectional	2006–2013	518	54.7 ± 12.8 (39.4)	>2 weeks	—	Hypovitaminosis (38.4%): insufficiency (35.1%); deficiency (3.3%) Osteopenia: lumbar area (8.1%); femoral area (55%) Osteoporosis: lumbar area (23.7%); femoral area (22%) Fractures (7.3%)
Férnandez Castillo R. et al. [20] 2018	Spain	Cross-sectional	—	119	–(41.2)	6 months	—	Osteopenia: lumbar area (32.9%); femoral area (49.3%) Osteoporosis: lumbar area (30.1%); femoral area (15.1%)
						12 months		Osteopenia: lumbar area (38.4%); femoral area (51.5%) Osteoporosis: lumbar area (30.8%); femoral area (16.7%)
Gregorini M. et al. [21] 2017	Italy	Cross-sectional	2000–2016	297	55.5 ± 12 (34.7)	24 months	—	Osteopenia: lumbar area (40.4%); femoral area (50.2%) Osteoporosis: lumbar area (13.8%); femoral area (20.9%) Fractures (12.1%)
Hamidian Jahromi A. et al. [22] 2009	England	Prospective longitudinal	2000–2002	121	35.5 ± 12.5 (30.6)	3 months	17.4 ± 6 months	Hypercalcemia (17.4%) Hyperparathyroidism (9.9%)
						12 months		Hypercalcemia (5.7%) Hyperparathyroidism (5.7%)
Jerman A. et al. [23] 2017	Slovenia	Cross-sectional	1976–2011	507	54.3 ± 12 (45)	116.4 months	63.4 ± 43.6 months	Fractures (12.6%)
Jørgensen HS. et al. [24] 2016	Norway	Cross-sectional	2006–2011	701	52.2 ± 14.7 (32.4)	2.5 months	13.8 (7.8–26.3) months	Osteopenia: lumbar area (35.7%); femoral area (51.8%) Osteoporosis: lumbar area (16.8%); femoral area (26%)

(Continued on following page)

TABLE 1 | (*Continued*) Characteristics of the studies included (*n* = 38).

Author and year	Country	Study design	Sample characteristics					Outcome (prevalence)
			Transplant year	n	Age and gender (% women)	Time since transplant	Time on haemodialysis prior to transplant	
Khosravi M. et al. [25] 2020	Iran	Cross-sectional	—	148	43.8 ± 12.7 (48)	67.59 ± 42.66 months	14.18 ± 16.05 months	Osteopenia: lumbar area (49.3%) Osteoporosis: lumbar area (18.9%)
Kim KM. et al. [26] 2010	South Korea	Cross-sectional	1990–2008	356	39.3 ± 10.3 (39.3)	102.63 ± 27.25 months	—	Hyperuricemia (15.4%)
Kosoku A. et al. [27] 2020	Japan	Cross-sectional	—	210	55 ± 10 (42)	85 (43–135) months	19 (6–67) months	Sarcopenia (11%)
Limirio LS. et al. [28] 2019	Brazil	Cross-sectional	—	127	47.6 ± 11.5 (31.5)	95.5 ± 78.2 months	55.4 ± 43.5 months	Sarcopenia (50.4%) Low muscle strength (80.3%) Low muscle mass (61.4%)
López Ruiz ML. et al. [29] 2015	Spain	Cross-sectional	2002–2009	306	46.9 ± 13.8 (37.6)	12 months	—	Osteopenia: lumbar area (14.4%); femoral area (19.6%) Osteoporosis: lumbar area (12.4%); femoral area (6.9%)
Malheiro J. et al. [30] 2012	Portugal	Cross-sectional	1983–2010	302	49.6 ± 13.4 (39.4)	91.2 (27.6–170.4) months	—	Hyperuricemia (42.1%)
Marcén R. et al. [31] 2009	Spain	Cross-sectional	—	509	45.4 ± 14.5 (42)	113 ± 76 months	—	Hypovitaminosis D (85.3%): insufficiency (47%); deficiency (38.3%)
Menna Barreto APM. et al. [32] 2019	Brazil	Cross-sectional	—	185	50 ± 7 (43)	117 (32–173) months	—	Sarcopenia (17.3%) Low muscle strength (45.9%) Low muscle mass (23.8%)
Muirhead N. et al. [33] 2014	Canada	Retrospective longitudinal	2003–2008	1,000	50 ± 12.5 (35.6)	12 months	—	Hypercalcemia (16.6%) Hyperparathyroidism (47.6%)
						24 months		Hypercalcemia (13.6%) Hyperparathyroidism (51.1%)
						36 months		Hypercalcemia (9.5%) Hyperparathyroidism (43.4%)
						48 months		Hypercalcemia (10.1%) Hyperparathyroidism (39.3%)
Ozkayar N. et al. [34] 2014	Turkey	Cross-sectional	—	166	37.9 ± 11.9 (41)	—	—	Sarcopenia (20.5%)
Park WY. et al. [35] 2017	United Kingdom	Prospective longitudinal	2011–2013	207	45 ± 11 (46.4)	12 months	25.3 months	Osteopenia: femoral area (40.1%) Osteoporosis: femoral area (47.3%)
Patel S. et al. [36] 2001	United Kingdom	Cross-sectional	1998	165	46 ± (42)	61.2 months	18 months	Osteopenia: lumbar area (30.9%); femoral area (40.6%) Osteoporosis: lumbar area (7.9%); femoral area (10.3%) Fractures (16.4%)
Savaj S. et al. [37] 2012	Iran	Cross-sectional	2010	113	46.1 ± 13.6 (51.3)	106.4 ± 77.0 months	147.1 ± 92.8 months	Hyperparathyroidism (76.1%) Hypovitaminosis D (94.7%): insufficiency (49.6%). deficiency (45.1%) Osteopenia: lumbar area (52.2%); femoral area (36.3%) Osteoporosis: lumbar area (12.4%); femoral area (45.1%)
Schreiber W. et al. [38] 2020	Switzerland	Prospective longitudinal	2008–2009	135	51 ± 11 (33.3)	6 months	—	Vitamin D deficiency (65.2%)
Segaud N. et al. [39] 2018	France	Prospective longitudinal	2005–2011	259	49.7 ± 12.1 (37.1)	8.8 ± 1.9 months	38.4 months	Osteopenia: femoral area (42.9%) Osteoporosis: femoral area (40.9%) Fractures (10.8%)
Simbolon FR. et al. [40] 2018	Taiwan	Retrospective longitudinal	1997–2010	5,917	45.1 ± 11.9 (48.6)	32.4 months	—	Gout (8.8%)
Stamp L. et al. [41] 2006	New Zealand	Cross-sectional	2004	202	53 (31.9)	>36 months	—	Gout (23.3%)

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TABLE 1 | (*Continued*) Characteristics of the studies included (*n* = 38).

Author and year	Country	Study design	Sample characteristics					Outcome (prevalence)
			Transplant year	n	Age and gender (% women)	Time since transplant	Time on haemodialysis prior to transplant	
Torres A. et al. [42] 2016	Spain	Cross-sectional	2008–2010	727	55 ± 13.6 (39.9)	>12 months	67 ± 29 months	Hypercalcemia (6.5%) Hypophosphatemia (6.2%) Hyperparathyroidism (76.9%) Hypovitaminosis D (83.2%): insufficiency (50.8%); deficiency (32.5%) Fractures (14.6%)
Velioglu A. et al. [43] 2021	Turkey	Cross-sectional	2017–2018	153	46.5 ± 11.9 (50.3)	86.4 months	35 months	Hyperparathyroidism (52.9%) Hypovitaminosis D (68.8%): insufficiency (49.7%); deficiency (19%) Osteopenia: lumbar area (28.1%); femoral area (41.2%) Osteoporosis: lumbar area (7.2%); femoral area (7.8%) Fractures (43.4%)
Vilarta CF. Et al [44]. 2017	Brazil	Cross-sectional	—	149	44 ± –(56.4)	72 months	—	Hypovitaminosis D (79.2%): insufficiency (37.6%); deficiency (41.6%) Fractures (10%)
Wang C. et al. [45] 2021	China	Cross-sectional	—	216	41.5 ± 9.9 (27.8)	—	15 months	Hypercalcemia (8.8%) Hypophosphatemia (3.7%) Hypovitaminosis D (78.7%): insufficiency (46.3%); deficiency (32.4%) Fractures (3.2%)
Weng SC. et al. [46] 2014	Taiwan	Prospective longitudinal	1999–2013	880	48.7 ± 12.3 (46.8)	—	—	Hyperuricemia (44.2%) Gout (17.7%)
Wolf M. et al. [47] 2016	United States	Prospective longitudinal	—	246	52.8 ± 13.4 (36.7)	—	42 ± 34.8 months	-Hypercalcemia (30.5%) -Hypophosphatemia (53.7%) -Hyperparathyroidism (89.4%)
Zhang K. et al. [48] 2015	China	Retrospective longitudinal	2008–2011	573	41.4 ± 9.5 (31.6)	1 month 3 months 24 months 36 months	—	-Hyperuricemia (16.2%) -Hyperuricemia (24.1%) -Hyperuricemia (30.9%) -Hyperuricemia (42.8%)

Reporting Items for Systematic Reviews and Meta-Analysis" (PRISMA) guidelines [10]. This study was registered in the International Prospective Register of Systematic Reviews (PROSPERO) with the registration number (CRD42023449171).

Search Strategy

A systematic search in MEDLINE (via PubMed), CINAHL, Cochrane Library, EMBASE (via Scopus), and Web of Science (WOS) was conducted from inception to June 2023. Gray literature and the references of selected studies were also reviewed to identify additional studies. The search strategy combined the following terms using Boolean operators: "post-kidney transplant," "post-renal transplant," "kidney transplant," "renal transplant," "musculoskeletal," "muscular pain," "muscle pain," sarcopenia, fibromyalgia, myopathy, "joint pain," fracture, fragility, "bone pain syndrome," "bone syndrome," "bone pain," "bone disease," "bone disorder," "lower limb pain," hyperparathyroidism, hypophosphatemia, gout, hyperuricemia, arthritis, "bone loss," osteoporosis, osteopenia, osteomalacia, "mineral disorder," hypercalcemia, "vitamin D," "hypovitaminosis D," "vitamin D deficiency." The references of the included studies were also checked. If the full text of a study was not available, the authors of the study were contacted. The systematic search was conducted independently by two investigators (AH-C and MG-M). The detailed search strategy is available in **Supplementary Table S1**.

Eligibility Criteria

Observational studies analysing musculoskeletal and metabolic disorders developed in kidney transplant patients were included. The inclusion criteria were as follows: 1) population: adult patients over 18 years of age; 2) study design: cross-sectional or baseline data from longitudinal studies without language restriction; and 3) outcome: primary outcomes including prevalence of musculoskeletal or metabolic disorders in kidney transplant recipients. Exclusion criteria were as follows: 1) ineligible publication types (clinical trials, literature reviews, commentaries, or letters to the editor); 2) patients with other

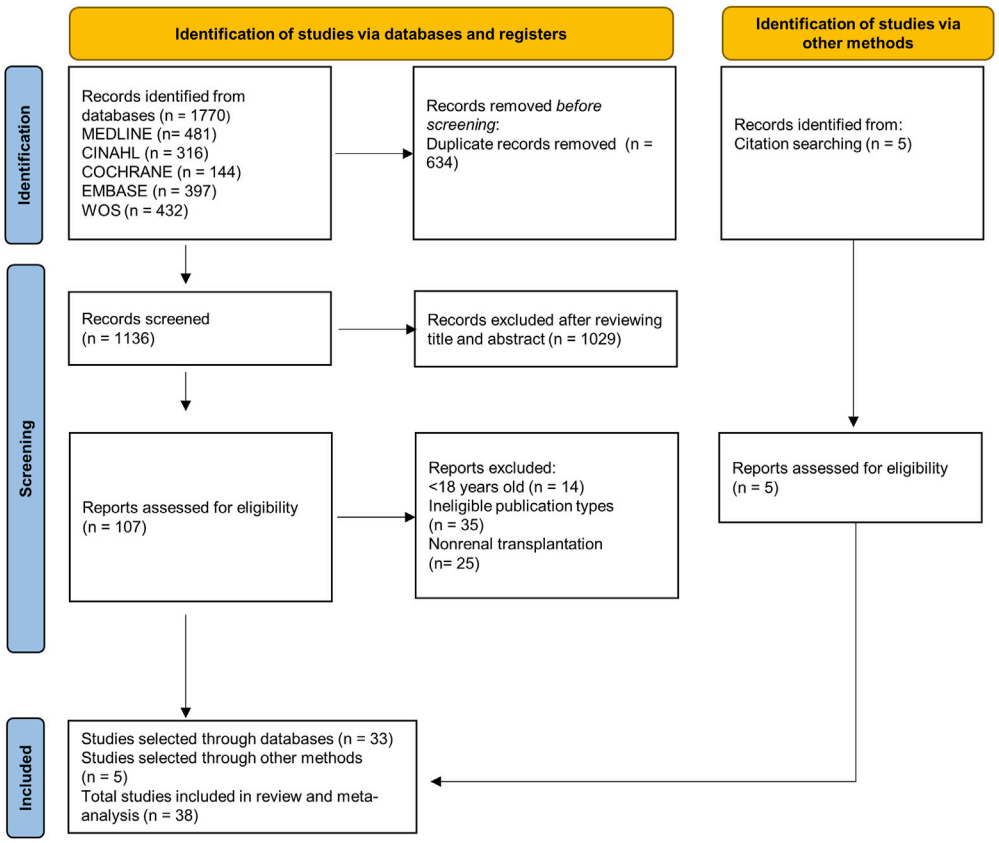

FIGURE 1 | PRISMA 2020 flowchart.

previous nonrenal transplants; 3) pregnant or breastfeeding women; and 4) no access to full text.

Data Extraction

After selecting the studies that met the inclusion criteria, the following data were collected and described in a descriptive table (**Table 1**): (a) first author and year of publication; (b) country; (c) study design; and (d) sample characteristics (year of transplantation, number of participants, age, and sex); and (e) outcome analysed. If more than one study provided data on the same sample, the study with the most detailed results and/or with the largest sample size was selected for data synthesis.

Two reviewers (AH-C and MG-M) independently conducted the data extraction, and disagreements were resolved by consensus or by a third reviewer (CB-M). Articles retrieved were imported and managed by Mendeley reference manager.

Methodological Quality Assessment

To assess the methodological quality of the studies included in this systematic review and meta-analysis, we used the Joanna Briggs Institute (JBI) tool *"Checklist for prevalence studies"* scale by Munn et al [49] for cross-sectional descriptive studies and the JBI tool *"Checklist for cohort studies"* scale by Moola et al [50] for longitudinal cohort studies. Both scales [49, 50] consist of 9 and 11 items, respectively. They are scored as "yes" (1), "no" (0), "not applicable" (NA) and "unclear" (?). The final score for each study therefore ranged from 0 to 9 or 11. Depending on this score, each study was classified as having a low (>7), moderate (4–6) or high (1–3) risk of bias [49, 50].

Both the data extraction and the quality assessment were performed independently by two reviewers (AH-C and MG-M), and disagreements were resolved by consensus or by involving a third reviewer (CB-M).

Statistical Analysis and Data Synthesis

Pooled prevalence estimates with their respective 95% confidence intervals (CIs) were calculated for each subgroup of musculoskeletal disorders (sarcopenia, low muscle strength, and low muscle mass, osteopenia, osteoporosis, fractures, and gout) and metabolic disorders subgroup (hypercalcemia, hypophosphatemia, hyperparathyroidism, hyperuricemia, and hypovitaminosis D). In addition, the overall pooled prevalence of both musculoskeletal and metabolic disorders was also estimated. DerSimonian and Laird random-effects method [51, 52] was used to calculate pooled prevalence estimates and their 95% CIs. Heterogeneity between studies

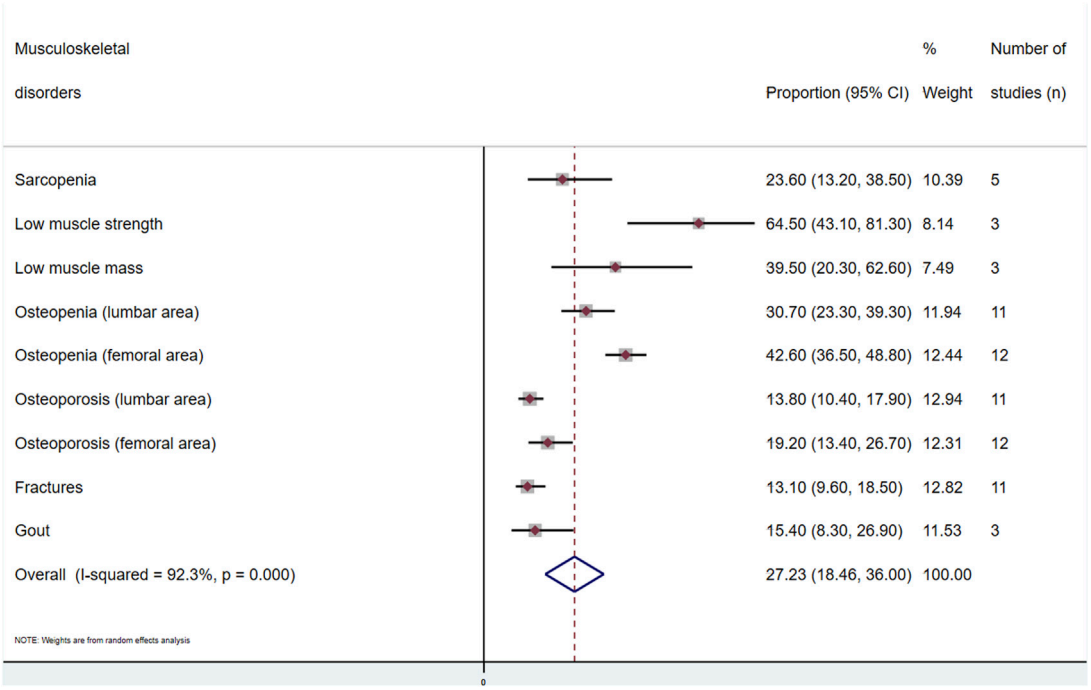

FIGURE 2 | Meta-analysis of the proportion of musculoskeletal disorders in kidney transplant recipients.

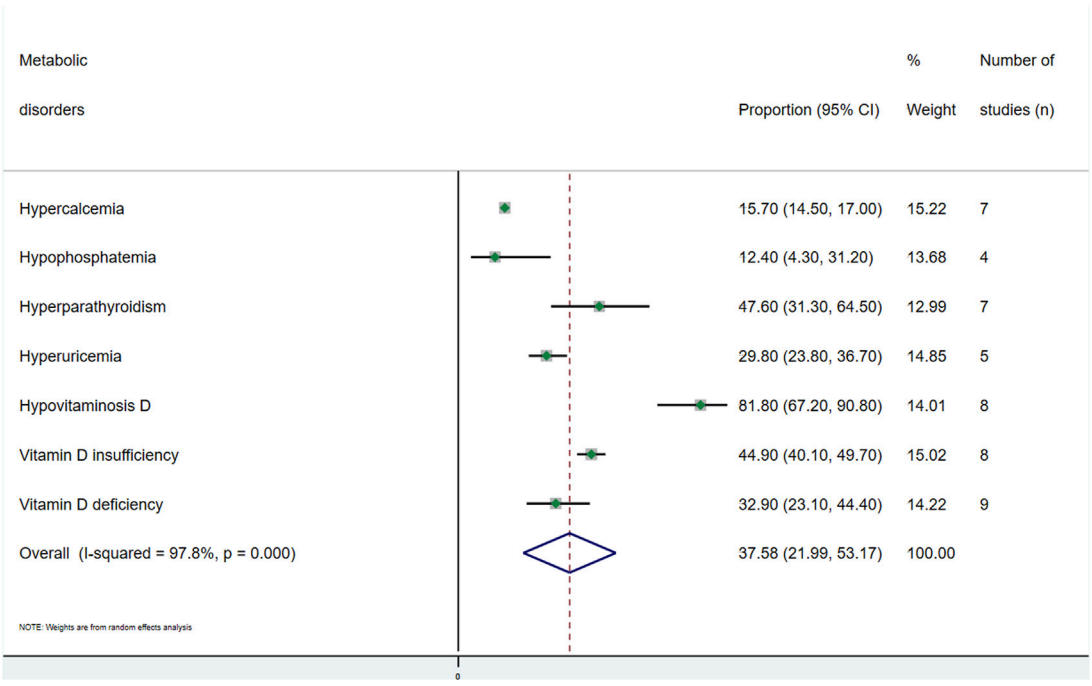

FIGURE 3 | Meta-analysis of the proportion of metabolic disorders in kidney transplant recipients.

was assessed using the I^2 statistic [53] with values considered as follows: not important (0%–40%), moderate (30%–60%), substantial (50%–90%) and considerable heterogeneity (75%–100%). The significance value of the pooled effect size was estimated based on the 95% CI. Two-sided p values of .05 or less were considered significant.

We conducted a sensitivity analysis to determine the robustness of the summary estimates by removing each included study from the analysis one by one. Furthermore, meta-regression models were performed considering mean age, percentage of women, time on hemodialysis prior to transplant, and time since transplant to determine their influence on prevalence estimates. Due to the limited number of studies included ($n < 10$) in each subgroup analysis, meta-regression analysis was only performed with the following outcome variables: osteopenia (lumbar area), osteopenia (femoral area), osteoporosis (lumbar area), osteoporosis (femoral area) and fractures.

Statistical analyses were performed using Stata SE software, version 15 (StataCorp) and Comprehensive Meta-Analysis V3. Global prevalence was estimated using the STATA metaprop statistical package.

RESULTS

Study Selection

A total of 1,770 articles were retrieved from the bibliographic search. After removing duplicates, a total of 38 articles [11–48] were selected for quantitative synthesis (**Figure 1**).

Characteristics of the Selected Studies

The characteristics of the studies selected for this systematic review and meta-analysis are detailed in **Table 1**. The study design was cross-sectional in 27 studies [11, 12, 14, 15, 18–21, 23–32, 34–37, 41–45] (71.1%) and longitudinal in 11 [13, 16, 17, 22, 33, 38–40, 46–48] (28.9%). All these articles were published between 2001 and 2021, and most of them were conducted in Europe [13, 14, 16, 19–24, 29–31, 35, 36, 38, 39, 42] (44.7%), although there were also studies from Asia [11, 18, 25–27, 34, 37, 40, 43, 45, 46, 48] (31.6%), America [15, 17, 28, 32, 33, 44, 47] (18.4%) and Oceania [12, 41] (5.3%).

A total of 21,879 patients (41.4% women) with a mean age of 45.4 years were analysed in this study. The kidney transplants were performed between 1971 and 2018. The mean time since transplantation was 41.3 months (3.4 years), and the mean time on dialysis before transplantation was 36.08 months (3 years).

The musculoskeletal disorders analysed in this review were sarcopenia, low muscle strength and low muscle mass, osteopenia and osteoporosis, bone fractures and gout. The outcomes analysed for metabolic disorders were hypercalcemia, hypophosphatemia, hyperparathyroidism, hyperuricemia and hypovitaminosis D.

Study Quality

Of the cross-sectional studies (**Supplementary Table S2**), 96.3% and 3.7% had a low and a moderate risk of bias, respectively. For longitudinal studies, 54.5% and 45.6% had a low and a moderate risk of bias, respectively (**Supplementary Table S3**). Considering all the studies, 84.2% and 15.8% had a low and moderate risk of bias, respectively.

Main Results

A general estimate of the outcomes regarding both musculoskeletal and metabolic disorders is shown in **Figures 2**, **3**, respectively. Each outcome was also independently analysed and is shown in **Supplementary Figures S1–S16**. The overall proportion of kidney transplant patients with musculoskeletal disorders was 27.2 (95% CI: 18.4–36.0; $I^2 = 92.3\%$) (**Figure 2**), and that with metabolic disorders was 37.6% (95% CI: 21.9–53.2; $I^2 = 97.8\%$) (**Figure 3**).

(i) Musculoskeletal disorders
   (a) Muscle disorders (sarcopenia, low muscle strength and low muscle mass): The prevalence of sarcopenia was analysed in five studies [16, 27, 28, 32, 34]. A total of 816 individuals were included, with an overall prevalence of 23.6% (95% CI: 13.2–38.5; $I^2 = 94.1$) (**Supplementary Figure S1**). Three studies [16, 28, 32], with 440 subjects, included the other two outcomes. For low muscle strength, the overall prevalence was 64.5% (95% CI: 43.1–81.3; $I^2 = 94.4$), and the prevalence of low muscle mass was 39.5% (95% CI: 20.3–62.6; $I^2 = 95.3$) (**Supplementary Figures S2, S3**).
   (b) Osteopenia and osteoporosis: Eleven articles [13, 15, 19–21, 24, 25, 29, 36, 37, 43] investigated the prevalence in the lumbar area, and 12 [13, 15, 19–21, 24, 29, 35–37, 39, 43] studied the prevalence in the femoral area, with 3,021 and 3,339 transplant recipients, respectively. The prevalence of osteopenia in the lumbar area was 30.7% (95% CI: 23.3–39.3; $I^2 = 95.1$). In the femoral area, it was 42.6% (95% CI: 36.5–48.8; $I^2 = 91.9$) (**Supplementary Figures S4, S5**). For lumbar osteoporosis, the prevalence was 13.8% (95% CI: 10.4–17.9; $I^2 = 88.2$). Finally, for the femoral area, the prevalence was 19.2% (95% CI: 13.4–26.7; $I^2 = 95.6$) (**Supplementary Figures S6, S7**).
   (c) Fractures: Eleven articles [15, 17, 19, 21, 23, 36, 39, 42–45] assessed this outcome. The prevalence in 3,736 patients was 13.1% (95% CI: 9.6–18.5; $I^2 = 93.1$) (**Supplementary Figure S8**).
   (d) Gout: Three studies [40, 41, 46] analysed the prevalence of this disorder in renal transplant recipients, including 6,999 participants, where the overall prevalence of gout was 15.4% (95% CI: 8.3–26.9; $I^2 = 97.3$) (**Supplementary Figure S9**).

(ii) Metabolic disturbances.
   (a) Hypercalcaemia: Seven studies [11, 12, 22, 33, 42, 45, 47] provided data on this disorder, with a total of 3,165 subjects analysed. The overall prevalence in this population was 15.7% (95% CI: 14.5–17.0; $I^2 = 91.3$) (**Supplementary Figure S10**).
   (b) Hypophosphatemia: Four studies [11, 42, 45, 47] analysed the prevalence of hypophosphatemia among kidney transplant recipients. The overall prevalence of 1,365 individuals was 12.4% (95% CI: 4.3–31.2; $I^2 = 98.1$) (**Supplementary Figure S11**).
   (c) Hyperparathyroidism: The prevalence of this disorder was obtained from seven studies [11, 22, 33, 37, 42, 43,

47]. The overall prevalence obtained in this population of 2,536 subjects was 47.6% (95% CI: 31.3–64.5; I^2 = 98.2) (**Supplementary Figure S12**).

(d) Hyperuricemia: Hyperuricemia was analysed in five studies [18, 26, 30, 46, 48], with a total of 6,328 subjects, with metabolic disorders having the largest population. The overall prevalence was 29.8% (95% CI: 23.8–36.7; I^2 = 96.8) (**Supplementary Figure S13**).

(e) Hypovitaminosis D: Eight studies [14, 19, 31, 37, 42–45] analysed the prevalence of this disorder. In this population of 2,495 people, the overall prevalence was 81.8% (95% CI: 67.2–90.8; I^2 = 98.2), with this metabolic disorder being the most common finding (**Supplementary Figure S14**). This alteration was divided into vitamin D insufficiency and vitamin D deficiency. For the former, the prevalence was 44.9% (95% CI: 40.1–49.7; I^2 = 80.7), and for vitamin D deficiency, it was 32.9% (95% CI: 23.1–44.4; I^2 = 96.3) (**Supplementary Figures S15, S16**).

These results obtained have been compared with the results of other studies [54–61] that analyse the same variables in the general population (who have not received a kidney transplant). Among metabolic disturbances, the comparison is as follows: 14.9% vs. 0.8% (general population) for hypercalcemia, 58.0% vs. 0.8% for hyperparathyroidism, 31.2% vs. 13.3% for hyperuricemia, and 81.8% vs. 15.7% for hypovitaminosis D. Regarding musculoskeletal disorders, the differences are as follows: 23.6% vs. 15.5% for sarcopenia, 39.5% vs. 27.0% for low muscle mass, 30.7/42.6% (lumbar/femoral area) vs. 40.4% for osteopenia, 13.8/19.2% (lumbar/femoral area) vs. 18.3% for osteoporosis, 14.2% vs. 1.1% for fractures, and 15.4% vs. 1.1% for gout. This comparison is shown in detail in **Supplementary Tables S4, S5**, and individually the comparison of each variable can be seen in **Supplementary Figures S17–S26**.

Sensitivity and Meta-Regression Analysis

When the impact of individual studies was examined by removing studies from the analysis one by one, the estimate of the proportion of sarcopenia changed after removing the Limirio LS. sample [28] (from 23.6% to 19.1%); Menna Barreto ANP. [32] for low muscle strength (from 64.5% to 71.4%); Limirio LS. [28] for low muscle mass (from 39.5% to 29.1%); Wang C. [45] for fractures (from 13.10% to 16.0%), and Simbolon FR. [40] for gout (from 15.4% to 19.9%) (**Supplementary Table S6**). On the other hand, regarding the estimated proportions of metabolic disorders, these were modified after removing the samples of Wolf M. [47] for hypercalcemia (from 15.7% to 13.0%) and hypophosphatemia (from 12.4% to 7.7%), and Evenepoel P. [19] for vitamin D deficiency (from 32.9% to 40.0%) (**Supplementary Table S7**).

Meta-regression models showed that all the variables considered (age, %females, time since transplant and time on haemodialysis prior to transplant) influenced the prevalence estimates of the outcome variables analysed (**Supplementary Table S8**).

DISCUSSION

The main objective of this systematic review and meta-analysis was to provide a complete synthesis of the prevalence of musculoskeletal disorders and metabolic disorders in kidney transplant patients. There is a wide range in the prevalence of metabolic disturbances and musculoskeletal disorders in this population. The most common metabolic disorders in this group of patients were hypovitaminosis D (81.8%), hyperparathyroidism (47.6%), and hyperuricemia (29.8%). Among the musculoskeletal disorders, the most common were low muscle strength (64.5%), femoral osteopenia (42.7%), and low muscle mass (39.5%).

Renal transplantation solves many problems of end-stage renal disease; however, certain metabolic disturbances may persist for some time. Hyperparathyroidism, hypercalcemia, hypophosphatemia, hypovitaminosis D, and hyperuricemia are common after transplantation, and they often occur simultaneously. In addition, together with other factors, they may be involved in the development of certain musculoskeletal disorders that affect the quality of life of the transplanted patient [62].

Despite the improvement in renal function after transplantation, hyperparathyroidism may develop due to a number of factors, including the high levels of parathyroid hormone (PTH) prior to transplantation, the prolonged period of renal disease and dialysis, the degree of hyperplasia of the parathyroid gland or the decrease in vitamin D [2, 62–64]. PTH levels begin to decline during the first 3–6 months after the procedure, but according to the article published by Hassan et al [6], high PTH levels can still be found in 30%–60% of patients 1 year after transplantation [6]. This alteration is also associated with hypercalcemia and hypophosphatemia, among others, which could lead to loss of BMD [2].

Approximately 15% of patients with hyperparathyroidism also have hypercalcemia [65]. PTH increases blood calcium levels by transporting calcium from the bones into the blood, facilitating calcium reabsorption in the kidneys and its absorption in the digestive system [62]. However, it is not the only factor that allows an increase in blood calcium. The increase in vitamin D levels after transplantation also increases calcium absorption in the intestine, as well as the bone resorption that can occur after the procedure [5, 62]. It is important to emphasize that hypercalcaemia is not a cause of BMD loss but rather a consequence [62]. This alteration is reported in 5%–15% of the transplanted population after the intervention, according to the article published by Bouquegneau et al [9], and is more common at 3–6 months, especially in patients with higher blood PTH levels [9]. This change may resolve in some patients 6–8 months after transplantation, but in others, it may take years [64]. Hypercalcemia may play a role in triggering nephrolithiasis, rejection, and dysfunction of the transplanted kidney [65].

Another change associated with hyperparathyroidism is hypophosphatemia. Like calcium, PTH is involved in the regulation of phosphorus in the body [62]. In addition, there is another hormone in the body called fibroblast growth factor 23

(FGF-23), which has been identified as the main phosphorus-regulating factor in the body. This hormone is secreted by bone cells, and its production is partially stimulated by PTH. It has also been described that excess FGF-23 [66] is produced in bone mineralization disorders. These two hormones contribute to a decrease in the reabsorption of phosphorus in the kidney, which is why kidney transplant recipients have low levels of this metabolite [64]. This alteration is common during the first 3 months after transplantation, and according to the study by Bouquegneau et al [9], it occurs in 50% of transplant recipients and stabilizes after 6–12 months [9, 67]. This alteration is associated with a decrease in osteoblast activity, resulting in deficient bone mineralization [9].

Vitamin D metabolism is also affected after renal transplantation. Hypovitaminosis D is associated with immunosuppressive therapy, residual renal function after transplantation, malabsorption, poor diet or reduced exposure to sunlight [62, 64]. The vitamin D status of the body is tested by blood levels of calcidiol, a precursor of this vitamin; hypovitaminosis D is therefore understood to be a blood level of calcidiol of less than 30 ng/mL, with vitamin D insufficiency being between 15 and 30 ng/mL and vitamin D deficiency being less than 15 ng/mL [44, 68]. According to the article published by Evenepoel et al, [68] the prevalence of hypovitaminosis D in the third month after renal transplantation is 78%, and according to the articles by Bouquegneau et al [9] and Alshayeb et al, [64] vitamin D deficiency would be present in 30% of the operated patients. As renal function recovers, vitamin D levels begin to rise, although they remain lower than those of the general population (**Supplementary Table S4**) [8, 62]. Hypovitaminosis D may be associated with lower transplant tolerance, worsening infections, and an upset in BMD [5].

This BMD alteration is associated with both osteopenia and osteoporosis, as mentioned above, with various metabolic disturbances that occur after renal transplantation [4]. Although there are also several risk factors, such as advanced age, sex, and ethnicity of the patient [4, 6], the main underlying factor for BMD loss is treatment with glucocorticoids after transplantation. Glucocorticoids inhibit bone tissue formation and increase osteoclast activity by decreasing the formation and differentiation of osteoblasts [69]. The difference between osteopenia and osteoporosis is the amount of BMD lost, with osteoporosis considered a more severe pathology than osteopenia. These disorders occur most frequently in the first 6–12 months after transplantation, with the greatest loss of BMD in the first 6 months [70]. In our meta-regression analysis, we have also shown a negative correlation between the time of transplantation and the prevalence of osteoporosis and osteopenia [20, 70]. After 6 months, this loss of bone mineral slows down, probably due to the decrease in glucocorticoid use and the gradual correction of the various metabolic disturbances associated with these disorders. According to the study published by Ebeling et al, [71] the presence of osteoporosis can be found in 17%–49% of kidney transplant recipients in the lumbar area and in 11%–56% of patients transplanted in the femoral area [71]. In our meta-analysis, the prevalence of osteoporosis in the lumbar area was 13.8%. In contrast, in the femoral area, the prevalence was 19.2%.

The main consequence of BMD loss is an increased risk of fractures [2], although there are several factors, such as advanced age, loss of muscle mass and reduced physical activity, that may increase the risk of this adverse event [4]. Approximately 22.5% of patients suffer at least one fracture in the first 5 years after transplantation, which means an incidence 4 times higher than that for the general population but lower than that in patients who remain on dialysis (**Supplementary Table S5**) [9]. Fractures are associated with increased hospitalization and mortality in kidney transplant recipients, with hip, ankle, and foot fractures being the most common, suggesting a large economic impact [9, 72, 73]. In our study, the prevalence of fractures after transplant was 14.2%.

Another factor that could increase the risk of fractures in transplant recipients is the loss of muscle mass and muscle strength associated with sarcopenia, which is associated with an increased risk of falls, physical disability, lower quality of life and greater morbidity and mortality [73]. The main risk factors for sarcopenia in kidney transplant recipients are vitamin D deficiency, physical inactivity, prolonged hospitalization, nutritional deficiencies, hyperparathyroidism, and proteinuria [7, 74]. The prevalence of this condition, whose occurrence in kidney transplant recipients is estimated at a younger age in comparison with the general population [75], varies greatly because there are no universal diagnostic criteria. In addition, together with osteopenia and osteoporosis, the risk of fracture in these patients increases considerably [7].

On the other hand, gout, which is a type of arthritis that occurs after the deposition of uric acid crystals in the joints, causing attacks of pain and inflammation, is another disorder that could be associated with kidney transplantation [76]. The main cause for this disorder is hyperuricemia, a metabolic disturbance that is common after renal transplantation. According to the article by Gupta et al, [77] hyperuricemia could reach a prevalence of 10%–84%, while gout could be present in 2%–28% of transplant recipients. In our study, the prevalence of gout was specifically 15.4%, although the differences in the date of transplantation between the three included studies may limit the generalisability of this estimate.

In order to compare these results, we have found a series of studies [54–61] that provide prevalence data for the variables analysed in the general population (**Supplementary Tables S4, S5**; **Supplementary Figures S17–S26**). It should be noted that for the variables of hypophosphatemia and low muscle strength, we have not been able to find any study providing prevalences in the general population. As for the other variables, we can observe the large difference in prevalence estimates between the transplanted population and the general population for hypercalcemia [54], hyperparathyroidism [55], hyperuricemia [55], hypervitaminosis D [56], sarcopenia [57], low muscle mass [58], fractures [59] and gout [55], indicating an increase in the prevalence of these metabolic disturbances and musculoskeletal disorders after kidney transplantation. In the case of osteopenia and osteoporosis, the difference in prevalence between the two populations is small, although it is true that in the studies [60,

61] we have found in the general population, it is not divided into zones, as it is in the studies [13, 15, 19–21, 24, 25, 29, 35–37, 39, 43] in the transplanted population, which divide the prevalence into lumbar and femoral areas, so that no conclusions could be drawn when comparing the two populations with regard to these two variables analysed.

This study has several potential limitations, and its findings should be interpreted with some caution. First, they are inherent to the conduct of a systematic review and meta-analysis (selection bias and limited information reported by original studies). Second, the design of most studies was retrospective and cross-sectional, which does not allow establishing a cause-effect relationship. Additionally, the heterogeneity of the results was high, which may limit the extrapolation of data to different populations. Thirdly, the results should be interpreted with caution, given the pooling of studies from different years and geographical locations, with different circumstances and sample characteristics. In this sense, studies were conducted in five different decades, in which the surgical techniques, metabolic goals, and available medications may have been different. On the other hand, there was variability in the time at which the different outcomes were measured after transplantation. Finally, despite the physiological link between hyperparathyroidism and hypercalcemia and hypophosphatemia, our results did not show a clear association between their prevalence estimates. The main reasons for this finding could be the coexistence of some lifestyle-related covariates that were not included in the original analyses, and the small number of studies (only three) that analysed the prevalence of the three outcomes (low muscle strength, low muscle mass and gout), whose sample sizes were not very large. However, the aim of this study was to show the prevalence of different kidney transplant-related disorders.

CONCLUSION

In conclusion, this systematic review and meta-analysis shows a high prevalence regarding the presence of certain musculoskeletal disorders and their related metabolic disorders in kidney transplant recipients. Hypovitaminosis D, hyperparathyroidism and hyperuricemia were the most common metabolic disturbances. In parallel, low muscle strength, femoral osteopenia and low muscle mass were the main musculoskeletal disorders. At a clinical level, knowledge of these data will allow us to improve the prevention, diagnosis, and treatment of these complications, increase patient wellbeing, reduce the recovery time after surgery and avoid increased hospitalizations, morbidity, and mortality in kidney transplant patients, although further research is needed using experimental designs to test the effectiveness of different therapeutic prevention strategies in this specific population.

AUTHOR CONTRIBUTIONS

AH-C had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. AH-C, MG-M, and CB-M designed the study; AH-C, MG-M, CB-M, and VM-V performed the data analysis, and AT-C, CP-M, LH-C, and IS-D interpreted the findings. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.12312/full#supplementary-material

REFERENCES

1. Valdivia AJ, Gutiérrez GC, Méndez FD, Delgado AE, Treto RJ, Fernández MI. Supervivencia en pacientes con trasplante renal. Factores pronósticos. *Invest Medicoquir* (2013) 5(2):253–75.
2. Mainra R, Elder G. Review Article: Managing Bone Complications After Kidney Transplantation. *Nephrology (Carlton)* (2009) 14(4):437–42. doi:10.1111/j.1440-1797.2009.01156.x
3. WHO. *Global Observatory on Donation and Transplantation*. Geneva: WHO-ONT (2007). Available from: http://www.transplant-observatory.org/export-database/ (Accessed April 12, 2022).
4. Bia M. Evaluation and Management of Bone Disease and Fractures Post Transplant. *Transpl Rev (Orlando)* (2008) 22(1):52–61. doi:10.1016/j.trre.2007.09.001
5. Khairallah P, Nickolas TL. Bone and Mineral Disease in Kidney Transplant Recipients. *Clin J Am Soc Nephrol* (2022) 17(1):121–30. doi:10.2215/CJN.03410321
6. Hassan AB, Ghalib KW, Jahrami HA, El-Agroudy AE. Prevalence of Musculoskeletal Manifestations in Adult Kidney Transplant's Recipients: A Systematic Review. *Medicina (Kaunas)* (2021) 57(6):525. doi:10.3390/medicina57060525
7. Yanishi M, Kinoshita H, Tsukaguchi H, Kimura Y, Koito Y, Sugi M, et al. Factors Related to Osteosarcopenia in Kidney Transplant Recipients. *Transpl Proc* (2018) 50(10):3371–5. doi:10.1016/j.transproceed.2018.04.032

8. Molnar MZ, Naser MS, Rhee CM, Kalantar-Zadeh K, Bunnapradist S. Bone and Mineral Disorders After Kidney Transplantation: Therapeutic Strategies. *Transpl Rev (Orlando)* (2014) 28(2):56–62. doi:10.1016/j.trre.2013.12.003
9. Bouquegneau A, Salam S, Delanaye P, Eastell R, Khwaja A. Bone Disease After Kidney Transplantation. *Clin J Am Soc Nephrol* (2016) 11(7):1282–96. doi:10.2215/CJN.11371015
10. 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. *Syst Rev* (2021) 10(1):89. doi:10.1186/s13643-021-01626-4
11. Alagoz S, Trabulus S. Long-Term Evaluation of Mineral Metabolism After Kidney Transplantation. *Transpl Proc* (2019) 51(7):2330–3. doi:10.1016/j.transproceed.2019.01.181
12. Amin T, Coates PT, Barbara J, Hakendorf P, Karim N. Prevalence of Hypercalcaemia in a Renal Transplant Population: A Single Centre Study. *Int J Nephrol* (2016) 2016:7126290. doi:10.1155/2016/7126290
13. Batteux B, Bodeau S, André C, Hurtel-Lemaire AS, Gras-Champel V, Desailly-Henry I, et al. Association Between Uremic Toxin Concentrations and Bone Mineral Density After Kidney Transplantation. *Toxins (Basel)* (2020) 12(11):715. doi:10.3390/toxins12110715
14. Berga JK, Crespo Albiach J, Beltran Catalan S, Gavela Martinez E, Sancho Calabuig A, Avila Bernabeu A, et al. Vitamin D Deficiency in a Renal Transplant Population: Safe Repletion With Moderate Doses of Calcidiol. *Transpl Proc* (2010) 42(8):2917–20. doi:10.1016/j.transproceed.2010.08.015
15. Júnior JWRB, Neves RM, Pinheiro MM, Frisoli Júnior A, Castro CH, Szejnfeld VL, et al. Prevalence of Low Trauma Fractures in Long-Term Kidney Transplant Patients With Preserved Renal Function. *Braz J Med Biol Res* (2006) 39(1):137–47. doi:10.1590/s0100-879x2006000100016
16. Chan W, Chin SH, Whittaker AC, Jones D, Kaur O, Bosch JA, et al. The Associations of Muscle Strength, Muscle Mass, and Adiposity With Clinical Outcomes and Quality of Life in Prevalent Kidney Transplant Recipients. *J Ren Nutr* (2019) 29(6):536–47. doi:10.1053/j.jrn.2019.06.009
17. Conley E, Muth B, Samaniego M, Lotfi M, Voss B, Armbrust M, et al. Bisphosphonates and Bone Fractures in Long-Term Kidney Transplant Recipients. *Transplantation* (2008) 86(2):231–7. doi:10.1097/TP.0b013e318176b40f
18. Einollahi B, Einollahi H, Nafar M, Rostami Z. Prevalence and Risk Factors of Hyperuricemia Among Kidney Transplant Recipients. *Indian J Nephrol* (2013) 23(3):201–5. doi:10.4103/0971-4065.111849
19. Evenepoel P, Claes K, Meijers B, Laurent MR, Bammens B, Naesens M, et al. Bone mineral Density, Bone Turnover Markers, and Incident Fractures in De Novo Kidney Transplant Recipients. *Kidney Int* (2019) 95(6):1461–70. doi:10.1016/j.kint.2018.12.024
20. Fernández Castillo R, Fernández Gallegos R, Peña Amaro MP, Esteban de la Rosa RJ. Valoración del perfil lipídico y de la densidad mineral ósea en pacientes trasplantados renales. *Nutr Hosp* (2015) 31(6):2503–10. doi:10.3305/nh.2015.31.6.8719
21. Gregorini M, Sileno G, Pattonieri EF, Corradetti V, Abelli M, Ticozzelli E, et al. Understanding Bone Damage After Kidney Transplantation: A Retrospective Monocentric Cross Sectional Analysis. *Transpl Proc* (2017) 49(4):650–7. doi:10.1016/j.transproceed.2017.02.023
22. Hamidian Jahromi A, Roozbeh J, Raiss-Jalali GA, Dabaghmanesh A, Jalaeian H, Bahador A, et al. Risk Factors of Post Renal Transplant Hyperparathyroidism. *Saudi J Kidney Dis Transpl* (2009) 20(4):573–6.
23. Jerman A, Lindič J, Škoberne A, Borštnar Š, Martinuč Bergoč M, Godnov U, et al. Prevalence and Risk Factors for Nonvertebral Bone Fractures in Kidney Transplant Recipients - A Single-Center Retrospective Analysis. *Clin Nephrol* (2017) 88(13):101–8. doi:10.5414/CNP88FX23
24. Jørgensen HS, Eide IA, Hartmann A, Åsberg A, Christensen JH, Schmidt EB, et al. Plasma N-3 Polyunsaturated Fatty Acids and Bone Mineral Density in Renal Transplant Recipients. *J Ren Nutr* (2016) 26(3):196–203. doi:10.1053/j.jrn.2015.11.007
25. Khosravi M, Soltanian N, Monfared A, Ghanbari A, Ramezanzade E, Kazemnezhad Leyli E. Bone Mineral Density and Related Factors in Renal Transplant Recipients, in the North of Iran. *Iran J Kidney Dis* (2020) 14(5):405–11.
26. Kim KM, Kim SS, Han DJ, Yang WS, Park JS, Park SK. Hyperuricemia in Kidney Transplant Recipients With Intact Graft Function. *Transpl Proc* (2010) 42(9):3562–7. doi:10.1016/j.transproceed.2010.07.104
27. Kosoku A, Uchida J, Nishide S, Kabei K, Shimada H, Iwai T, et al. Association of Sarcopenia With Phase Angle and Body Mass index in Kidney Transplant Recipients. *Sci Rep* (2020) 10(1):266. doi:10.1038/s41598-019-57195-z
28. Limirio LS, Santos HO, Dos Reis AS, de Oliveira EP. (Dis) Agreement Between the First and the Recent European Consensus on Definition and Diagnosis for Sarcopenia in Kidney Transplant Patients. *Eur J Clin Nutr* (2020) 74(7):1104–8. doi:10.1038/s41430-019-0535-5
29. López Ruiz MC, Ortega Martínez AR, Fernández Castillo R, Esteban de la Rosa RJ, Bravo Soto JA. Osteoporosis e índice de masa corporal en el trasplantado renal. *Nutr Hosp* (2015) 32(2):872–7. doi:10.3305/nh.2015.32.2.9166
30. Malheiro J, Almeida M, Fonseca I, Martins LS, Pedroso S, Dias L, et al. Hyperuricemia in Adult Renal Allograft Recipients: Prevalence and Predictors. *Transpl Proc* (2012) 44(8):2369–72. doi:10.1016/j.transproceed.2012.07.033
31. Marcén R, Ponte B, Rodríguez-Mendiola N, Fernández-Rodriguez A, Galeano C, Villafruela JJ, et al. Vitamin D Deficiency in Kidney Transplant Recipients: Risk Factors and Effects of Vitamin D3 Supplements. *Transpl Proc* (2009) 41(6):2388–90. doi:10.1016/j.transproceed.2009.06.050
32. Menna BAPM, Barreto Silva MI, Pontes KSDS, Costa MSD, Rosina KTC, Souza E, et al. Sarcopenia and Its Components in Adult Renal Transplant Recipients: Prevalence and Association With Body Adiposity. *Br J Nutr* (2019) 122(12):1386–97. doi:10.1017/S0007114519002459
33. Muirhead N, Zaltman JS, Gill JS, Churchill DN, Poulin-Costello M, Mann V, et al. Hypercalcemia in Renal Transplant Patients: Prevalence and Management in Canadian Transplant Practice. *Clin Transpl* (2014) 28(2):161–5. doi:10.1111/ctr.12291
34. Ozkayar N, Altun B, Halil M, Kuyumcu ME, Arik G, Yesil Y, et al. Evaluation of Sarcopenia in Renal Transplant Recipients. *Nephrourol Mon* (2014) 6(4):e20055. doi:10.5812/numonthly.20055
35. Park WY, Han S, Choi BS, Park CW, Yang CW, Kim YS, et al. Progression of Osteoporosis After Kidney Transplantation in Patients With End-Stage Renal Disease. *Transpl Proc* (2017) 49(5):1033–7. doi:10.1016/j.transproceed.2017.03.038
36. Patel S, Kwan JT, McCloskey E, McGee G, Thomas G, Johnson D, et al. Prevalence and Causes of Low Bone Density and Fractures in Kidney Transplant Patients. *J Bone Miner Res* (2001) 16(10):1863–70. doi:10.1359/jbmr.2001.16.10.1863
37. Savaj S, Ghods FJ. Vitamin D, Parathyroid Hormone, and Bone Mineral Density Status in Kidney Transplant Recipients. *Iran J Kidney Dis* (2012) 6(4):295–9.
38. Schreiber PW, Kusejko K, Bischoff-Ferrari HA, Boggian K, Bonani M, van Delden C, et al. Vitamin D Deficiency Is Common in Kidney Transplant Recipients, But Is Not Associated With Infections After Transplantation. *Clin Transpl* (2020) 34(2):e13778. doi:10.1111/ctr.13778
39. Segaud N, Legroux I, Hazzan M, Noel C, Cortet B. Changes in Bone Mineral Density After Kidney Transplantation: 2-Year Assessment of a French Cohort. *Osteoporos Int* (2018) 29(5):1165–75. doi:10.1007/s00198-018-4383-2
40. Simbolon FR, Lee SS, Tsai YC, Tsai WC, Lin GT, Tung YC, et al. Risk of Incident Gout in Kidney Transplant Recipients: A Retrospective Cohort Study. *Int J Rheum Dis* (2018) 21(11):1993–2001. doi:10.1111/1756-185X.13393
41. Stamp L, Ha L, Searle M, O'Donnell J, Frampton C, Chapman P. Gout in Renal Transplant Recipients. *Nephrology (Carlton)* (2006) 11(4):367–71. doi:10.1111/j.1440-1797.2006.00577.x
42. Torres A, Torregrosa V, Marcen R, Campistol JM, Arias M, Hernández D, et al. Mineral Metabolism Disorders, Vertebral Fractures and Aortic Calcifications in Stable Kidney Transplant Recipients: The Role of Gender (EMITRAL Study). *Nefrologia* (2016) 36(3):255–67. doi:10.1016/j.nefro.2016.03.004
43. Velioglu A, Kaya B, Aykent B, Ozkan B, Karapinar MS, Arikan H, et al. Low Bone Density, Vertebral Fracture and FRAX Score in Kidney Transplant Recipients: A Cross-Sectional Cohort Study. *PLoS One* (2021) 16(4):e0251035. doi:10.1371/journal.pone.0251035
44. Vilarta CF, Unger MD, Dos Reis LM, Dominguez WV, David-Neto E, Moysés RM, et al. Hypovitaminosis D in Patients Undergoing Kidney Transplant: The Importance of Sunlight Exposure. *Clinics (Sao Paulo)* (2017) 72(7):415–21. doi:10.6061/clinics/2017(07)05
45. Wang C, Huo Y, Li X, Lin A, Hu Q, Xiong C, et al. Factors Related to Bone Metabolism in Kidney Transplant Recipients. *Mediators Inflamm* (2021) 2021:6679095. doi:10.1155/2021/6679095

46. Weng SC, Shu KH, Tarng DC, Cheng CH, Chen CH, Yu TM, et al. Uric Acid Is Highly Associated With Kidney Allograft Survival in a Time-Varying Analysis. *Transpl Proc* (2014) 46(2):505–10. doi:10.1016/j.transproceed.2013.09.038
47. Wolf M, Weir MR, Kopyt N, Mannon RB, Von Visger J, Deng H, et al. A Prospective Cohort Study of Mineral Metabolism After Kidney Transplantation. *Transplantation* (2016) 100(1):184–93. doi:10.1097/TP.0000000000000823
48. Zhang K, Gao B, Wang Y, Wang G, Wang W, Zhu Y, et al. Serum Uric Acid and Renal Transplantation Outcomes: At Least 3-Year Post-Transplant Retrospective Multivariate Analysis. *PLoS One* (2015) 10(7):e0133834. doi:10.1371/journal.pone.0133834
49. Munn Z, Moola S, Lisy K, Riitano D, Tufanaru C. Methodological Guidance for Systematic Reviews of Observational Epidemiological Studies Reporting Prevalence and Cumulative Incidence Data. *Int J Evid Based Healthc* (2015) 13(3):147–53. doi:10.1097/XEB.0000000000000054
50. Moola S, Munn Z, Tufanaru C, Aromataris E, Sears K, Sfetcu R, et al. Chapter 7: Systematic Reviews of Etiology and Risk. In: Aromataris E, Munn Z, editors. *JBI Manual for Evidence Synthesis*. Australia: JBI (2020). Available from: https://synthesismanual.jbi.global (Accessed May 20, 2022).
51. Mantel N, Haenszel W. Statistical Aspects of the Analysis of Data From Retrospective Studies of Disease. *J Natl Cancer Inst* (1959) 22:719–48.
52. DerSimonian R, Kacker R. Random-Effects Model for Meta-Analysis of Clinical Trials: An Update. *Contemp Clin Trials* (2007) 28:105–14. doi:10.1016/j.cct.2006.04.004
53. Higgins J, Thompson SG. Quantifying Heterogeneity in a Meta-Analysis. *Stat Med* (2002) 21:1539–58. doi:10.1002/sim.1186
54. Lindner G, Felber R, Schwarz C, Marti G, Leichtle AB, Fiedler GM, et al. Hypercalcemia in the ED: Prevalence, Etiology, and Outcome. *Am J Emerg Med* (2013) 31(4):657–60. doi:10.1016/j.ajem.2012.11.010
55. Soto-Pedre E, Newey PJ, Leese GP. Stable Incidence and Increasing Prevalence of Primary Hyperparathyroidism in a Population-Based Study in Scotland. *J Clin Endocrinol Metab* (2023) 108(10):e1117–e1124. doi:10.1210/clinem/dgad201
56. Cui A, Zhang T, Xiao P, Fan Z, Wang H, Zhuang Y. Global and Regional Prevalence of Vitamin D Deficiency in Population-Based Studies From 2000 to 2022: A Pooled Analysis of 7.9 Million Participants. *Front Nutr* (2023) 10:1070808. doi:10.3389/fnut.2023.1070808
57. Sepúlveda-Loyola W, Osadnik C, Phu S, Morita AA, Duque G, Probst VS. Diagnosis, Prevalence, and Clinical Impact of Sarcopenia in COPD: A Systematic Review and Meta-Analysis. *J Cachexia Sarcopenia Muscle* (2020) 11(5):1164–76. doi:10.1002/jcsm.12600
58. Petermann-Rocha F, Balntzi V, Gray SR, Lara J, Ho FK, Pell JP, et al. Global Prevalence of Sarcopenia and Severe Sarcopenia: A Systematic Review and Meta-Analysis. *J Cachexia Sarcopenia Muscle* (2022) 13(1):86–99. doi:10.1002/jcsm.12783
59. Court-Brown CM, Caesar B. Epidemiology of Adult Fractures: A Review. *Injury* (2006) 37(8):691–7. doi:10.1016/j.injury.2006.04.130
60. Xiao PL, Cui AY, Hsu CJ, Peng R, Jiang N, Xu XH, et al. Global, Regional Prevalence, and Risk Factors of Osteoporosis According to the World Health Organization Diagnostic Criteria: A Systematic Review and Meta-Analysis. *Osteoporos Int* (2022) 33(10):2137–53. doi:10.1007/s00198-022-06454-3
61. Salari N, Ghasemi H, Mohammadi L, Behzadi MH, Rabieenia E, Shohaimi S, et al. The Global Prevalence of Osteoporosis in the World: A Comprehensive Systematic Review and Meta-Analysis. *J Orthop Surg Res* (2021) 16:609. doi:10.1186/s13018-021-02772-0
62. Kalantar-Zadeh K, Molnar MZ, Kovesdy CP, Mucsi I, Bunnapradist S. Management of Mineral and Bone Disorder After Kidney Transplantation. *Curr Opin Nephrol Hypertens* (2012) 21(4):389–403. doi:10.1097/MNH.0b013e3283546ee0
63. Neves CL, dos Reis LM, Batista DG, Custodio MR, Graciolli FG, Martin RC, et al. Persistence of Bone and Mineral Disorders 2 Years After Successful Kidney Transplantation. *Transplantation* (2013) 96(3):290–6. doi:10.1097/TP.0b013e3182985468
64. Alshayeb HM, Josephson MA, Sprague SM. CKD-Mineral and Bone Disorder Management in Kidney Transplant Recipients. *Am J Kidney Dis* (2013) 61(2):310–25. doi:10.1053/j.ajkd.2012.07.022
65. Sakhaee K. Osteoporosis Following Organ Transplantation: Pathogenesis, Diagnosis and Management. *Expert Rev Endocrinol Metab* (2011) 6(2):157–76. doi:10.1586/eem.10.86
66. Sánchez-González MC, Salanova L, Ruano P. FGF-23: Solo regulador del metabolismo del fósforo o algo más? *Reumatol Clin* (2011) 7(2):5–7. doi:10.1016/j.reuma.2011.05.009
67. Hirukawa T, Kakuta T, Nakamura M, Fukagawa M. Mineral and Bone Disorders in Kidney Transplant Recipients: Reversible, Irreversible, and De Novo Abnormalities. *Clin Exp Nephrol* (2015) 19(4):543–55. doi:10.1007/s10157-015-1117-z
68. Evenepoel P, Naesens M, Claes K, Kuypers D, Vanrenterghem Y. Tertiary 'Hyperphosphatoninism' Accentuates Hypophosphatemia and Suppresses Calcitriol Levels in Renal Transplant Recipients. *Am J Transpl* (2007) 7(5):1193–200. doi:10.1111/j.1600-6143.2007.01753.x
69. Silkensen JR. Long-Term Complications in Renal Transplantation. *J Am Soc Nephrol* (2000) 11(3):582–8. doi:10.1681/ASN.V113582
70. Pérez-Sáez MJ, Prieto-Alhambrab D, Díez-Pérez A, Pascua J. Advances in the Evaluation of Bone Health in Kidney Transplant Patients. *Nefro (Madrid)* (2018) 38(1):27–33. doi:10.1016/j.nefro.2017.04.002
71. Ebeling PR. Transplantation Osteoporosis. *Curr Osteoporos Rep* (2007) 5(1):29–37. doi:10.1007/BF02938620
72. Naylor KL, Li AH, Lam NN, Hodsman AB, Jamal SA, Garg AX. Fracture Risk in Kidney Transplant Recipients: A Systematic Review. *Transplantation* (2013) 95(12):1461–70. doi:10.1097/TP.0b013e31828eead8
73. Williams SA, Daigle SG, Weiss R, Wang Y, Arora T, Curtis JR. Economic Burden of Osteoporosis-Related Fractures in the US Medicare Population. *Ann Pharmacother* (2021) 55(7):821–9. doi:10.1177/1060028020970518
74. Gandolfini I, Regolisti G, Bazzocchi A, Maggiore U, Palmisano A, Piotti G, et al. Frailty and Sarcopenia in Older Patients Receiving Kidney Transplantation. *Front Nutr* (2019) 6:169. doi:10.3389/fnut.2019.00169
75. Martins CA, França AKTC, Dias RSC, Costa RCO, Lemos APL, Santos AMD, et al. Prevalence of Sarcopenia in Kidney Transplants and Their Association With Determinant Factors of Muscle Homeostasis. *Rev Assoc Med Bras* (1992) 2020) 66(9):1235–40. doi:10.1590/1806-9282.66.9.1235
76. Pascual Gómez E, Sivera Mascaró F. Hyperuricemia Y Gout. *IT Del SNS* (2009) 33(4):110–5.
77. Gupta G, Unruh ML, Nolin TD, Hasley PB. Primary Care of the Renal Transplant Patient. *J Gen Intern Med* (2010) 25(7):731–40. doi:10.1007/s11606-010-1354-5

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ORIGINAL RESEARCH
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Disulfiram, an Anti-alcoholic Drug, Targets Macrophages and Attenuates Acute Rejection in Rat Lung Allografts

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Macrophages contribute to post-transplant lung rejection. Disulfiram (DSF), an anti-alcoholic drug, has an anti-inflammatory effect and regulates macrophage chemotactic activity. Here, we investigated DSF efficacy in suppressing acute rejection post-lung transplantation. Male Lewis rats (280–300 g) received orthotopic left lung transplants from Fisher 344 rats (minor histocompatibility antigen-mismatched transplantation). DSF (0.75 mg/h) monotherapy or co-solvent only (50% hydroxypropyl-β-cyclodextrin) as control was subcutaneously administered for 7 days (n = 10/group). No post-transplant immunosuppressant was administered. Grades of acute rejection, infiltration of immune cells positive for CD68, CD3, or CD79a, and gene expression of monocyte chemoattractant protein and pro-inflammatory cytokines in the grafts were assessed 7 days post-transplantation. The DSF-treated group had significantly milder lymphocytic bronchiolitis than the control group. The infiltration levels of $CD68^+$ or $CD3^+$ cells to the peribronchial area were significantly lower in the DSF than in the control groups. The normalized expression of chemokine ligand 2 and interleukin-6 mRNA in allografts was lower in the DSF than in the control groups. Validation assay revealed interleukin-6 expression to be significantly lower in the DSF than in the control groups. DSF can alleviate acute rejection post-lung transplantation by reducing macrophage accumulation around peripheral bronchi and suppressing pro-inflammatory cytokine expression.

Keywords: lung transplantation, acute lung rejection, macrophage, disulfiram, rodent study

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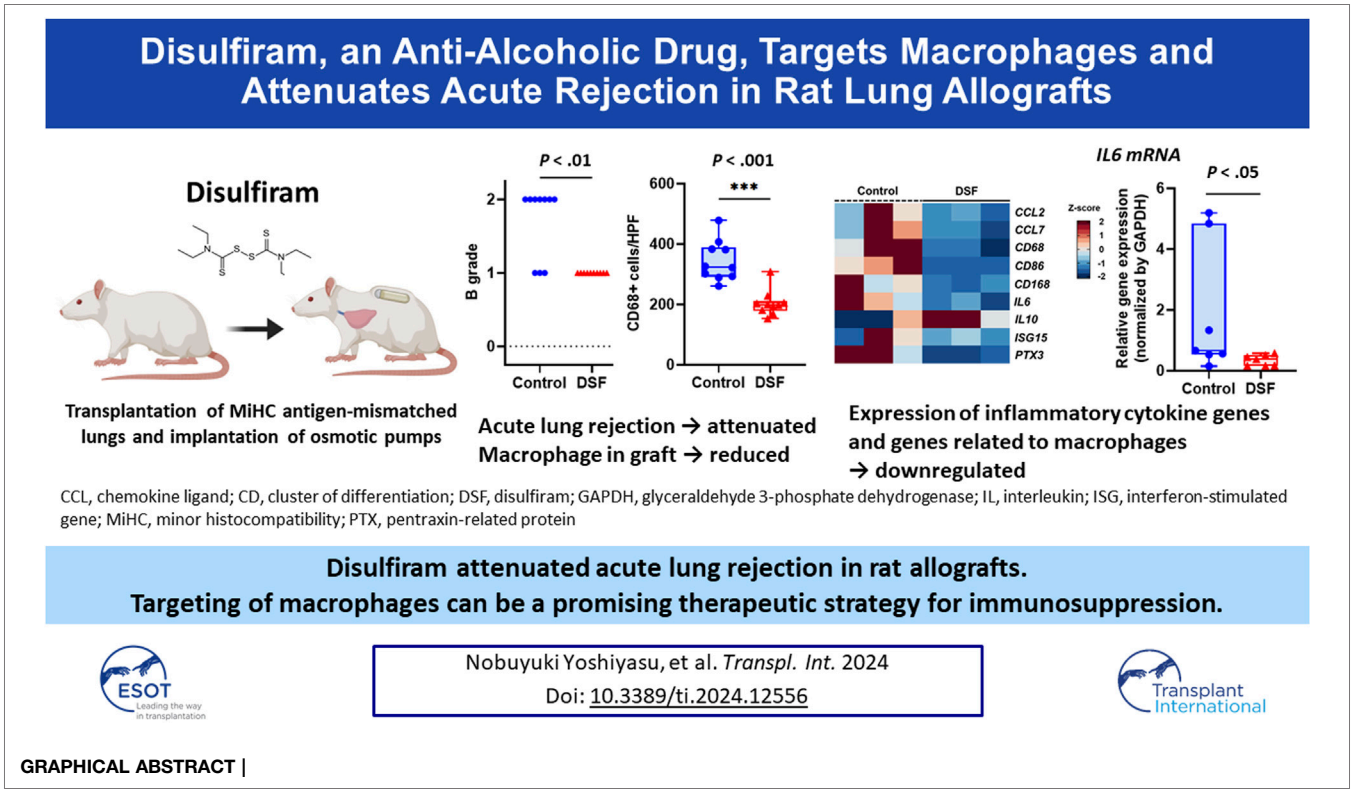
Abbreviations: CD, cluster of differentiation; COVID-19, coronavirus disease 2019; HIV, human immunodeficiency virus; ISG, interferon-stimulated gene; PTX, pentraxin-related protein; RLE, relative log expression; BALF, bronchoalveolar lavage fluid; CCL, chemokine ligand; CLAD, chronic lung allograft dysfunction; DEG, differentially expressed gene; DSF, disulfiram; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HBC, hydroxypropyl-β-cyclodextrin; IL, interleukin; IFN-γ, interferon-gamma; MiHC, minor histocompatibility; NF, nuclear factor; TCC, total cell count; TNF-α, tumor necrosis factor α.



GRAPHICAL ABSTRACT |

INTRODUCTION

Lung transplantation is an established therapy for end-stage lung disease. However, lung rejection remains the most challenging complication after transplantation. Acute lung rejection can be a risk factor for developing chronic lung allograft dysfunction (CLAD) even after a single episode [1, 2]. Thus, preventing acute rejection may improve long-term survival by preventing the development of CLAD. Despite using common maintenance immunosuppressive drugs, such as calcineurin inhibitors, anti-metabolites, and steroids, over 25% of lung transplant recipients experience acute rejection at least once within a year after transplantation [1, 3]. In recent years, a few research groups have proposed that not only T cells, but also macrophages are involved in the development of acute lung rejection [4–6]. Cell profiles during acute rejection obtained using single-cell RNA sequencing (RNA-Seq) of human samples provide evidence of macrophage involvement [7].

Disulfiram (DSF), a well-known anti-alcoholic drug [8], also shows other pharmacological effects, such as anti-inflammatory and anti-cancer effects [9–12]. Furthermore, DSF inhibits the activity of the cytoplasmic protein FROUNT, which regulates the chemotactic signals of macrophages [12, 13]. Owing to the broad therapeutic potential of DSF, repositioning of DSF has garnered interest recently. Drug repositioning is the process of discovering new indications for approved or failed drugs [14]. Clinical trials on the use of DSF for various diseases, such as coronavirus disease 2019, human immunodeficiency virus infection, and treatment-refractory multiple myeloma have been conducted or are ongoing [15]. However, there are no reports on the therapeutic efficacy of DSF in acute post-transplant rejection. We hypothesized that DSF could attenuate acute lung rejection by suppressing the chemotaxis of macrophages to allografts after lung transplantation. Therefore, in this study, we aimed to investigate the efficacy of DSF in a rat model of acute rejection after orthotopic lung transplantation.

MATERIALS AND METHODS

Animal Models

This study was approved by the Experimental Animal Ethics Committee of the University of Tokyo under license number H20-204 (issued January 19, 2021). All procedures complied with the Institutional Animal Care and Use Committee Guidelines of the University of Tokyo. Specific-pathogen-free inbred male rats were purchased from Japan SLC, Inc. (Hamamatsu, Japan). All rats (age: 12–13 weeks; weight: 280–300 g) received adequate care according to the animal study protocols. The animal experiments were conducted using Lewis (LEW; $RT1^{l}$) and Fischer 344 (F344; $RT1^{lv1}$) rats in accordance with the guidelines. Allogenic orthotopic left lung transplantation was performed using the modified cuff technique as reported previously [16]. F344 rats were used as donors, whereas LEW rats were used as recipients in the minor histocompatibility (MiHC) antigen-mismatched transplantation procedure.

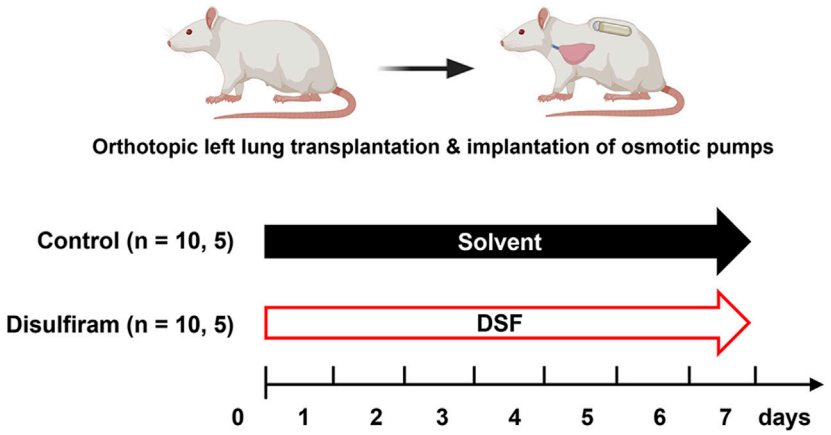


FIGURE 1 | Treatment intervention protocols with osmotic pumps for 7 days. Minor histocompatibility complex-mismatched left lung transplantation was performed from Fisher 344 rats to Lewis rats. The control group was administered 50% hydroxypropyl-β-cyclodextrin as a solvent and disulfiram was administered to the treatment group. Samples such as lung tissues (n = 10/group) and bronchoalveolar lavage fluid (n = 5/group) were collected on post-operative day 7. DSF, disulfiram.

Preparation of DSF Solution

DSF (Mitsubishi Tanabe Pharma, Osaka, Japan) was dissolved in 50% hydroxypropyl-β-cyclodextrin (HBC) (Tokyo Chemical Industry, Tokyo, Japan) with agitation to a final concentration of 37.5 mg/mL, and it was stored at 4 °C under a light shield. ALZET osmotic pumps (model 2ML1; DURECT, Cupertino, CA, United States), which deliver solutions continuously at a rate of 10 μL/h for 7 days, were filled with 2 mL of the DSF solution or 50% HBC per piece just before implantation. The pumps were unlabeled; hence, the operator was blinded to the content of each pump.

Treatment Protocols

The treatment protocols for the recipients are summarized in **Figure 1**. Prior to making the skin incision, methylprednisolone sodium (10 mg per animal unit; SHIONOGI, Osaka, Japan) and cefazoline sodium (10 mg per animal unit; Nipro Medical, Osaka, Japan) were injected subcutaneously or peritoneally into the recipients to prevent reperfusion injury and infection, respectively. These injections were administered under general anesthesia. It is important to note that the recipients did not receive any post-transplant immunosuppressive drugs. After reperfusion, two osmotic pumps, primed with 50% HBC (control group, n = 10) or DSF solution (DSF group, n = 10), were subsequently embedded under the skin of each recipient. The recipients were euthanized on day 7 post-transplantation. The DSF group rats were administered 18 mg DSF/day until sacrifice, equivalent to approximately 600 mg/day in humans. All rats had *ad libitum* access to water throughout the study. Recipient feeding was fixed at 200 g for 7 days and body weight was measured daily.

Histopathological Evaluation and Immunohistochemical Staining

The cranial sections (approximately two-thirds) of the allograft were fixed in 10% formalin (FUJIFILM Wako Pure Chemical, Osaka, Japan) and embedded in paraffin. The sections were stained with hematoxylin–eosin. According to the criteria of the International Society for Heart and Lung Transplantation for acute lung rejection, expert pathologists (M.S. and A.U.) graded sections A (subtypes: 0–4, X) when they observed infiltration of perivascular mononuclear cells or B (subtypes: 0–2R, X) when they observed lymphocytic bronchiolitis, in a double-blinded fashion (**Figure 2**) [17]. The extent of perivascular inflammation, referred to as A-grade, is determined by examining the infiltration of mononuclear cells around vascular structures, within the interstitial spaces of the submucosa, and along the alveolar partitions. This is systematically categorized into various levels: A0 (none), A1 (minimal), A2 (mild), A3 (moderate), A4 (severe), and AX (ungradable). Additionally, the evaluation of airway inflammation, designated as B-grade rejection, focuses on the lymphocytic activity within the bronchiole submucosa. The extent of this response is classified into the following distinct categories: B0 (none), B1R (low grade), B2R (high grade), and BX (ungradable). Particularly, when lymphocyte infiltration beyond the basement membrane was observed, the more advanced stage B2R was graded. For immunohistochemistry (IHC), sections were deparaffinized and incubated with 0.1% pepsin for 40 min at 37 °C for CD3 and CD68 staining, and with 0.01 M citrate buffer at a pH of 6.0 for 20 min at 120 °C for CD68 staining. This was followed by overnight incubation with the following primary antibodies: anti-CD3 (rabbit polyclonal; 1:300; DAKO, Tokyo, Japan), anti-CD79a (mouse monoclonal; 1:100; Biocare Medical, Pacheco, CA, United States), and anti-CD68 (mouse monoclonal; 1:1000; BMA Biomedicals, Augst, Switzerland). Histofine Simple Stain Rat MAX PO (MULTI; Nichirei Bioscience, Tokyo, Japan) was used as the secondary antibody, and 3,3′-diaminobenzidine (DOJINDO, Kumamoto, Japan) was used for detection. The primary antibodies were omitted to serve as negative controls for each CD staining, and assessments were conducted to detect false positives. The sections were counterstained with hematoxylin. In the IHC evaluation, six high-power field images (magnification ×400) were randomly chosen from each section

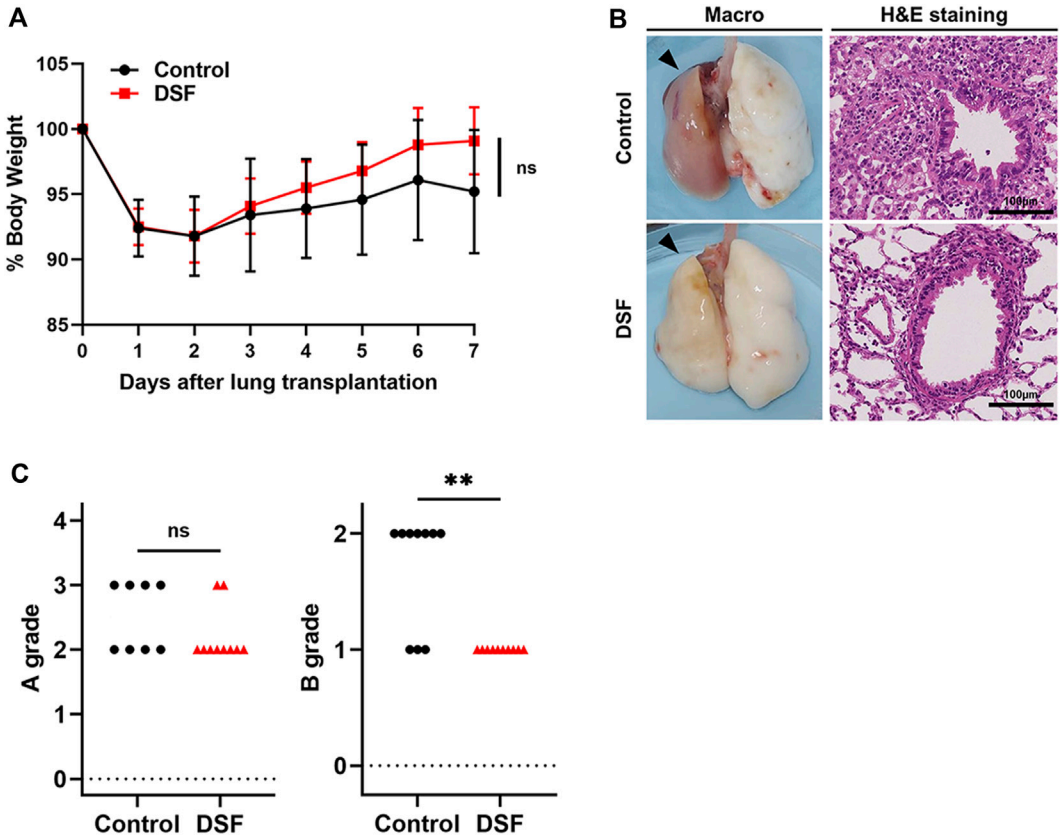


FIGURE 2 | Physical and histopathological effects of disulfiram. **(A)** % Body weight. Daily body weight was measured, and it is indicated as a percent of that right after surgery. **(B)** Macroscopic (black arrows denote allografts) and microscopic images (H&E staining; high-power field, magnification ×400). Scale bar: 100 μm. **(C)** A/B grading of acute lung rejection. The scores were settled according to the criteria of the International Society for Heart and Lung Transplantation. Two cases in the control group were excluded because of AX. ns, not significant. $^{**}p < 0.01$. DSF, disulfiram; H&E, hematoxylin and eosin.

and the positive cell counts per field were automatically determined using a hybrid cell count application (BZ-H4C; KEYENCE, Osaka, Japan) in BZ-X Analyzer software (BZ-H4A; KEYENCE). We separately conducted our analyses of the perivascular/peribronchiolar area when grading the extent of rejection or the alveolar area without vascular and bronchial structures (**Figure 3**).

Transcriptome Analysis via RNA-Seq

We selected representative rejection cases based on histopathological findings (n = 3/group) for RNA-Seq. Total RNA was extracted from the frozen samples, that is, the caudal one-third of the allografts, using ISOSPIN Cell and Tissue RNA (Nippon Gene, Tokyo, Japan). RNA quality was checked using Agilent 4150 TapeStation (Agilent Technologies, Santa Clara, CA, United States). A strand-specific RNA library was prepared using 1 μg of each sample with the NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB, Ipswich, MA, United States) and NEBNext Ultra II Directional RNA Library Prep Kit (NEB). RNA sequences were obtained using paired-end reads (150 bp × 2) on the NovaSeq 6000 platform (Illumina, San Diego, CA, United States). Differentially expressed genes (DEGs) between the control and DSF groups were identified with the cut-off criteria $|\log_2$ fold change$| > 1$ and Q-value <0.05 using DESeq2 software[1]. Raw read counts were normalized using the relative log expression method (**Figure 4**). Heat maps with z-scores of the normalized gene expression were created using all genes that matched the criteria[2]. Ward's clustering method and correlation distances were also used to generate hierarchical clusters of genes from the generated heat maps. To further examine the potential biological roles of the DEGs affected by DSF, we conducted a Gene Ontology (GO) term enrichment analysis using the DAVID WebService package[3].

Validation Using Real-Time Quantitative Polymerase Chain Reaction

The remaining samples (n = 7/group) were used to validate the transcription levels obtained via RNA-seq using real-time

[1]https://bioconductor.org/packages/release/bioc/html/DESeq2.html
[2]https://www.rdocumentation.org/packages/stats/versions/3.6.2
[3]https://www.rdocumentation.org/packages/RDAVIDWebService/versions/1.10.0

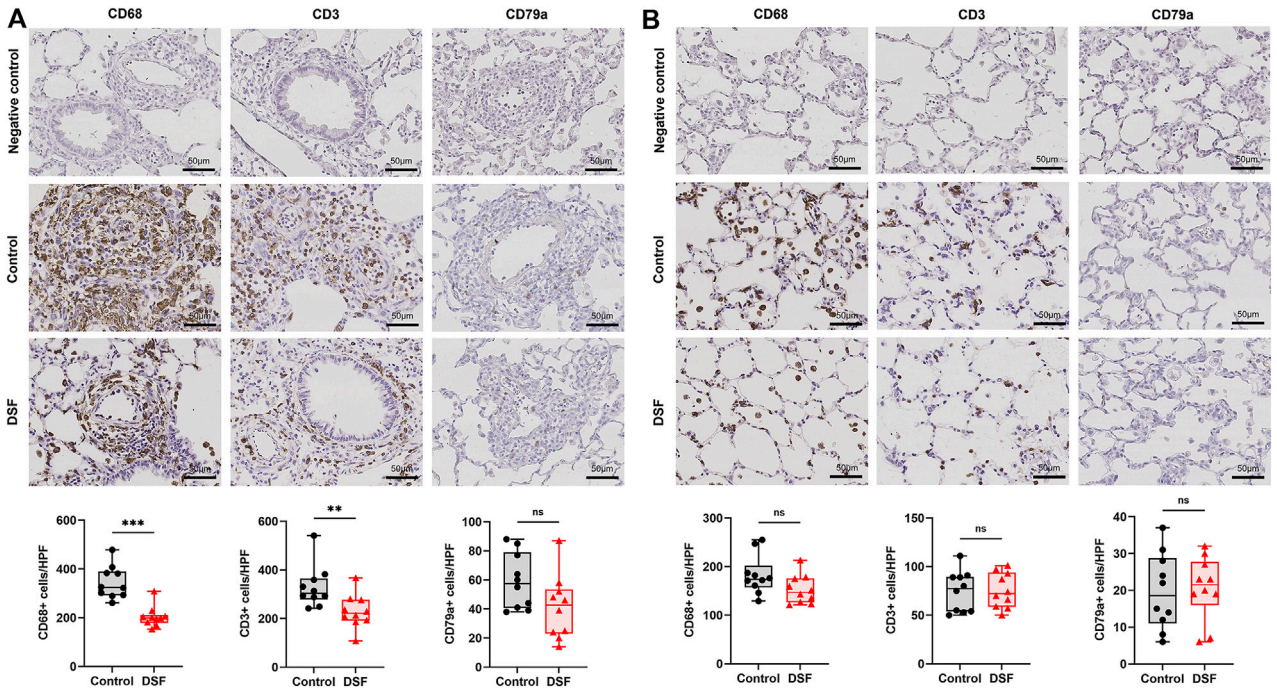


FIGURE 3 | Immunohistochemical staining. The plots show the counts of CD68-, CD3-, and CD79a-positive cells in the negative control, control, and disulfiram (DSF) groups per high-power field (HPF; magnification, ×400). **(A)** Perivascular/peribronchiolar area. **(B)** Alveolar area. The box-and-whiskers dot plots represent the medians and interquartile ranges with the minimum and maximum values. ns, not significant. $^{**}p < 0.01$; $^{***}p < 0.001$.

quantitative polymerase chain reaction (RT-qPCR). Relative expression of *CCL2* and *IL-6* in both groups was normalized against the expression level of the internal control gene glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). Total RNA (1 μg) isolated from samples was used for reverse transcription with the High-Capacity RNA-to-cDNA™ Kit (Thermo Fisher Scientific, Waltham, MA, United States) in a 20 μL volume. The protocol involved incubating at 37 °C for 60 min, followed by heating to 95 °C for 5 min, and finally cooling to 4 °C. RT-qPCR was then performed on the Applied Biosystems® 7500 System (Thermo Fisher Scientific) using 100 ng of cDNA and the TaqMan™ Gene Expression Master Mix (Thermo Fisher Scientific). Each sample was processed in duplicate. The PCR conditions were as follows: an initial 2-min step at 50 °C, a 10-min step at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C, concluding with a cooldown to 25 °C. Data analysis was conducted using the 7500 System SDS Software Version 1.4 (Thermo Fisher Scientific). The following probes were used for RT-qPCR: *CCL2* (NM_031530), *GAPDH* (NM_017008), and *IL-6* (NM_012589). For the negative controls, a no-template control from the RT reaction and a no-template control from the RT-qPCR reaction were used. Relative gene expression was calculated using the comparative ΔΔCT method [18].

Bronchoalveolar Lavage Fluid (BALF) Collection

Additional rats (n = 5/group) were subjected to left lung transplantation and implantation of osmotic pumps to obtain BALF samples. Briefly, their tracheas were cannulated, and lungs were lavaged thrice with 3 mL of phosphate-buffered saline on day 7 post-transplantation. The LUNA-FL Dual Fluorescence Cell Counter (Logos Biosystems, Gyeonggi-do, South Korea) was used to measure total cell count (TCC). Smears stained with Diff Quick were then used by pulmonologists to assess cell fractions in a double-blinded manner.

Determination of Pro-inflammatory Cytokine Levels and Potent Chemokines for Macrophages

On post-operative day (POD) 7, blood samples (3 mL) were collected from the inferior vena cava of the rats before heparinization and centrifuged to obtain sera (2,500 *g*, 10 min, 21 °C). The sera (n = 10 each), and the remaining BALF (n = 5 each; additional rats) after centrifugation (3,200 *g*, 20 min, 4 °C) were preserved at −80 °C. A MILLIPLEX MAP Kit Rat Cytokine/Chemokine Magnetic Bead Panel (MilliporeSigma, Burlington, VT, United States) was used to measure the protein concentrations in the serum and BALF. The levels of the following cytokines and chemokines were measured: chemokine ligand (CCL)2, interleukin (IL)-1β, IL-6, interferon-γ, and tumor necrosis factor-α.

Statistical Analysis

Continuous variables are presented as medians and interquartile ranges, except for % body weight, which is presented as the mean ± standard deviation due to its normal distribution.

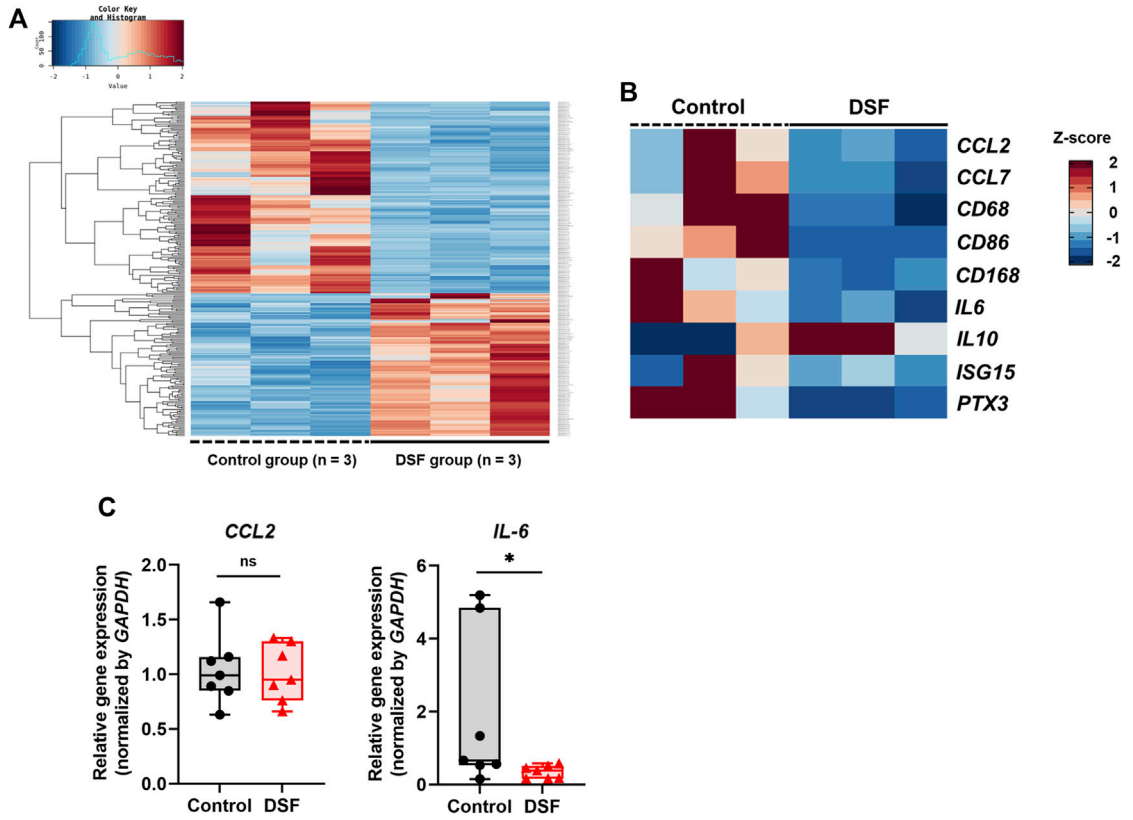


FIGURE 4 | Gene expression analyses using RNA sequencing (RNA-Seq) and real-time quantitative polymerase chain reaction (RT-qPCR). **(A)** Heat map based on the z-scores of differentially expressed genes between the control and disulfiram (DSF) groups (n = 3/group). **(B)** Extracted heat map focusing on the genes related to macrophages and acute rejection. **(C)** RT-qPCR analysis to validate the RNA-Seq results. The expression of *CCL2* and *IL-6* (n = 7 each) was normalized with that of *GAPDH*. The box-and-whiskers dot plots represent the medians and interquartile ranges with the minimum and maximum values. ns, not significant. *$p < 0.05$.

Mann–Whitney *U* test or Student's t-test was used to compare the values, respectively. Analyses were performed using R software (version 4.2.1; R Foundation for Statistical Computing, Vienna, Austria). The Benjamini–Hochberg method was used to identify DEGs. GraphPad Prism (version 9; GraphPad Software, San Diego, CA, United States) was used for creating figures. $p < 0.05$ or $Q < 0.05$ indicated significant differences in two-tailed tests.

RESULTS

Weight Changes

The percentage of rats' weights after treatment to the baseline value (% body weight) is shown in **Figure 2**. Both groups showed weight loss for 2 days with gradual recovery thereafter. Specifically, the % body weight was 95.2% ± 4.7% in the control group and 99.1% ± 3.6% in the DSF group on POD 7 ($p = 0.052$; **Figure 2A**).

Histological Findings

On POD 7, allogenic transplanted lungs in rats treated with DSF had a more whitish appearance and milder rejection than the control (**Figure 2B**). Perivascular lymphocytic infiltration ($p = 0.321$; **Figure 2C**) was not significantly altered, while lymphocytic bronchiolitis ($p = 0.0031$; **Figure 2C**) was significantly milder in the DSF group than in the control group. In the perivascular/peribronchiolar area, the infiltration of $CD68^+$ and $CD3^+$ cells was significantly inhibited after DSF treatment ($p = 0.0001$ and $p = 0.0029$, respectively; **Figure 3A**). In the alveolar area, the proportions of infiltrating $CD68^+$, $CD3^+$, and CD79a+ cells were not reduced after DSF treatment (**Figure 3B**). No false positives were observed in any of the CD staining instances.

Differential Gene Expression Analysis

In the DEG analysis between the control and DSF groups, 258 genes that matched the cut-off criteria ($|\log_2$ fold change$| > 1.0$, and $Q < 0.05$) were identified from RNA-Seq analysis. The expression heat map of DEGs indicated that 134 genes were downregulated after DSF treatment (**Figure 4A**). Among them, the expression of genes associated with macrophages and acute lung rejection was downregulated in the DSF group compared with that in the control group (**Figure 4B**). The expression of *CD86* and *CD163* was significantly downregulated in the DSF group compared with that in the control group ($Q = 0.002$ and $Q = 0.014$, respectively; **Supplementary Figure S1**). The expression of *IL-6* was significantly

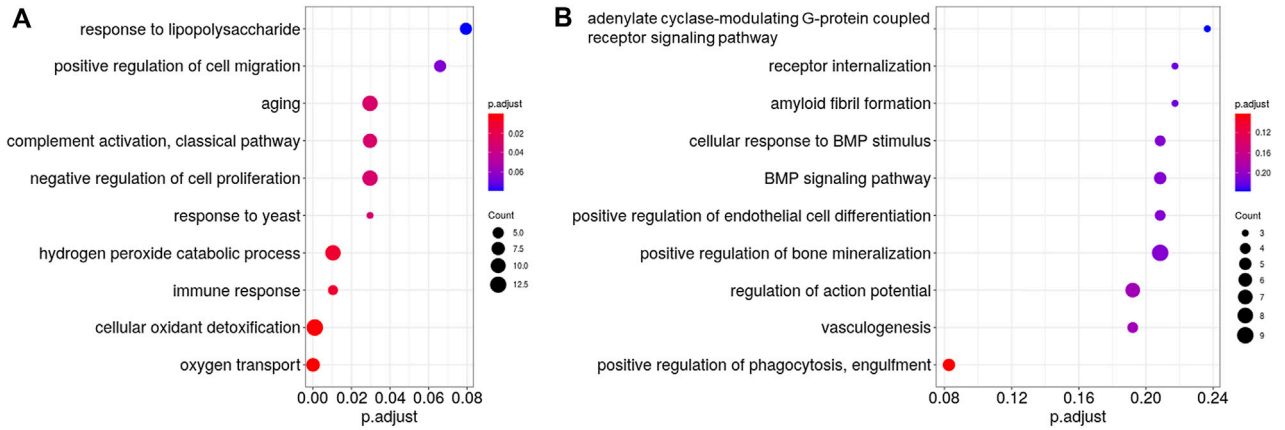


FIGURE 5 | Gene Ontology (GO) analysis of the differentially expressed genes between the control and disulfiram (DSF) groups. The top 10 most enriched GO terms (biological process) of **(A)**, downregulated genes and **(B)**, upregulated genes after DSF treatment. The vertical axis shows the GO terms, whereas the horizontal axis shows the adjusted *p*-values (*Q*-values). Gradations are applied according to the adjusted *p*-values. Circles represent the gene counts related to each GO term. If the GO terms had the same adjusted *p*-value, they are listed alphabetically from top to bottom.

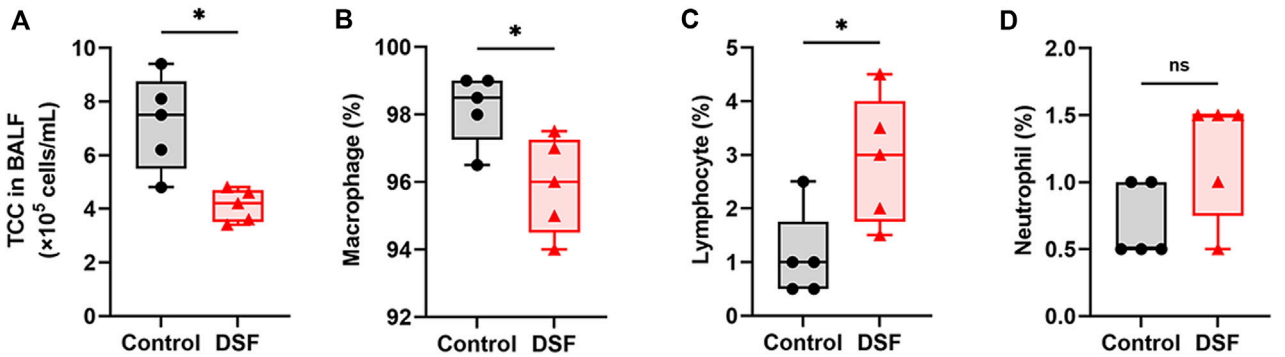


FIGURE 6 | Bronchoalveolar lavage fluid and their cell profiles. **(A)** Total cell count per milliliter of bronchoalveolar lavage fluid. **(B)** Macrophages (%), **(C)**, lymphocytes (%), and **(D)**, neutrophils (%). The box-and-whiskers dot plots represent the medians and interquartile ranges with the minimum and maximum values. ns, not significant. *$p < 0.05$. BALF, bronchoalveolar lavage fluid; DSF, disulfiram; TCC, total cell count.

downregulated in the DSF group compared with that in the control group ($Q = 0.037$; **Supplementary Figure S1**), and these findings are consistent with the RT-qPCR results ($p = 0.047$; **Figure 4C**). Additionally, the expression of the monocyte chemotactic protein CCL2 was lower in the DSF group than in the control group ($Q = 0.100$; **Supplementary Figure S1**); however, the RT-qPCR analysis showed only a slight change in its expression in both groups ($p = 0.874$; **Figure 4C**). When the cut-off value was increased to 1.5-fold higher expression ($|\log_2$ fold change$| > 0.6$), upregulation of IL-10 expression was observed in the DSF group, but the difference between the groups was not significant ($Q = 0.654$; **Supplementary Figure S1**). No-template controls exhibited undetermined Ct values, indicating the absence of detectable amplification.

GO Analysis

The downregulated genes in the DSF group were significantly enriched in eight biological process terms (**Figure 5A**), the top five being oxygen transport (GO:015671; $Q < 0.001$), cellular oxidant detoxification (GO:0098869; $Q < 0.001$), hydrogen peroxide catabolic process (GO:0042744; $Q = 0.0104$), immune response (GO:0006955; $Q = 0.0104$), and aging (GO:0007568; $Q = 0.0296$). In contrast, the upregulated genes were not significantly enriched in any biological process (**Figure 5B**).

TCC and Cell Fractionation in the BALF

The TCC in the BALF was markedly lower in the DSF group than in the control group ($p = 0.0159$; **Figure 6A**). The cell profile of the DSF group showed that the proportion of macrophages significantly decreased ($p = 0.032$; **Figure 6B**), whereas the percentage of lymphocytes significantly increased ($p = 0.024$; **Figure 6C**) compared with that in the control group. There was no difference in the proportion of neutrophils between the groups ($p = 0.143$; **Figure 6D**). In terms of the absolute counts in the BALF (**Supplementary Figure S2**), macrophages in the DSF

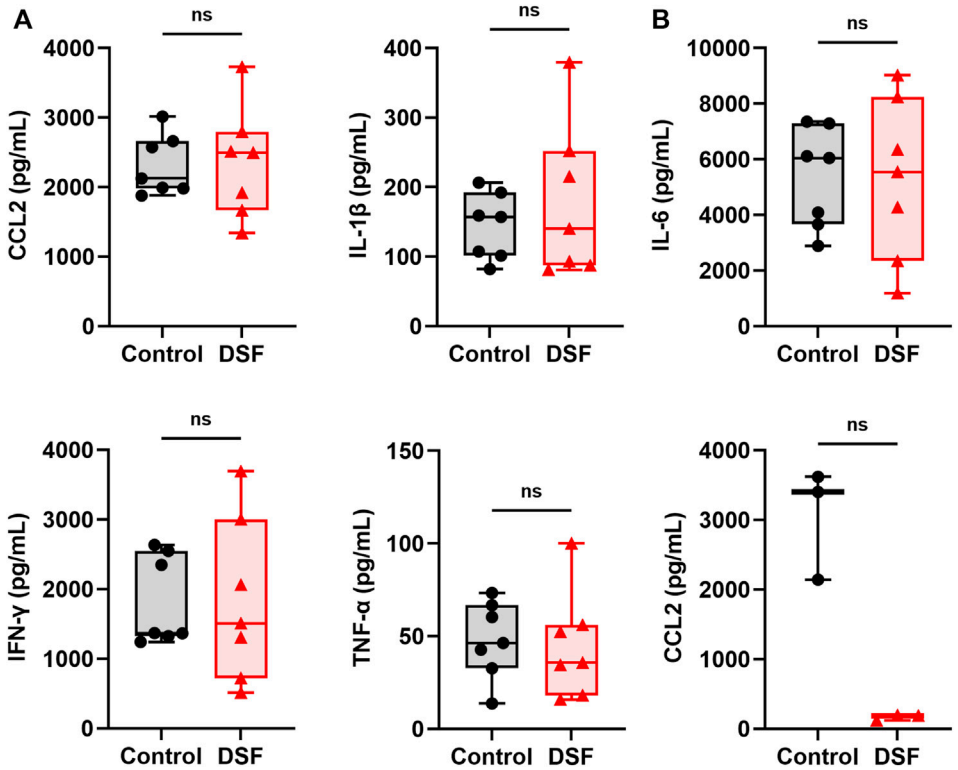


FIGURE 7 | Cytokines and chemokines in **(A)**, serum (n = 7 each) and **(B)**, bronchoalveolar lavage fluid (n = 3 each). The box-and-whiskers dot plots represent the medians and interquartile ranges with the minimum and maximum values. ns, not significant. CCL2, chemokine ligand 2; DSF, disulfiram; IFN-γ, interferon-gamma; IL-1β, interleukin-1 beta; IL-6, interleukin-6; TNF-α, tumor necrosis factor α.

group significantly decreased compared to those in the control group (median: 3.8×10^5 vs. 7.3×10^5 cells/mL; $p = 0.008$). Conversely, there was no significant difference in lymphocyte counts between the DSF and control groups (median: 9.9×10^3 vs. 4.0×10^3 cells/mL; $p = 0.151$).

Protein Concentrations in the Serum and BALF

The median concentration of CCL2 in the serum was 2,123 pg/mL in the control group and 2,493 pg/mL in the DSF group, and the difference between the groups was not significantly different ($p = 0.805$; **Figure 7A**). Among the measurable samples (n = 3 each), the CCL2 level in the BALF was relatively lower in the DSF group than in the control group (median: 3,400 pg/mL vs. 189 pg/mL; $p = 0.100$; **Figure 7B**). The levels of other cytokines in the serum did not significantly change after DSF treatment (**Figure 7A**), and they were undetectable in the BALF of both groups.

DISCUSSION

In this study, we demonstrated that DSF could attenuate acute rejection after MiHC lung transplantation in rats without using immunosuppressants. DSF reduced the accumulation of macrophages and T cells around the bronchioles in allografts, which might contribute to the prevention of bronchiolitis obliterans (BO). Furthermore, the expression of genes associated with macrophages and inflammatory cytokines in the lungs was downregulated after DSF treatment. These results support our hypothesis that macrophages are involved in acute rejection after lung transplantation and that DSF suppresses their chemotaxis.

The direct allorecognition of T cells is generally observed in acute lung rejection cases [19, 20]. DSF may have the potential to inhibit allorecognition and suppress macrophage migration and activation. Following lung transplantation, cells of the acquired immune system in recipients are mobilized to the graft by recognizing alloantigens presented by the donor's antigen-presenting cells [21]. In addition, monocyte-derived macrophages could migrate and cause injury to the graft together with T cells because they depend on the microenvironment and are particularly plastic [6, 22]. The present study showed that the post-operative regimen of DSF monotherapy for 7 days resulted in a reduction of lymphocytic bronchiolitis and decrease in the number of $CD68^+$ and $CD3^+$ cells in the perivascular/parabronchial area. The reduced accumulation of immunocompetent cells was presumably associated with the DSF-induced inhibition of their mobilization from circulation. Similarly, some animal studies have also shown that inhibiting macrophage

migration to the allograft suppressed acute lung rejection [4, 5, 23]. Furthermore, single-cell RNA-Seq data of the BALF from humans with acute rejection of the lungs and biopsy samples of lungs with chronic rejection suggested the involvement of macrophages [7]. These findings strongly indicate the involvement of macrophages in lung transplant rejection.

Our group reported that DSF inhibits the expression of the cytoplasmic protein FROUNT in macrophages, suppressing their migration and activation [12, 13], whose effect may have decreased the proportion of $CD68^+$ cells in the grafts. We did not observe a decrease in CCL2 levels in the serum or lung tissues in this study, and this is consistent with the fact that DSF has been shown to inhibit intracellular signaling between FROUNT and chemokine receptors (CCR2 or CCR5) on macrophages [24]. A previous study showed that when CCR2-positive cells accumulated in the inflamed lung, CCL2 was consumed in the serum and lung tissue [25]. Conversely, another study reported that CCL2 levels in the serum and grafts were higher in CCR2-deficient recipients than in wild-type recipients [26]. The results of these studies support a part of our results.

Repositioning for DSF has been proposed since it also has other therapeutic benefits, such as anti-inflammatory and anti-cancer effects [9–12]. However, there have been no reports on the preventive effect of DSF on rejection after solid organ transplantation. In a previous study, RNA-Seq of samples of rodents infected with severe acute respiratory syndrome coronavirus 2 and then administered DSF revealed the downregulation of the immunity pathway and complement and coagulation cascade [27]. The GO analysis in the present study also showed similar findings. Therefore, DSF is considered to have the potential to downregulate the immune response, attenuating organ and tissue rejection.

The number of macrophages in the alveolar area was not significantly different after DSF treatment compared with that in the perivascular/peribronchial area. This may have the advantage of maintaining their activity against bacteria and viruses to alveolar invasion. The imbalance in drug efficacy between areas can result from the main rejection site being a perivascular/peribronchial area and the differences in the turnover rates of tissue-resident and monocyte-derived macrophages. Monocyte-derived macrophages are produced from the bone marrow and have a short half-life, whereas tissue-derived macrophages exist in the lungs from early embryonic development and survive for long periods through self-renewal [28–30].

The inhibitory effect of DSF on lymphocytic bronchiolitis observed in this study may contribute to the prevention of BO because lymphocytic bronchiolitis is regarded as its precursor lesion [31]. IL-6, a pro-inflammatory cytokine, is strongly implicated in acute rejection after lung transplantation [32, 33]. The suppression of IL-6 signaling reportedly inhibits the development of BO [34]. As the expression of *IL-6* in allografts was downregulated in the DSF group in our study, DSF may be able to inhibit BO. Furthermore, we also hypothesized that DSF suppresses inflammation in the bronchi because of the decrease in the TCC in the BALF. In the BO lesions of human lung tissue, phosphorylation-induced activation of nuclear factor (NF)-κB and STAT3 and an increase in the proportions of $CD4^+$ T cells and macrophages have been reported [34]. As there is some evidence that DSF inhibits the NF-κB pathway [35, 36], it can be expected to prevent not only acute lung rejection but also BO and subsequent CLAD development.

There were a few limitations to this study. First, the effect of administering DSF via the oral route was not investigated. To stabilize DSF concentrations in the blood and prevent aspiration related to dosing and handling, we implanted osmotic pumps and administered the drug solutions subcutaneously. Second, the immune system varies from species to species. In this study, we employed the combination of F344 and Lewis rat strains, which is characterized by a minor mismatch in the MHC class I region. We acknowledge that this model does not fully represent the genetic diversity usually observed between human lung transplant donors and recipients. Furthermore, rodents, including the strains used in our study, are generally more likely to develop spontaneous tolerance compared to humans. However, we chose this model because it enables us to achieve relatively uniform levels of acute rejection within the same groups, without the complicating effects of intense post-transplant immunosuppression required in major mismatch models. Further studies are needed to confirm our findings in a large animal model before clinical trials. Third, this study focused on whether DSF can prevent acute lung rejection; therefore, the mechanism of drug action was not clarified. Although our group has previously revealed a part of its mechanism of action [12, 24], additional studies should be conducted to clarify its molecular mechanism in a rat lung transplantation model. The pharmacokinetics and safety profile of DSF are also well-known because the Food and Drug Administration approved it approximately 70 years ago [8].

In conclusion, DSF inhibited acute rejection after rat MiHC lung transplantation through an anti-immune response effect, especially involving macrophages. Targeting macrophages using DSF can be a new immunotherapeutic option to attenuate the rejection of allografts.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was approved by the Experimental Animal Ethics Committee of The University of Tokyo. The study was conducted in accordance with the local legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

Participated in research design: NY, MSa, HU, YuT, and JN. Participated in the writing the paper: NY and MSa. Participated in the performance of the research: NY, RM, ET, YaT, MSu, and AS-

U. Contributed new reagents or analytic tools: ET and YuT. Participated in data analysis: NY, MSa, HU, and JN.

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CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.12556/full#supplementary-material

REFERENCES

1. DeVito DA, Hoffman LA, Iacono AT, Wells CL, Grgurich W, Zullo TG, et al. Pattern and Predictors of Early Rejection After Lung Transplantation. *Am J Crit Care* (2003) 12:497–507. doi:10.4037/ajcc2003.12.6.497
2. Hachem RR, Khalifah AP, Chakinala MM, Yusen RD, Aloush AA, Mohanakumar T, et al. The Significance of a Single Episode of Minimal Acute Rejection After Lung Transplantation. *Transplantation* (2005) 80:1406–13. doi:10.1097/01.tp.0000181161.60638.fa
3. Todd JL, Neely ML, Kopetskie H, Sever ML, Kirchner J, Frankel CW, et al. Risk Factors for Acute Rejection in the First Year After Lung Transplant: A Multicenter Study. *Am J Respir Crit Care Med* (2020) 202:576–85. doi:10.1164/rccm.201910-1915OC
4. Hirschburger M, Zakrzewicz A, Kummer W, Padberg W, Grau V. Nicotine Attenuates Macrophage Infiltration in Rat Lung Allografts. *J Heart Lung Transpl* (2009) 28:493–500. doi:10.1016/j.healun.2009.02.005
5. Schmidt A, Sucke J, Fuchs-Moll G, Freitag P, Hirschburger M, Kaufmann A, et al. Macrophages in Experimental Rat Lung Isografts and Allografts: Infiltration and Proliferation *In Situ*. *J Leukoc Biol* (2007) 81:186–94. doi:10.1189/jlb.0606377
6. Chiu S, Bharat A. Role of Monocytes and Macrophages in Regulating Immune Response Following Lung Transplantation. *Curr Opin Organ Transpl* (2016) 21:239–45. doi:10.1097/MOT.0000000000000313
7. Moshkelgosha S, Duong A, Wilson G, Andrews T, Berra G, Renaud-Picard B, et al. Interferon-Stimulated and Metallothionein-Expressing Macrophages Are Associated With Acute and Chronic Allograft Dysfunction After Lung Transplantation. *J Heart Lung Transpl* (2022) 41:1556–69. doi:10.1016/j.healun.2022.05.005
8. Suh JJ, Pettinati HM, Kampman KM, O'Brien CP. The Status of Disulfiram: A Half of a Century Later. *J Clin Psychopharmacol* (2006) 26:290–302. doi:10.1097/01.jcp.0000222512.25649.08
9. Hu JJ, Liu X, Xia S, Zhang Z, Zhang Y, Zhao J, et al. FDA-Approved Disulfiram Inhibits Pyroptosis by Blocking Gasdermin D Pore Formation. *Nat Immunol* (2020) 21:736–45. doi:10.1038/s41590-020-0669-6
10. Custodio MM, Sparks J, Long TE. Disulfiram: A Repurposed Drug in Preclinical and Clinical Development for the Treatment of Infectious Diseases. *Antiinfect Agents* (2022) 20:e040122199856. doi:10.2174/2211352520666220104104747
11. Kona FR, Buac D, Burger AM. Disulfiram, and Disulfiram Derivatives as Novel Potential Anti-Cancer Drugs Targeting the Ubiquitin-Proteasome System in Both Preclinical and Clinical Studies. *Curr Cancer Drug Targets* (2011) 11:338–46. doi:10.2174/156800911794519798
12. Terashima Y, Toda E, Itakura M, Otsuji M, Yoshinaga S, Okumura K, et al. Targeting FROUNT With Disulfiram Suppresses Macrophage Accumulation and Its Tumor-Promoting Properties. *Nat Commun* (2020) 11:609. doi:10.1038/s41467-020-14338-5
13. Toda E, Sawada A, Takeuchi K, Wakamatsu K, Ishikawa A, Kuwahara N, et al. Inhibition of the Chemokine Signal Regulator FROUNT by Disulfiram Ameliorates Crescentic Glomerulonephritis. *Kidney Int* (2022) 102:1276–90. doi:10.1016/j.kint.2022.07.031
14. Ashburn TT, Thor KB. Drug Repositioning: Identifying and Developing New Uses for Existing Drugs. *Nat Rev Drug Discov* (2004) 3:673–83. doi:10.1038/nrd1468
15. ClinicalTrials. *ClinicalTrials.gov* (2023). Available from: https://clinicaltrials.gov/search?intr=Disulfiram (Accessed October 3, 2023).
16. Tian D, Shiiya H, Sato M, Nakajima J. Rat Lung Transplantation Model: Modifications of the Cuff Technique. *Ann Transl Med* (2020) 8:407. doi:10.21037/atm.2020.02.46
17. Stewart S, Fishbein MC, Snell GI, Berry GJ, Boehler A, Burke MM, et al. Revision of the 1996 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Lung Rejection. *J Heart Lung Transpl* (2007) 26:1229–42. doi:10.1016/j.healun.2007.10.017
18. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clin Chem* (2009) 55:611–22. doi:10.1373/clinchem.2008.112797
19. Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, et al. Structure of M(Pro) From SARS-CoV-2 and Discovery of Its Inhibitors. *Nature* (2020) 582:289–93. doi:10.1038/s41586-020-2223-y
20. Rogers NJ, Lechler RI. Allorecognition. *Am J Transpl* (2001) 1:97–102. doi:10.1034/j.1600-6143.2001.10201.x
21. Sivaganesh S, Harper SJ, Conlon TM, Callaghan CJ, Saeb-Parsy K, Negus MC, et al. Copresentation of Intact and Processed MHC Alloantigen by Recipient Dendritic Cells Enables Delivery of Linked Help to Alloreactive CD8 T Cells by Indirect-Pathway CD4 T Cells. *J Immunol* (2013) 190:5829–38. doi:10.4049/jimmunol.1300458
22. Mosser DM, Edwards JP. Exploring the Full Spectrum of Macrophage Activation. *Nat Rev Immunol* (2008) 8:958–69. doi:10.1038/nri2448
23. Oyaizu T, Okada Y, Shoji W, Matsumura Y, Shimada K, Sado T, et al. Reduction of Recipient Macrophages by Gadolinium Chloride Prevents Development of Obliterative Airway Disease in a Rat Model of Heterotopic Tracheal Transplantation. *Transplantation* (2003) 76:1214–20. doi:10.1097/01.TP.0000088672.48259.F1
24. Toda E, Terashima Y, Sato T, Hirose K, Kanegasaki S, Matsushima K. FROUNT Is a Common Regulator of CCR2 and CCR5 Signaling to

Control Directional Migration. *J Immunol* (2009) 183:6387–94. doi:10.4049/jimmunol.0803469
25. Maus UA, Wellmann S, Hampl C, Kuziel WA, Srivastava M, Mack M, et al. CCR2-Positive Monocytes Recruited to Inflamed Lungs Downregulate Local CCL2 Chemokine Levels. *Am J Physiol Lung Cel Mol Physiol* (2005) 288: L350–8. doi:10.1152/ajplung.00061.2004
26. Gelman AE, Okazaki M, Sugimoto S, Li W, Kornfeld CG, Lai J, et al. CCR2 Regulates Monocyte Recruitment as Well as CD4 T1 Allorecognition After Lung Transplantation. *Am J Transpl* (2010) 10:1189–99. doi:10.1111/j.1600-6143.2010.03101.x
27. Adrover JM, Carrau L, Daßler-Plenker J, Bram Y, Chandar V, Houghton S, et al. Disulfiram Inhibits Neutrophil Extracellular Trap Formation and Protects Rodents From Acute Lung Injury and SARS-CoV-2 Infection. *JCI Insight* (2022) 7:e157342. doi:10.1172/jci.insight.157342
28. Landsman L, Jung S. Lung Macrophages Serve as Obligatory Intermediate Between Blood Monocytes and Alveolar Macrophages. *J Immunol* (2007) 179: 3488–94. doi:10.4049/jimmunol.179.6.3488
29. Misharin AV, Morales-Nebreda L, Mutlu GM, Budinger GR, Perlman H. Flow Cytometric Analysis of Macrophages and Dendritic Cell Subsets in the Mouse Lung. *Am J Respir Cel Mol Biol* (2013) 49:503–10. doi:10.1165/rcmb.2013-0086MA
30. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate Mapping Reveals Origins and Dynamics of Monocytes and Tissue Macrophages Under Homeostasis. *Immunity* (2013) 38:79–91. doi:10.1016/j.immuni.2012.12.001
31. Glanville AR, Aboyoun CL, Havryk A, Plit M, Rainer S, Malouf MA. Severity of Lymphocytic Bronchiolitis Predicts Long-Term Outcome After Lung Transplantation. *Am J Respir Crit Care Med* (2008) 177:1033–40. doi:10.1164/rccm.200706-951OC
32. Iacono A, Dauber J, Keenan R, Spichty K, Cai J, Grgurich W, et al. Interleukin 6 and Interferon-Gamma Gene Expression in Lung Transplant Recipients With Refractory Acute Cellular Rejection: Implications for Monitoring and Inhibition by Treatment With Aerosolized Cyclosporine. *Transplantation* (1997) 64:263–9. doi:10.1097/00007890-199707270-00015
33. Yoshida Y, Iwaki Y, Pham S, Dauber JH, Yousem SA, Zeevi A, et al. Benefits of Posttransplantation Monitoring of Interleukin 6 in Lung Transplantation. *Ann Thorac Surg* (1993) 55:89–93. doi:10.1016/0003-4975(93)90479-2
34. Lee J, Nakagiri T, Kamimura D, Harada M, Oto T, Susaki Y, et al. IL-6 Amplifier Activation in Epithelial Regions of Bronchi After Allogeneic Lung Transplantation. *Int Immunol* (2013) 25:319–32. doi:10.1093/intimm/dxs158
35. Celik O, Ersahin A, Acet M, Çelik N, Baykuş Y, Deniz R, et al. Disulfiram, as a Candidate NF-Kb and Proteasome Inhibitor, Prevents Endometriotic Implant Growing in a Rat Model of Endometriosis. *Eur Rev Med Pharmacol Sci* (2016) 20:4380–9.
36. Zha J, Chen F, Dong H, Shi P, Yao Y, Zhang Y, et al. Disulfiram Targeting Lymphoid Malignant Cell Lines Via ROS-JNK Activation as Well as Nrf2 and NF-kB Pathway Inhibition. *J Transl Med* (2014) 12:163. doi:10.1186/1479-5876-12-163

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Proteomic Analysis of Primary Graft Dysfunction in Porcine Lung Transplantation Reveals Alveolar-Capillary Barrier Changes Underlying the High Particle Flow Rate in Exhaled Breath

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Primary graft dysfunction (PGD) remains a challenge for lung transplantation (LTx) recipients as a leading cause of poor early outcomes. New methods are needed for more detailed monitoring and understanding of the pathophysiology of PGD. The measurement of particle flow rate (PFR) in exhaled breath is a novel tool to monitor and understand the disease at the proteomic level. In total, 22 recipient pigs underwent orthotopic left LTx and were evaluated for PGD on postoperative day 3. Exhaled breath particles (EBPs) were evaluated by mass spectrometry and the proteome was compared to tissue biopsies and bronchoalveolar lavage fluid (BALF). Findings were confirmed in EBPs from 11 human transplant recipients. Recipients with PGD had significantly higher PFR [686.4 (449.7–8,824.0) particles per minute (ppm)] compared to recipients without PGD [116.6 (79.7–307.4) ppm, p = 0.0005]. Porcine and human EBP proteins recapitulated proteins found in the BAL, demonstrating its utility instead of more invasive techniques. Furthermore, adherens and tight junction proteins were underexpressed in PGD tissue. Histological and proteomic analysis found significant

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Abbreviations: AQP-5, Aquaporin-5; BALF, Bronchoalveolar lavage fluid; BOS, Bronchiolitis obliterans syndrome; CLAD, Chronic lung allograft dysfunction; DIA, Data-independent acquisition; EBP, Exhaled breath particles; ECM, Extracellular matrix; GSEA, Gene set enrichment analysis; GO, Gene ontology; H&E, Hematoxylin and eosin; ICU, Intensive care unit; JAM-1, Junctional adhesion molecule-1; ISHLT, International Society for Heart and Lung Transplantation; LEA, Lycopersicon Esculentum lectin; LTx, Lung transplantation; MQ, Morphological quotient; PANTHER, Protein analysis through evolutionary relationships; PExA, Particles in exhaled air; PFR, Particle flow rate; PGD, Primary graft dysfunction; PPM, Particles per minute; PPP2CA, Serine/threonine-protein phosphatase 2A catalytic subunit; RTLF, Respiratory tract lining fluid.

changes to the alveolar-capillary barrier explaining the high PFR in PGD. Exhaled breath measurement is proposed as a rapid and non-invasive bedside measurement of PGD.

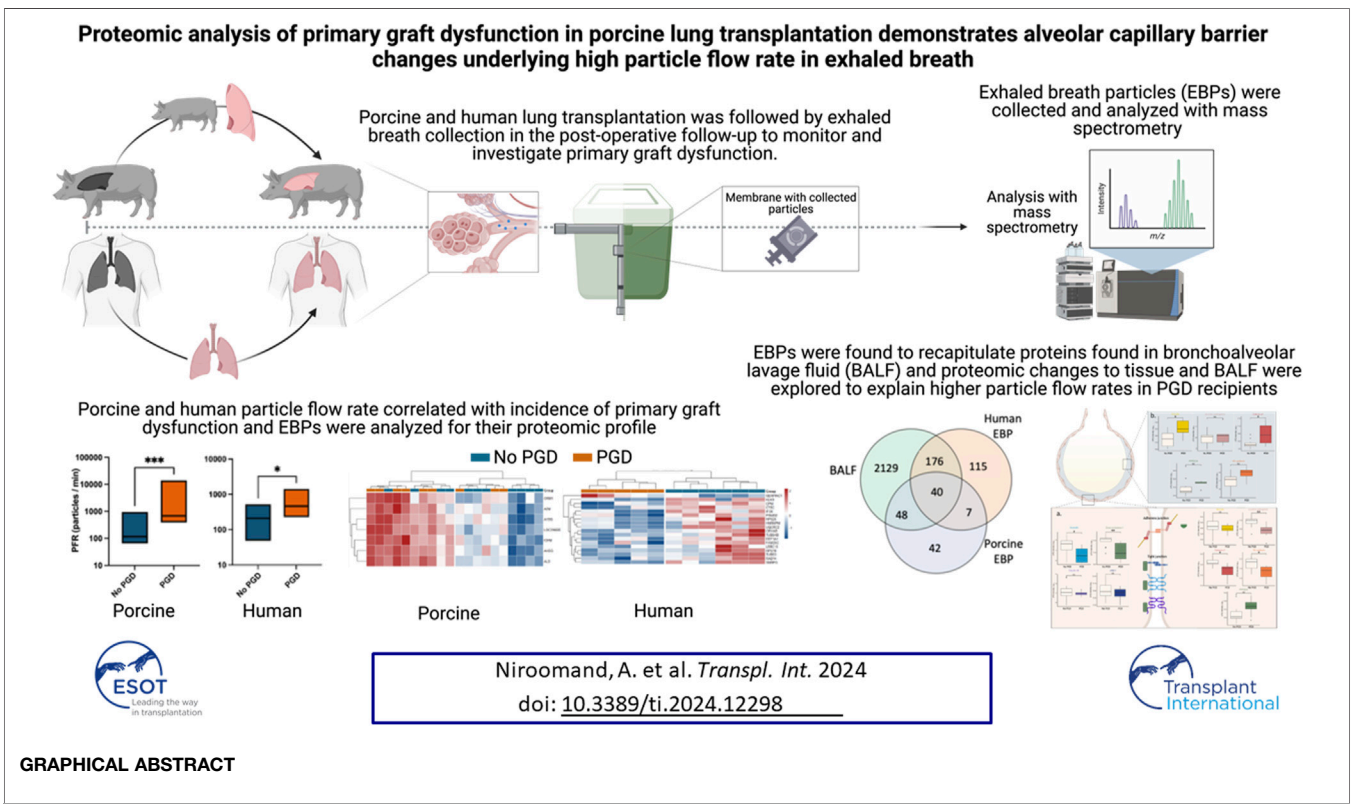
Keywords: primary graft dysfunction, lung transplantation, particle flow rate, exhaled breath particles, mass spectrometry

INTRODUCTION

Primary graft dysfunction (PGD) remains a challenge in the postoperative management of lung transplantation (LTx) with an estimated incidence rate of up to 25% of all cases [1]. Recognition and appropriate management are particularly important as PGD grade 3 correlates with increased mortality and rates of chronic lung allograft dysfunction (CLAD) [2–4].

While PGD is readily diagnosed by the PaO_2/FiO_2 ratio and chest imaging, current diagnostic tools do not necessarily indicate early onset, offer a means of non-invasive bedside detection, or provide a more detailed view of disease pathology. Chest x-rays, while non-invasive and readily available, are not necessarily specific and do not preclude the existence of other processes. Advanced techniques for patient evaluation such as bronchoalveolar lavage and transbronchial biopsy are invasive and have associated risks. Sampling of exhaled breath particles (EBPs), in contrast, utilizes a non-invasive device connected to the mechanical ventilation circuit with no additional safety considerations. The benefit of this form of sampling would be the ability to quickly identify PGD while being able to analyze the patient's condition from a more granular perspective given the downstream analyses available for EBP collection.

The efficacy of EBP collection as a methodology has previously been demonstrated in patients in intensive care units (ICUs) and post-transplant patients on mechanical ventilation to show feasibility and lack of adverse effects [5–8]. Porcine models have also demonstrated a relationship between lung injury and particle flow rate [9]. EBPs are thought to originate from the distal respiratory tract lining fluid (RTLF) as the small airways open and close [10–12] and share a similar composition to bronchoalveolar lavage fluid or BALF [10, 11, 13]. While the safety of EBP collection has been proven, its proteomic composition and the mechanism by which disease processes lead to higher PFR have not yet been elucidated, motivating the current study. Additionally, there are few proteomic studies of lung transplantation in general and PGD in particular, with the majority focusing on biomarkers in the blood or BALF [14–17]. Even without considering PGD, studies of the proteome in lung tissue specifically following transplantation are severely limited, with a literature search revealing only one other study examining proteins in post-



GRAPHICAL ABSTRACT

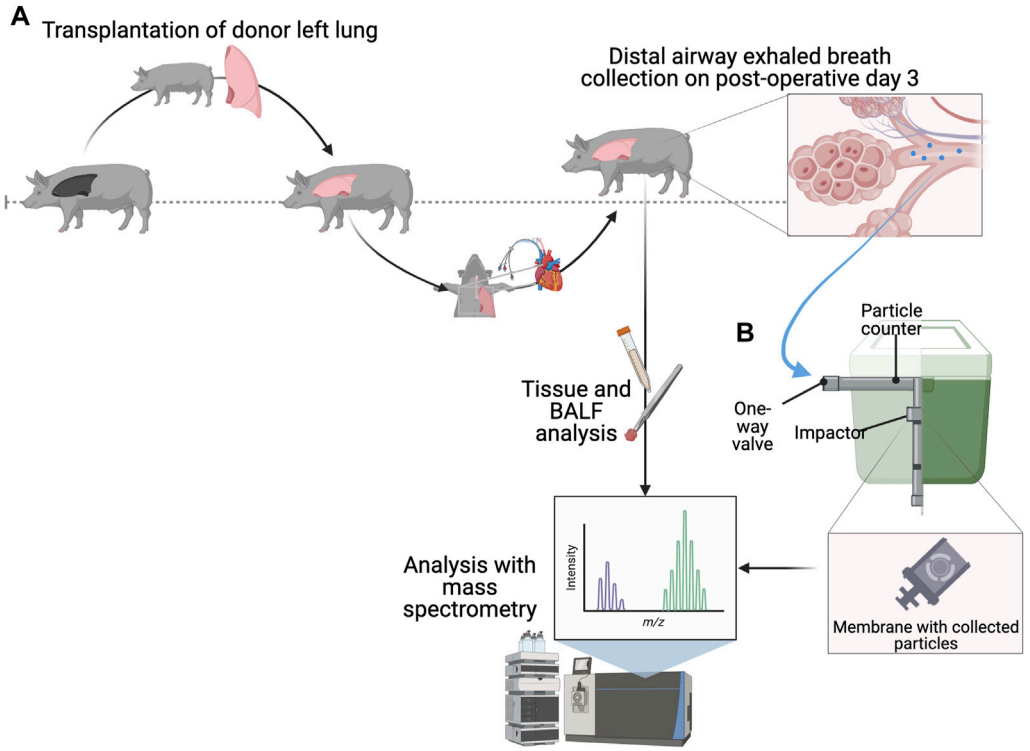


FIGURE 1 | Experimental overview and observed particle flow rate (PFR) in recipients with primary graft dysfunction (PGD). **(A)** Overview of transplantation followed by the right pneumonectomy and distal airway exhaled breath collection on postoperative day 3. **(B)** Schematic illustrating the collection of particles from the distal airways, where exhaled breath travels through a one-way valve in the particles in exhaled air (PExA) device, which counts them prior to impaction. Samples including tissue, bronchoalveolar lavage fluid (BALF) and collected exhaled breath particles were then analyzed for protein identity using mass spectrometry. Figure created in biorender.com.

transplant porcine tissue [18]. Consequently, there is a great need to gain a more detailed understanding of PGD using a variety of profiles sourced from tissue, BALF, and exhaled breath, all of which would be valuable in understanding disease pathogenesis. The comparison of EBP proteins with BALF and tissue proteomes would also validate the collection of exhaled breath as a clinically valuable monitoring tool.

In this study, we utilized a pig lung transplantation model as a platform to study PGD. We applied particle flow rate (PFR) measurement and EBP collection to postoperative mechanical ventilation and correlated PFR with disease occurrence. We then isolated and identified the proteins found in the EBP, validating the methodology for the detection of PGD and compared BALF and tissue proteins to porcine and human lung transplant EBPs. We utilized the proteomic findings to understand the mechanism of higher PFR in PGD. We hypothesized that the epithelial and endothelial damage that occurs in PGD underlies the particle accumulation in the RTLF behind the higher PFR in the disease state.

MATERIALS AND METHODS

Further details are provided in the online **Supplementary Materials and Methods**.

Ethical Considerations for Porcine Experiments

The study was approved by the local Animal Research Ethics Committee (Dnr 5.2.18-4903/16, and Dnr 5.2.18-8927/16) at Lund University. All animals received care according to the USA Principles of Laboratory Animal Care of the National Society for Medical Research, Guide for the Care and Use of Laboratory Animals, National Academies Press (1996). All human patients signed written informed consent and approval was obtained from the Ethics Committee for Research (Dnr 2017/396).

Animal Preparation

An overview is provided in **Figures 1A, B**. All donors (n = 22) and recipients (n = 22) were premedicated with xylazine (Rompun® vet. 20 mg/mL; Bayer AG, Leverkusen, Germany; 2 mg/kg) and ketamine (Ketaminol® vet. 100 mg/mL; Farmaceutici Gellini S.p.A., Aprilia, Italy; 20 mg/kg). All animals were placed under general anesthesia with ketamine (Ketaminol® vet, 100 mg/mL; Farmaceutici Gellini S.p.A., Aprilia, Italy; 20 mg/kg), midazolam (Midazolam Panpharma®, Oslo, Norway) and fentanyl (Leptanal®, Lilly, France). A pulmonary artery catheter (Swan-Ganz CCOmbo V and Introflex, Edwards Lifesciences Services GmbH, Unterschleissheim, Germany) was inserted into the right internal jugular vein and an arterial line (Secalon-T™, Merit Medical

Ireland Ltd., Galway, Ireland) was placed in the right common carotid artery.

Lung Transplantation and Monitoring

Lung harvesting from the donor and transplantation into the recipient followed the previous descriptions [19]. Recipient care followed clinical standards and immunosuppression, infection prophylaxis and ventilatory strategies are described in the **Supplementary Methods**. On day 3 post-transplantation, a right pneumonectomy (including the accessory lobe) allowed for assessment of the transplanted left lung (**Figure 1A**). The recipient was followed for an additional 4 h under one-lung ventilation, with tidal volume and respiratory rate adjusted to maintain a peak pressure <30 cmH_2O. All recipients were monitored throughout the post-transplantation period with hemodynamic parameters and arterial blood gases (ABL 90 FLEX blood gas analyzer, Radiometer Medical ApS, Brønshøj, Denmark) analyzed hourly.

Particles in Exhaled Air (PExA) and Exhaled Breath Particles (EBP)

Following the right pneumonectomy, a customized PExA 2.0 device (PExA, Gothenburg, Sweden) was connected to the expiratory limb of the ventilator, as previously described [6, 9] to measure PFR (particles per minute or ppm) and deposit particles on a membrane (**Figure 1B**). Membranes were kept frozen at −80°C until analysis.

Staging of Primary Graft Dysfunction

PGD was staged on postoperative day 3 based on the PaO_2/FiO_2 ratio according to the guidelines of the International Society for Heart and Lung Transplantation (ISHLT) [20]. Chest imaging was performed with a mobile C-arm x-ray machine (Siemens, Munich, Germany).

Histopathological and Immunofluorescence Analyses

Baseline biopsies were taken from the right lower lobe after intubation and from the transplanted left lung at the completion of the experiment. All biopsies were fixed in a 10% neutral buffered formalin solution (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). For histopathological analysis, sections were stained with hematoxylin and eosin (Merck Millipore, Darmstadt, Germany). Images from each recipient were assessed by two blinded scorers to report a lung injury score. For immunofluorescence imaging, sections stained with 4′,6-diamidino-2-phenylindole (DAPI), Lycopersicon Esculentum lectin (LEA) DyLight-488 and aquaporin-5 (AQP-5) were imaged on a Nikon A1RHD confocal microscopy platform (Nikon, Tokyo, Japan). Alveoli were individually imaged at random locations and analyzed using Fiji software [21]. A morphological quotient or MQ was calculated by dividing the alveolar circularity by its wall thickness.

Collection of EBPs From Human Lung Transplantation Patients

Membranes with EBPs were collected from 11 lung transplant recipients in the ICU using a modified PExA 2.0 instrument, as previously described [5]. PGD was graded according to the ISHLT guidelines based on arterial blood gas measurements, ventilator settings, and imaging. All patients arrived at the ICU after transplantation with a 7.5-mm tracheal tube and were ventilated according to unit guidelines, including a tidal volume of 6 mL/kg, positive end-expiratory pressure of 5 cmH_2O, end-inspiratory pressure of less than 25 cmH_2O, and an inspiratory to expiratory ratio of 1:2 (Maquet Servo I, Getinge, Solna, Sweden). EBPs were collected during measurements taken over 2 h from the second or third post-operative day based on the last measurement possible while the patient was on mechanical ventilation.

Mass Spectrometry Analysis

Proteins were extracted from porcine tissue, porcine BALF, porcine EBP membranes, and human EBP membranes. Mass spectra were acquired using a data-independent acquisition (DIA) method and analyzed with DIA-NN v 1.8.1 [22]. After quality control, two porcine EBP membranes, and two porcine BALF samples were excluded from further analysis. Differentially expressed proteins were determined with a threshold p-value <0.05 using the log2-transformed label-free quantification (LFQ) intensities and fold-change thresholds estimated from bootstrapping procedures. p-values were adjusted to determine an FDR-adjusted q-value with a significance level of 0.05. Hierarchical clustering in the heat map was performed on normalized, log2-transformed LFQ intensities (z-scores). The log2-fold change was used as the differential rank statistic for the gene set enrichment analysis (GSEA). A protein analysis through evolutionary relationships (PANTHER) overrepresentation test[1] of all EBP proteins was performed to look for statistically significantly enriched gene ontology (GO) terms under the biological process ontology.

Calculations and Statistics

Continuous variables were reported as median and interquartile range (IQR). Statistically significant differences were tested with the Student's t-test and ANOVA for normally distributed data and with the Mann-Whitney test and the Kruskal-Wallis tests for non-normally distributed data. A Chi-squared test was performed to analyze the observed frequencies of categorical variables. All statistical analyses were performed using GraphPad Prism 9.1 and R Studio (version 4.2.2). Significance was defined as $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*), and $p > 0.05$ (not significant).

RESULTS

Human Patient Demographics and Characteristics

Within the cohort, four patients were transplanted for idiopathic pulmonary fibrosis, four for chronic obstructive pulmonary disease, and another three for cystic fibrosis; none had an

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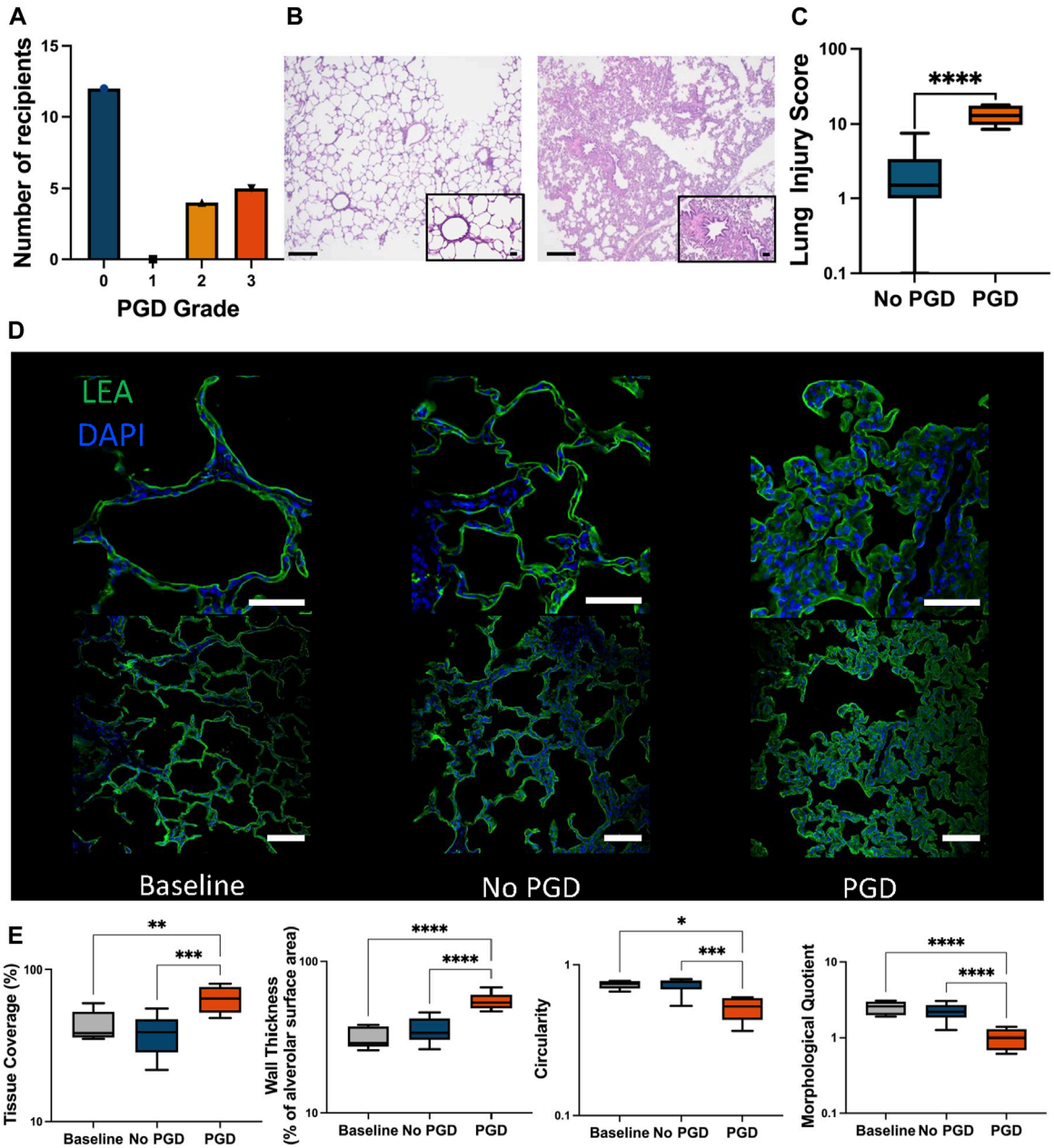

FIGURE 2 | Incidence of primary graft dysfunction (PGD) in lung transplant recipients. **(A)** PGD grade was determined according to the ISHLT guidelines and recipients were graded 0–3 based on the PaO_2/FiO_2 ratio and chest imaging. **(B)** Representative images of hematoxylin and eosin (H&E) staining of biopsies taken at the experimental endpoint show the differences between tissue from recipients without PGD (left) and those with PGD grades 2-3 (right). The scale bar in the larger image represents 0.5 mm and the callout shows a magnified portion of tissue where the bar represents 0.2 mm. **(C)** Blinded scoring of the H&E biopsies taken at the experimental endpoint. **(D)** Representative images of immunofluorescence staining for Lycopersicon esculentum lectin (LEA, green) and 4′,6-diamidino-2-phenylindole (DAPI, blue) at the lung biopsies taken at baseline prior to transplantation (left), at the experimental endpoint from a recipient without PGD (center) and at the experimental endpoint from a recipient with PGD (right). The top row shows a magnified portion of the tissue in the bottom row. The scale bar in the top row represents 50 μm while the bar in the bottom row represents 100 μm. **(E)** Morphological analysis of the immunofluorescence staining was conducted on the biopsies taken at baseline prior to transplantation, and the experimental endpoints for PGD and no PGD recipients post-transplantation on LEA and DAPI staining, showing the percent of tissue coverage in each image field of view (far left), in addition to the average thickness of individual alveoli (second from left), the calculated alveolar circularity (third from left), and the morphological quotient calculated based on the wall thickness and circularity (right). Plots represent samples taken from baseline biopsies ($n = 5$), the experimental endpoint for recipients with PGD ($n = 9$) or without PGD ($n = 13$). Values represent the median and interquartile range (box) with minimum and maximum values (whiskers). For statistical comparisons between two groups the two-tailed Mann-Whitney test was used. For comparisons between more than two groups a one-way ANOVA or Kruskal-Wallis test was used, followed by Tukey's or Dunn's *post hoc* tests, respectively. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

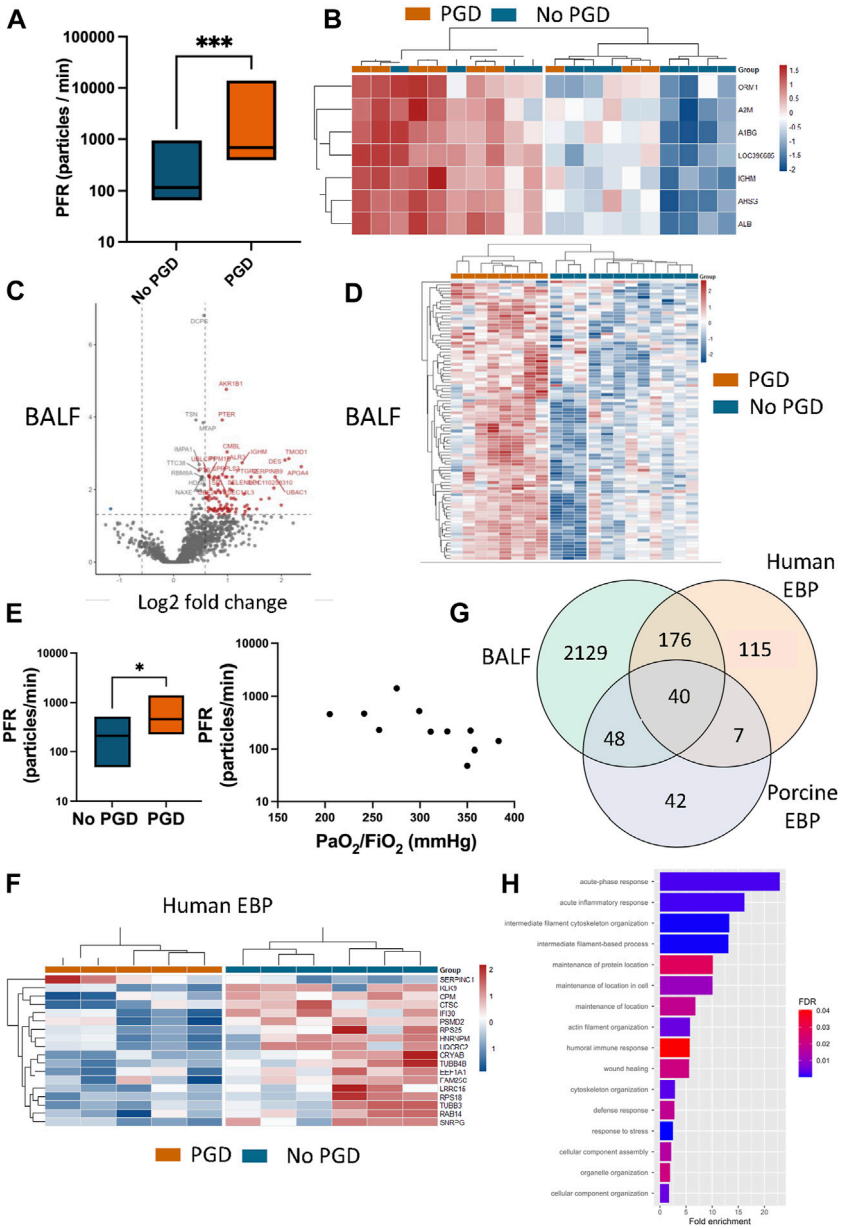

FIGURE 3 | Particles in the exhaled breath of porcine and human transplant recipients with and without primary graft dysfunction (PGD) exhibited significant differences in both particle flow rate and protein identity and recapitulated identities found in the bronchoalveolar lavage fluid (BALF). **(A)** Particle flow rate (PFR) relative to the grade of PGD determined for the porcine lung transplant recipients. Plots represent measurements taken at the experimental endpoint for recipients with PGD (n = 9) or without PGD (n = 13). **(B)** Proteins from the exhaled breath particles (EBP, n = 20) were isolated and analyzed using mass spectrometry and a heat map was made of differentially expressed proteins when the PGD porcine recipient group was compared to the non-PGD group. **(C)** Proteins were also identified from within the bronchoalveolar lavage fluid (BALF) samples of the porcine transplant recipients with the volcano plot showing the differentially expressed proteins with significantly higher expressed proteins in red. **(D)** A heat map was generated from the differentially expressed BALF proteins showing the grouping of the PGD and non-PGD porcine recipients. **(E)** The PFR was also measured within human transplant recipients (n = 11) between groups (left) and per recipient in correlation to the recipient's PaO_2/FiO_2 ratio. **(F)** Isolated proteins from the human exhaled breath particles (n = 11) were analyzed by mass spectrometry and a heat map was generated from the differentially expressed proteins. **(G)** A Venn diagram shows the overlap of protein identities isolated from the porcine bronchoalveolar lavage (BAL) fluid compared to the porcine EBPs and the human EBPs. **(H)** Gene Ontology (GO) term analysis was performed on the human EBPs to identify the biological processes from within the samples, with relevant terms highlighted in the plot demonstrating the fold enrichment and the false discovery rate (FDR)-corrected p-value in the bar color. An enlarged version can be found in the **Supplementary Materials** (**Supplementary Figure S1**). Plots represent measurements taken at the experimental endpoint for porcine recipients with PGD (n = 9) or without PGD (n = 13) and for human recipients with PGD (n = 5) and without PGD (n = 6). Statistically significant differences between groups were tested with the two-tailed Mann-Whitney test. *p < 0.05, ***p < 0.001. CPM, carboxypeptidase M; CRYAB, α-crystallin B chain; EEF1A1, elongation factor 1-α 1; HNRNPM, heterogeneous nuclear ribonucleoprotein M; IFI30, γ -interferon-inducible lysosomal thiol reductase; KLK9, kallikrein-9; LRRC15, leucine-rich

(*Continued*)

FIGURE 3 | repeat-containing protein 15; PSMD2, 26S proteasome non-ATPase regulatory subunit 2; RAB14, ras-related protein Rab-14; RPS18, 40S ribosomal protein S18; RPS25, 40S ribosomal protein S25; SERPINC1, antithrombin-III; SNRPG, small nuclear ribonucleoprotein G; TUBB3, tubulin β-3 chain; TUBB4B, tubulin β-4B chain; UQCRC2, cytochrome b-c1 complex subunit 2.

active smoking status. Following transplantation, 6 patients had PGD grade 0 while 5 patients had PGD grade 2. In the non-PGD group, the median age was 61 years (56–64.5) and all were men. The non-PGD group had a median pH of 7.44 (7.40–7.46), lactate of 2.1 (1.85–2.9), and ventilation with a median tidal volume of 522 mL (497–571), a median minute ventilation of 9.8 (8.9–10.8), and a median PEEP of 5 cmH_2O (5–5). In the PGD group, the median age was 56 years (55–59) and 3 of the 5 patients were women. The PGD group had a median pH of 7.40 (7.37–7.44), lactate of 1.6 (1.4–2.3), and was ventilated with a median tidal volume of 454 mL (444–548), median minute ventilation of 9.0 (8.1–10.8), and a median PEEP of 5 cmH_2O (5-5).

Primary Graft Dysfunction After Porcine Lung Transplantation Correlates With Histologic Analysis

All porcine recipients underwent a left LTx and were monitored for 3 days, after which a right pneumonectomy was performed to monitor the isolated left transplanted lung. Porcine recipients were assessed for PGD according to ISHLT guidelines. Severe PGD with grades 2 and 3 was detected in nine recipients while the remaining twelve had PGD grade 0 (**Figure 2A**).

PGD grades were correlated with the histological examination of end-experiment lung biopsies (**Figure 2B**). PGD samples received a lung injury score of 13.00 (9.75–17.75) compared to 1.50 (1.00–3.38) in non-PGD samples ($p < 0.0001$, **Figure 2C**). Signs of damage including immune cell infiltration, alveolar wall thickening, and capillary congestion were noted.

Immunofluorescence morphological examination showed increased damage in PGD (**Figure 2D**). Tissue coverage was significantly higher in the PGD group compared to the non-PGD group and the pre-transplant baseline ($p < 0.0001$ and $p < 0.0001$ respectively, **Figure 2E**). Alveolar walls were significantly thicker and alveolar circularity was significantly decreased ($p < 0.0001$, $p = 0.0009$, **Figure 2E**). The morphologic quotient (MQ) which takes into account the contribution of structural changes in the alveolar wall and circularity showed significant damage compared to the baseline and the non-PGD groups ($p < 0.0001$ and $p < 0.0001$ respectively, **Figure 2E**). The MQ of the non-PGD group did not significantly differ from the baseline ($p = 0.506$).

PGD Incidence Correlates With Higher Rates of Particle Flow and Particles Revealed a Proteomic Profile Similar to BALF

Using the custom PExA device connected to the expiratory limb of the ventilator, PFR was measured in the post-transplant recipients and found to be significantly higher in those with PGD with a rate of 686.4 (449.7–8,824.0) ppm. Those without PGD had a rate of 116.6 (79.7–307.4) ppm ($p = 0.0003$, **Figure 3A**). The EBPs were collected on a membrane from which the proteins were extracted. After filtering for those present in at least 60% of the samples per group, 137 proteins were analyzed, of which 7 were significantly overexpressed in PGD. Hierarchical clustering of the differentially expressed proteins showed a clear separation of the PGD group from the non-PGD group (**Figure 3B**). In BALF collected from all recipients, from which 2,418 proteins were identified after filtering for proteins in at least 65% of the samples. Of these, 91 proteins were overexpressed in PGD samples compared to 55 in non-PGD samples (**Figure 3C**). Again, hierarchical clustering differentiated between the two recipient groups (**Figure 3D**).

These results were additionally compared to findings collected from human EBPs from transplant recipients. As previously reported [5], the PFR in human recipients with PGD was higher than in non-PGD recipients. When comparing recipients, the PGD recipients had a significantly higher PFR (461.2 with IQR 284–1,177 ppm) compared to the non-PGD group (210.5 ppm with IQR 95–220.2, $p = 0.0424$; **Figure 3E**). When PFR was plotted against each recipient's PaO_2/FiO_2 ratio, there was a significant correlation (Spearman $r = -0.7364$, $p = 0.0128$, **Figure 3E**). While this relationship between disease and PFR has been noted previously, the proteins within this exhaled breath have never been analyzed. Within the human samples, 338 proteins were found after filtering, of which 18 were significantly differentially expressed in PGD and hierarchical clustering showed separation of the PGD and non-PGD samples (**Figure 3F**). Proteins found in the EBPs of both the human and porcine samples overlapped with those identified in BALF. When comparing the porcine EBP proteins to BALF, there was an overlap of 88 protein identities, representing 64.2% of all EBP proteins (**Figure 3G**). When comparing human EBPs to BALF, there was an overlap of 216 proteins or 63.9% of human EBP proteins (**Figure 3G**). A PANTHER overrepresentation test was performed to examine the significantly enriched pathways and 16 terms were identified (**Figure 3H**). Within the PGD group, acute phase and acute inflammatory responses were highlighted, in addition to several terms related to cytoskeletal and filament organization.

To understand how these processes are important in the production of EBPs and to highlight the mechanism by which EBPs are increased in the PGD group, further analysis of both the tissue and BALF was performed to demonstrate the alterations in the alveolar-capillary barrier.

Proteins in the Adherens and Tight Junctions Are Underexpressed in Tissue and Overexpressed in BALF

Analysis of the lung tissue identified 5,206 proteins, of which 302 were significantly overexpressed in the PGD group and

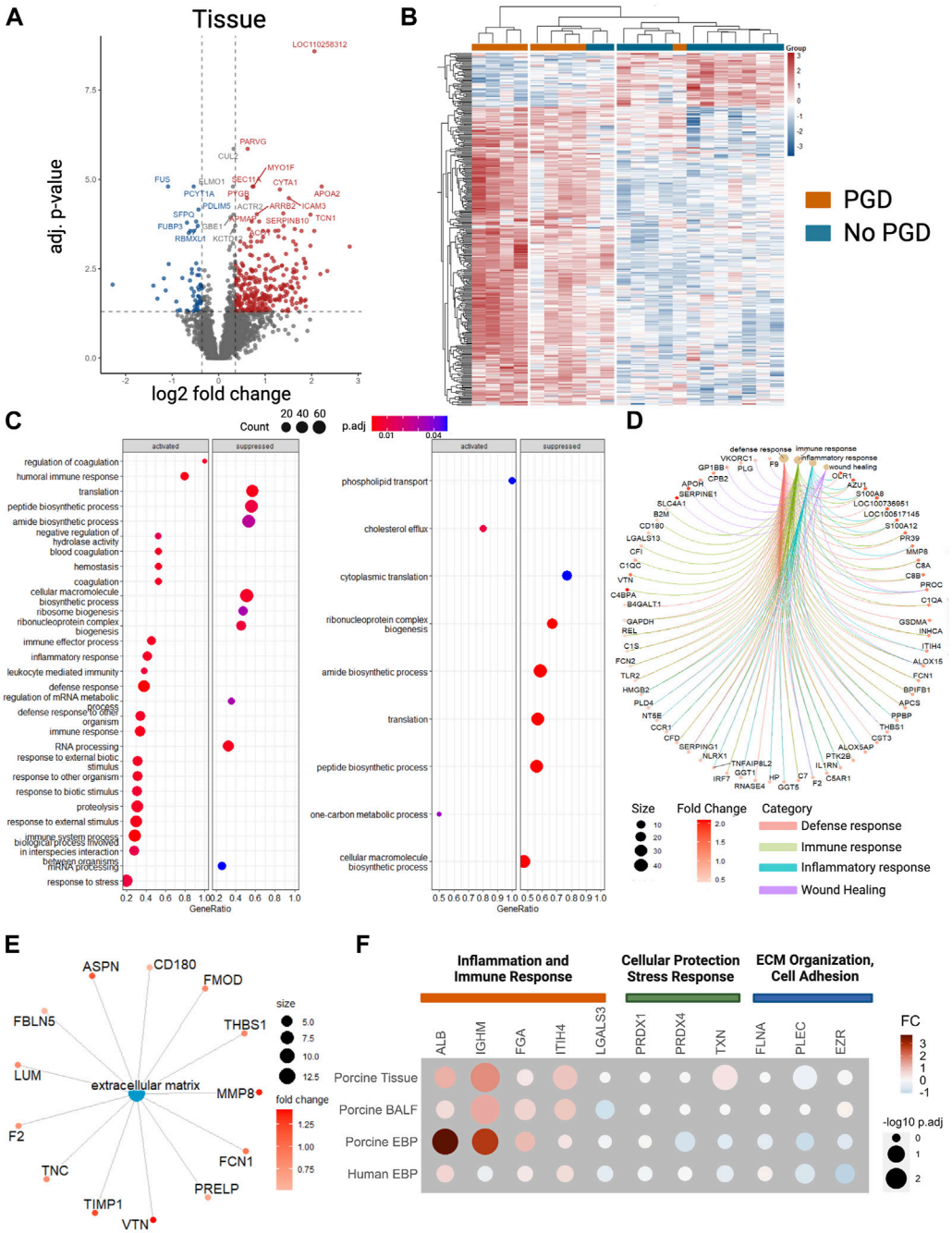

FIGURE 4 | Proteins identified in the tissue between primary graft dysfunction (PGD) and no PGD samples showed differences in biological processes as identified by gene ontology analysis. **(A)** Volcano plots of differentially expressed proteins detected by mass spectrometry of porcine lung tissue samples at the experimental endpoint. Blue dots indicate proteins that were underexpressed in PGD samples while red dots are those that were overexpressed in the samples. **(B)** Heat maps of hierarchical clustering performed on porcine tissue samples on differentially expressed proteins with blue representing underexpression and red representing overexpression. **(C)** Gene set enrichment analysis (GSEA) was performed on the differentially expressed proteins to show the statistically significant biological processes found in the tissue (left) and BALF (right) samples represented as dot plots. An enlarged version can be found in the **Supplementary Material** (**Supplementary Figure S2**). **(D)** Gene-concept network plot showing the association between proteins and biological processes found in the GSEA. Refer to **Supplementary Tables S1, S2** for a list of protein names. **(E)** Concept network plot for the analysis of the gene ontology (GO) term of the extracellular matrix. A list of protein names is in **Supplementary Table S3**. Enlarged versions of 4d and 4e can be found in the **Supplementary Materials** (**Supplementary Figure S3**). **(F)** The proteins identified were additionally compared between porcine tissue, BALF, and EBP samples and human EBP samples with a dot plot showing the fold change (FC) in color and the adjusted q-value in dot size. Proteins were grouped according to the GO term to which they belonged. Plots represent measurements taken at the experimental endpoint for recipients with PGD (n = 9) or without PGD (n = 13). Values represent the median and interquartile range (box) with minimum and maximum values (whiskers). Statistically significant differences between groups were tested with the two-tailed Mann-Whitney test. ***$p < 0.001$. ALB, albumin; EZR, ezrin; FGA, fibrinogen α chain; FLNA, filamin-A; IGHM,

(*Continued*)

FIGURE 4 | Immunoglobulin heavy constant mu; ITIH4, inter-α-trypsin inhibitor heavy chain H4; LGALS3, galectin; PLEC, plectin; PRDX1, peroxiredoxin-1; PRDX4, peroxiredoxin-4; TXN, thioredoxin.

55 were underexpressed (**Figure 4A**). As with other sample types like EBP and BALF, the hierarchical grouping of differentially expressed proteins showed a clear clustering of the PGD and non-PGD (**Figure 4B**). A GSEA was performed within the PGD group and showed that the enriched biological processes in the PGD samples included regulatory pathways of coagulation, wound healing, and responses to inflammation (**Figure 4C**). By isolating the specific pathways of defense responses, immune responses, inflammatory responses, and wound healing, enriched protein identities could be mapped to their biological processes (**Figure 4D**). Within the extracellular matrix, eleven proteins were significantly enriched, including known regulators such as metalloproteinase 8 (**Figure 4E**). Proteins identified in the tissue and BALF were additionally further compared to those found in the human and porcine EBPs (**Figure 4F**), demonstrating similarities in both the protein identities and relative fold changes. The identified proteins were grouped according to their corresponding GO biological processes.

To elucidate how there was a greater amount of exhaled breath particles in the respiratory tract lining fluid in the setting of PGD, the cell-cell adhesion proteins were examined. In the adherens junctions in the tissue samples (**Figure 5A**), junctional plakoglobin [log2 (FC) = −0.42, q = 0.005], catenin-α 1 [log2 (FC) = −0.36, q = 0.01], and vascular endothelial cadherin [log2 (FC) = −0.37, q = 0.02] were significantly underexpressed in the PGD group. Serine/threonine-protein phosphatase 2A catalytic subunit (PPP2CA) was higher in tissue from PGD samples, although not to a statistically significant degree. Zona occludens-1 and occludens of the tight junction were significantly underexpressed in PGD {log2 (FC) = −0.46, q = 0.005; [log2(FC) = −0.83, q = 0.04] respectively}, and others showed lower but not statistically significant levels, including junctional adhesions molecule-1 (JAM-1), claudin-18 and vinculin.

Within BALF (**Figure 5B**), adherens junction proteins were significantly higher in PGD samples. These included vinculin [log2 (FC) = 0.74, q = 0.04] and catenin-α 1 [log2 (FC) = 0.65, q = 0.03]. Junctional plakoglobin, PPP2CA, and vascular endothelial cadherin all showed increased but not statistically significant levels.

BALF was further examined to confirm the proteomic findings of alterations in the alveolar-capillary barrier. Total protein content in BALF showed an increasing trend toward the PGD group [2.0 (1.8–10.0) mg/mL in the PGD group, 2.2 (1.2–5.9) mg/mL in the non-PGD group, $p = 0.1213$, **Figure 6A**]. Albumin and IgM protein were overexpressed in the PGD BALF, demonstrating leakage of large molecular weight serum proteins, an established measure of alveolar-capillary barrier dysfunction [23] (**Figure 6B**). Other signs of alveolar-capillary barrier changes were found on histological examination, including H&E staining showing erythrocytes in the airspace in 6 of 9 PGD recipients (**Figure 6C**). Expression of aquaporin-5 (AQP-5), which is differentially localized to the apical membrane of the superficial epithelium in the airways, was decreased in tissue from the PGD group [log2 (FC) = −0.46, q = 0.04], which was also observed qualitatively by immunofluorescence imaging (**Figures 6D, E**).

DISCUSSION

Despite improvements in transplantation, PGD remains a threat to the postoperative recipient. Current methods of clinical appraisal could be supported by a non-invasive bedside approach to diagnostic surveillance. From this perspective, the addition of EBP analysis provides a novel means to monitor PGD, both from the rapidity of flow rate measurements that can be performed at the bedside and from the granular data that can be gathered from the in-depth analysis possible with collected particles. This study demonstrates that high EBP flow rates are significantly correlated with PGD and that the evaluation of proteins found in these EBPs can offer a window into the pathophysiological study of the distal airways without the need for more invasive bronchoscopy or tissue sampling. In this study, porcine and human EBP collections not only showed a correlation between high PFR and PGD incidence, but the proteins identified within the EBP samples reflected the BALF contents. Analysis of the tissue and BALF showed a disease-based difference in protein expression demonstrating alveolar-capillary barrier changes that explain the mechanism behind high PFR in PGD recipients.

Using the PExA device, breath particles are impacted according to their inertia, allowing both quantification and collection on a membrane housed within the device. In this study of a porcine lung transplant, PFR was correlated with the development of PGD at postoperative day 3. Previous studies have shown that PFR correlates with lung injury [8, 9] and a pilot study showed higher PFR in human lung transplant recipients with PGD [5]. In that report, we had previously reported that in human lung transplant recipients, particle counts measured on days 0, 1, 2, and 3 were increased in recipients with PGD compared to those without PGD [5]. In the present study, we aimed to build on these findings by identifying the proteins collected in exhaled breath and the mechanism by which PFR is higher in this state of injury, which has not been shown before. This work is novel not only for its proteomic profiling of the proteins captured by exhaled breath, but also because it aims to substantiate the hypothesis that changes to the alveolar-capillary barrier contribute to the PGD disease state and higher PFR through the analysis of both BALF and tissue samples. The evidence that porcine and human EBP particles recapitulate proteins found in BALF emphasizes that EBP collection can be a non-invasive means of sampling the distal airway without having to resort to bronchoalveolar lavage.

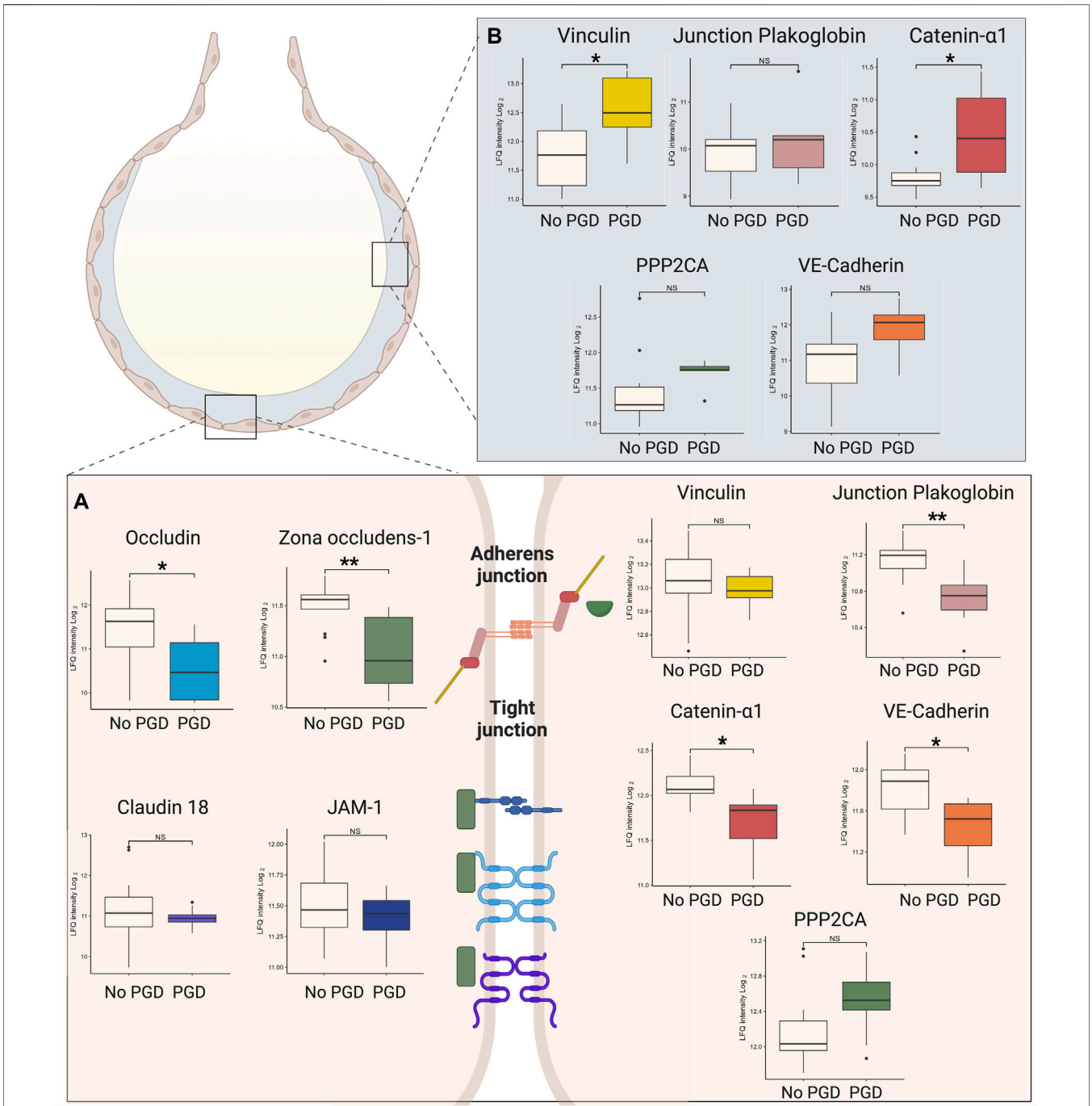

FIGURE 5 | Differential expression of junctional proteins in the adherens junctions and the tight junctions in the tissue from porcine recipients with primary graft dysfunction (PGD). **(A)** Plots represent the differences found within individual analyses of protein expression in porcine tissue samples from no PGD and PGD samples with respect to tight junction (left) and adherens junction (right) proteins. **(B)** Plots of the differences found within individual analyses of protein expression in bronchoalveolar lavage fluid (BALF) from no PGD and PGD samples. Plots represent measurements taken at the experimental endpoint for recipients without PGD (n = 13) or with PGD (n = 9). Values represent the median and interquartile range (box) with minimum and maximum values (whiskers). Statistically significant differences are reported as FDR corrected p-values (q-values) using log (2)-fold change differences between groups (see **Supplementary Methods**), *q < 0.05, NS. JAM-1, junctional adhesion molecule-1; VE-cadherin, vascular endothelial cadherin; PPP2CA, serine/threonine-protein phosphatase 2A-catalytic subunit. Figure created in biorender.com.

To understand why PFR was higher in the PGD group, an analysis of the alveolar-capillary barrier was pursued to demonstrate how injury status correlates with leakage of proteins into the respiratory tract lining fluid. While other forms of acute lung injury have demonstrated changes to the endothelial and epithelial barriers [23], this type of damage has

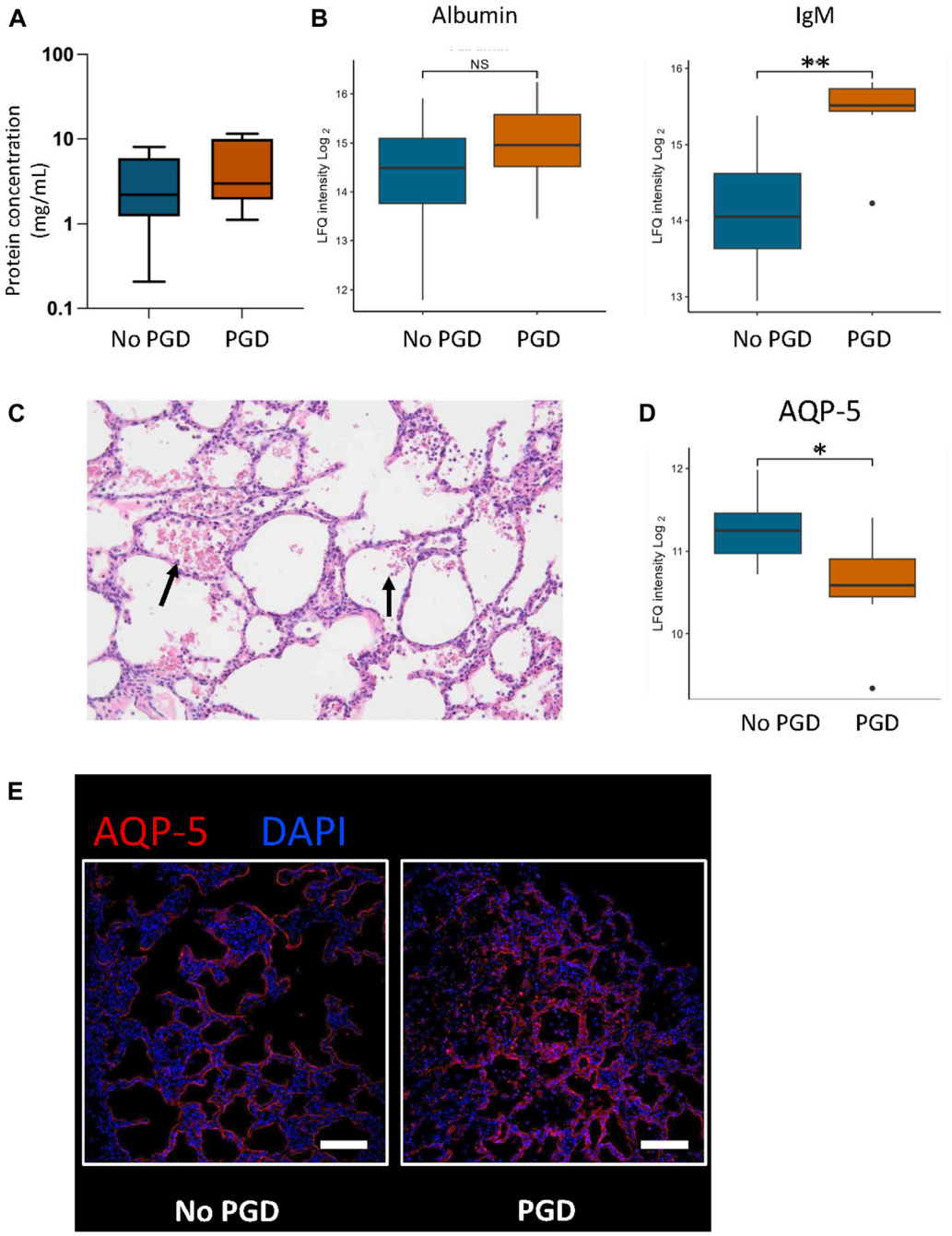

FIGURE 6 | Changes in the alveolar-capillary barrier compared between porcine recipients with and without primary graft dysfunction (PGD). **(A)** Total protein concentration in bronchoalveolar lavage fluid (BALF) was measured in the recipients at the end of the experiment. **(B)** Expression levels of albumin and IgM were measured using mass spectrometry in BALF samples. **(C)** Representative image of red blood cells seen in the airspace (black arrows) of hematoxylin and eosin (H&E) stained lung tissue from a recipient with primary graft dysfunction. **(D)** Aquaporin-5 (AQP-5) was measured by mass spectrometry in the tissue (left) and then visualized by immunofluorescence staining. **(E)** shows representative images from a no PGD sample (left) and PGD sample (right) using immunofluorescence staining (4′,6-diamidino-2-phenylindole or DAPI in blue, AQP-5 in magenta). The scale bar represents 100 μm. Plots represent measurements taken at the experimental endpoint for recipients with PGD (n = 9) or without PGD (n = 13). Values represent the median and interquartile range (box) with minimum and maximum values (whiskers). Statistically significant differences are reported as FDR corrected p-values (q-values) using log (2)-fold change differences between groups (see **Supplementary Methods**)**q < 0.05, NS.

not been as clearly established in studies of PGD, largely due to the lack of proteomic profiling in this disease. Other studies of the alveolar-capillary barrier have shown that tight and adherens junctions are important in maintaining alveolar permeability, with claudin-18 knockouts showing increased paracellular alveolar permeability [24, 25] and loss of vascular endothelial cadherin (VE-cadherin) implicated as a major mechanism of increased permeability in acute respiratory distress syndrome (ARDS) [26]. In this study, zona occludens, an important member of tight junctions, was significantly underexpressed in PGD tissue. Additionally, from the adherens junctions, VE-cadherin and its associated proteins including vinculin, junctional plakoglobin, catenin- α 1 and serine/threonine phosphatase 2 catalytic subunits were decreased in the PGD tissue in this study. This was accompanied by a concomitant increase in their BALF levels. The GO term "adherens junction assembly" was also statistically enriched within the EBP analysis.

Further evidence of alveolar-capillary barrier breakdown was found in the increased protein content of BALF and the presence of high molecular weight proteins, a recognized sign of barrier breakdown [23]. On H&E staining, erythrocytes were found in the airspace of the majority of PGD samples, further demonstrating barrier breakdown. AQP-5 levels were significantly reduced in the PGD group, which is important due to the specific localization of the protein to the apical surface of the lung epithelium, specifically within alveolar type 1 cell [27]. These results show that PGD status is correlated with significant changes in alveolar-capillary permeability. Combined with the findings of significant changes in tight and adherens junction components, the results that the alveolar-capillary barrier was significantly damaged in PGD can then be correlated to explain the finding of higher particle flow in the lining fluid of these recipients. This establishes the mechanism behind the higher PFR within this injury state.

There are few other proteomic studies of PGD in lung transplantation either in humans or in large animal models. Previous studies have primarily focused on the search for relevant biomarkers, which are typically measured in plasma or BALF. Individual proteins have been singled out instead [14, 28, 29], and thus this study demonstrates a novel use of proteomic profiling using mass spectrometry as a means to investigate the disease state through broader changes within the proteome. Mass spectrometry has rarely been utilized in lung transplantation research, with the exception of a few studies focusing on long-term outcomes in bronchiolitis obliterans syndrome [17, 30, 31]. This study therefore represents a novel approach to the study of PGD. Given the suggestion that there may be different phenotypes of PGD with different mechanisms underlying lung pathology, broader proteomic views such as those provided by EBP analysis may give a more detailed understanding of PGD pathophysiology [32]. Future endeavors with EBP collection and analysis could focus on expanding the findings within our porcine and human lung transplant recipients to look at proteomic changes in larger cohorts. This would be particularly valuable to increase the generalizability of the results as the current study only included human PGD grade 2 recipients. As implementation moves forward, EBP collection may become a complement to diagnostic techniques, but would need consideration and exercise of clinical judgment as an alternative to some techniques such as bronchoscopy, which, for example, may still be indicated for other reasons, such as mucus clearance and viral and bacterial sampling.

In conclusion, the use of exhaled breath particles allows for the rapid detection of PGD in lung transplant recipients by PFR measurement and may facilitate more in-depth analyses to investigate disease pathology by proteomic analysis of the distal airways. The higher PFR in this study in the PGD group coupled with the results showing the overlap between proteins captured by the EBPs compared to BALF sampling demonstrates that EBP collection can be an important diagnostic tool in the postoperative recipient. The advantages of such a technique include the ease with which the device can be connected to mechanical ventilation in addition to its lack of invasiveness, which is an improvement over traditional bronchoscopy. This technique can be implemented in clinical settings as a bedside diagnostic tool, thus allowing a transplant recipient to be monitored for the development of PGD in a convenient manner which can be leveraged for both rapid detection and more time-consuming but in-depth proteomic analysis.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: http://www.proteomexchange.org/, PXD046365.

ETHICS STATEMENT

The studies involving humans were approved by the Lund University Ethics Committee for Research. 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. The animal study was approved by Lund University Ethics Committee for Animal Research. The study was conducted in accordance with the local legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

Conceptualization: SL. Animal experiments: AN, GH, NB, HG, MS, EB, LP, SH, FO, and SL. Imaging: AN and NB. Mass spectrometry: AN, GH, SK, and MI. Funding acquisition: SK and SL. Writing–original draft: AN and SL. Writing–review and editing: AN, GH, FO, and SL. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.12298/full#supplementary-material

REFERENCES

1. Cantu E, Diamond JM, Cevasco M, Suzuki Y, Crespo M, Clausen E, et al. Contemporary Trends in PGD Incidence, Outcomes, and Therapies. *J Heart Lung Transpl* (2022) 41(12):1839–49. doi:10.1016/j.healun.2022.08.013
2. Huang HJ, Yusen RD, Meyers BF, Walter MJ, Mohanakumar T, Patterson GA, et al. Late Primary Graft Dysfunction After Lung Transplantation and Bronchiolitis Obliterans Syndrome. *Am J Transpl* (2008) 8(11):2454–62. doi:10.1111/j.1600-6143.2008.02389.x
3. Prekker ME, Nath DS, Walker AR, Johnson AC, Hertz MI, Herrington CS, et al. Validation of the Proposed International Society for Heart and Lung Transplantation Grading System for Primary Graft Dysfunction after Lung Transplantation. *J Heart Lung Transpl* (2006) 25(4):371–8. doi:10.1016/j.healun.2005.11.436
4. Whitson BA, Prekker ME, Herrington CS, Whelan TPM, Radosevich DM, Hertz MI, et al. Primary Graft Dysfunction and Long-Term Pulmonary Function After Lung Transplantation. *J Heart Lung Transpl* (2007) 26(10):1004–11. doi:10.1016/j.healun.2007.07.018
5. Broberg E, Hyllen S, Algotsson L, Wagner DE, Lindstedt S. Particle Flow Profiles from the Airways Measured by PExA Differ in Lung Transplant Recipients Who Develop Primary Graft Dysfunction. *Exp Clin Transpl* (2019) 17(6):803–12. doi:10.6002/ect.2019.0187
6. Broberg E, Andreasson J, Fakhro M, Olin AC, Wagner D, Hyllén S, et al. Mechanically Ventilated Patients Exhibit Decreased Particle Flow in Exhaled Breath as Compared to Normal Breathing Patients. *ERJ Open Res* (2020) 6(1):00198. doi:10.1183/23120541.00198-2019
7. Broberg E, Pierre L, Fakhro M, Algotsson L, Malmsjö M, Hyllén S, et al. Different Particle Flow Patterns From the Airways after Recruitment Manoeuvres Using Volume-Controlled or Pressure-Controlled Ventilation. *Intensive Care Med Exp* (2019) 7(1):16. doi:10.1186/s40635-019-0231-8
8. Hallgren F, Stenlo M, Niroomand A, Broberg E, Hyllén S, Malmsjö M, et al. Particle Flow Rate from the Airways as Fingerprint Diagnostics in Mechanical Ventilation in the Intensive Care Unit: A Randomised Controlled Study. *ERJ Open Res* (2021) 7(3):00961. doi:10.1183/23120541.00961-2020
9. Stenlo M, Hyllen S, Silva IAN, Bölükbas DA, Pierre L, Hallgren O, et al. Increased Particle Flow Rate from Airways Precedes Clinical Signs of ARDS in a Porcine Model of LPS-Induced Acute Lung Injury. *Am J Physiol Lung Cel Mol Physiol* (2020) 318(3):L510–L517. doi:10.1152/ajplung.00524.2019
10. Almstrand AC, Bake B, Ljungstrom E, Larsson P, Bredberg A, Mirgorodskaya E, et al. Effect of Airway Opening on Production of Exhaled Particles. *J Appl Physiol* (2010) 108(3):584–8. doi:10.1152/japplphysiol.00873.2009
11. Almstrand AC, Ljungstrom E, Lausmaa J, Bake B, Sjovall P, Olin AC. Airway Monitoring by Collection and Mass Spectrometric Analysis of Exhaled Particles. *Anal Chem* (2009) 81(2):662–8. doi:10.1021/ac802055k
12. Beck O, Olin AC, Mirgorodskaya E. Potential of Mass Spectrometry in Developing Clinical Laboratory Biomarkers of Nonvolatiles in Exhaled Breath. *Clin Chem* (2016) 62(1):84–91. doi:10.1373/clinchem.2015.239285
13. Bredberg A, Gobom J, Almstrand AC, Larsson P, Blennow K, Olin AC, et al. Exhaled Endogenous Particles Contain Lung Proteins. *Clin Chem* (2012) 58(2):431–40. doi:10.1373/clinchem.2011.169235
14. Hamilton BC, Kukreja J, Ware LB, Matthay MA. Protein Biomarkers Associated With Primary Graft Dysfunction Following Lung Transplantation. *Am J Physiol Lung Cel Mol Physiol* (2017) 312(4):L531–L541. doi:10.1152/ajplung.00454.2016
15. 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
16. 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 Cardiothorac Surg* (2020) 58(2):379–88. doi:10.1093/ejcts/ezaa043
17. Kosanam H, Sato M, Batruch I, Smith C, Keshavjee S, Liu M, et al. Differential Proteomic Analysis of Bronchoalveolar Lavage Fluid From Lung Transplant Patients With and Without Chronic Graft Dysfunction. *Clin Biochem* (2012) 45(3):223–30. doi:10.1016/j.clinbiochem.2011.11.015
18. Stone JP, Ball AL, Crichley W, Yonan N, Liao Q, Sjöberg T, et al. *Ex Vivo* Lung Perfusion Improves the Inflammatory Signaling Profile of the Porcine Donor Lung Following Transplantation. *Transplantation* (2020) 104(9):1899–905. doi:10.1097/TP.0000000000003338
19. 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
20. 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. *J Heart Lung Transpl* (2017) 36(10):1097–103. doi:10.1016/j.healun.2017.07.021
21. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: An Open-Source Platform for Biological-Image Analysis. *Nat Methods* (2012) 9(7):676–82. doi:10.1038/nmeth.2019
22. Demichev V, Messner CB, Vernardis SI, Lilley KS, Ralser M. DIA-NN: Neural Networks and Interference Correction Enable Deep Proteome Coverage in High Throughput. *Nat Methods* (2020) 17(1):41–4. doi:10.1038/s41592-019-0638-x
23. Kulkarni HS, Lee JS, Bastarache JA, Kuebler WM, Downey GP, Albaiceta GM, et al. Update on the Features and Measurements of Experimental Acute Lung Injury in Animals: An Official American Thoracic Society Workshop Report. *Am J Respir Cel Mol Biol* (2022) 66(2):e1–e14. doi:10.1165/rcmb.2021-0531ST
24. Li G, Flodby P, Luo J, Kage H, Sipos A, Gao D, et al. Knockout Mice Reveal Key Roles for Claudin 18 in Alveolar Barrier Properties and Fluid Homeostasis. *Am J Respir Cel Mol Biol* (2014) 51(2):210–22. doi:10.1165/rcmb.2013-0353OC
25. LaFemina MJ, Sutherland KM, Bentley T, Gonzales LW, Allen L, Chapin CJ, et al. Claudin-18 Deficiency Results in Alveolar Barrier Dysfunction and Impaired Alveologenesis in Mice. *Am J Respir Cel Mol Biol* (2014) 51(4):550–8. doi:10.1165/rcmb.2013-0456OC

26. Herwig MC, Tsokos M, Hermanns MI, Kirkpatrick CJ, Muller AM. Vascular Endothelial Cadherin Expression in Lung Specimens of Patients With Sepsis-Induced Acute Respiratory Distress Syndrome and Endothelial Cell Cultures. *Pathobiology* (2013) 80(5):245–51. doi:10.1159/000347062
27. Yadav E, Yadav N, Hus A, Yadav JS. Aquaporins in Lung Health and Disease: Emerging Roles, Regulation, and Clinical Implications. *Respir Med* (2020) 174: 106193. doi:10.1016/j.rmed.2020.106193
28. Moreno I, Vicente R, Ramos F, Vicente JL, Barbera M. Determination of Interleukin-6 in Lung Transplantation: Association With Primary Graft Dysfunction. *Transpl Proc* (2007) 39(7):2425–6. doi:10.1016/j.transproceed.2007.07.056
29. Pelaez A, Force SD, Gal AA, Neujahr DC, Ramirez AM, Naik PM, et al. Receptor for Advanced Glycation End Products in Donor Lungs Is Associated With Primary Graft Dysfunction After Lung Transplantation. *Am J Transpl* (2010) 10(4):900–7. doi:10.1111/j.1600-6143.2009.02995.x
30. Cagnone M, Piloni D, Ferrarotti I, Di Venere M, Viglio S, Magni S, et al. A Pilot Study to Investigate the Balance Between Proteases and α1-Antitrypsin in Bronchoalveolar Lavage Fluid of Lung Transplant Recipients. *High Throughput* (2019) 8(1):5. doi:10.3390/ht8010005
31. Muller C, Rosmark O, Ahrman E, Brunnström H, Wassilew K, Nybom A, et al. Protein Signatures of Remodeled Airways in Transplanted Lungs With Bronchiolitis Obliterans Syndrome Obtained Using Laser-Capture Microdissection. *Am J Pathol* (2021) 191(8):1398–411. doi:10.1016/j.ajpath.2021.05.014
32. Shah RJ, Diamond JM, Cantu E, Lee JC, Lederer DJ, Lama VN, et al. Latent Class Analysis Identifies Distinct Phenotypes of Primary Graft Dysfunction After Lung Transplantation. *Chest* (2013) 144(2):616–22. doi:10.1378/chest.12-1480

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Implications of High Sensitivity Troponin Levels After Lung Transplantation

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Trends in high-sensitivity cardiac troponin I (hs-cTnI) after lung transplant (LT) and its clinical value are not well stablished. This study aimed to determine kinetics of hs-cTnI after LT, factors impacting hs-cTnI and clinical outcomes. LT recipients from 2015 to 2017 at Toronto General Hospital were included. Hs-cTnI levels were collected at 0–24 h, 24–48 h and 48–72 h after LT. The primary outcome was invasive mechanical ventilation (IMV) >3 days. 206 patients received a LT (median age 58, 35.4% women; 79.6% double LT). All patients but one fulfilled the criteria for postoperative myocardial infarction (median peak hs-cTnI = 4,820 ng/mL). Peak hs-cTnI correlated with right ventricular dysfunction, >1 red blood cell transfusions, bilateral LT, use of EVLP, kidney function at admission and time on CPB or VA-ECMO. IMV>3 days occurred in 91 (44.2%) patients, and peak hs-cTnI was higher in these patients (3,823 vs. 6,429 ng/mL, $p < 0.001$ after adjustment). Peak hs-cTnI was higher among patients with had atrial arrhythmias or died during admission. No patients underwent revascularization. In summary, peak hs-TnI is determined by recipient comorbidities and perioperative factors, and not by coronary artery disease. Hs-cTnI captures patients at higher risk for prolonged IMV, atrial arrhythmias and in-hospital death.

Keywords: lung transplant, troponin, primary graft dysfunction, mechanical ventilation, arrhythmia

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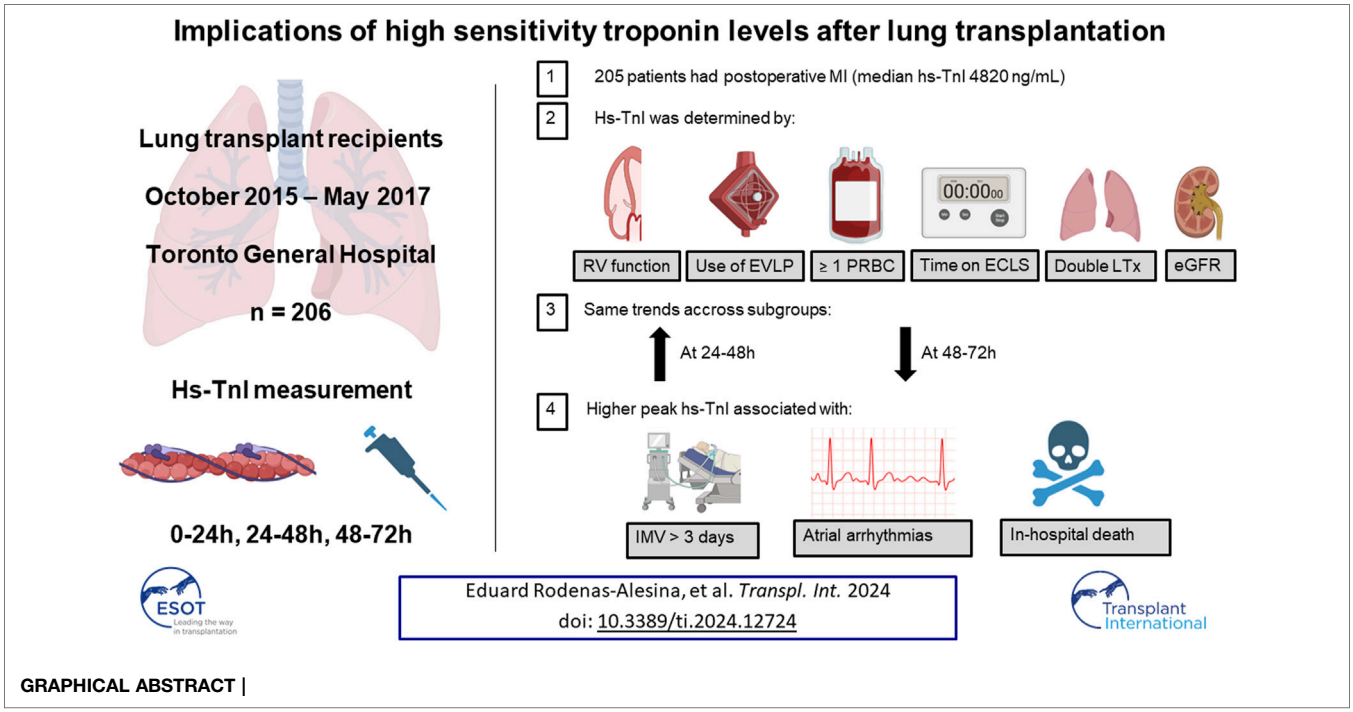
Abbreviations: AUC, area under the curve; IMV, invasive mechanical ventilation; LTx, lung transplant; VV-ECMO, veno-venous extracorporeal membrane oxygenation; BMI, body mass index; CABG, coronary artery bypass graft; CAD, coronary artery disease; CI, confidence interval; COPD, chronc obstructive pulmonary disease; CPB, cardiopulmonary bypass; eGFR, estimated glomerular filtration rate; EVLP, *ex-vivo* lung perfusion; hs-cTnI, high-sensitivity cardiac troponin I; ICU, intensive care unit; MCS, mechanical circulatory support; MI, myocardial infarction; OR, odds ratio; PCI, percutaneous coronary intervention; PGD, primary graft dysfunction; ROC, receiver-operating characteristic; RV, right ventricular; VA-ECMO, veno-arterial extracorporeal membrane oxygenation.



GRAPHICAL ABSTRACT |

INTRODUCTION

The use of high sensitivity cardiac troponin I (hs-cTnI) measurement after cardiac surgery is well stablished, and is associated with higher risk of death, prolonged invasive mechanical ventilation (IMV), and prolonged length of stay. In cardiac surgery, there are multiple factors involved in postoperative hs-cTnI levels, such as ischemia-reperfusion injury, cannulation and manipulation, type of cardioplegia used, inflammation and elevation of filling pressures, with direct damage to the coronary artery tree being rare [1]. Despite its recognized predictive value, routinary measurement on hs-TnI immediately after surgery is not useful in certain settings, such as in heart transplantation [2].

In non-cardiac surgery, hs-cTnI is commonly used to rule out perioperative myocardial injury following non-cardiac surgery (MINS), which has been associated with higher mortality, atrial arrhythmias and admissions for heart failure [3–5]. The mechanisms leading to hs-cTnI rise are not fully elucidated, but seem to be related as well with perioperative conditions and baseline characteristics, and consistently associated with worse outcomes irrespective of the presence of ischemic symptoms or ECG changes [6], with a dose-graded response based on degree of post-operative hs-cTnI elevation [7]. With the development newer hs-cTnI assays, the incidence of perioperative MINS will likely increase as it is now recommended to screen high-risk patients postoperatively [7].

The use of hs-cTnI in the lung transplant (LTx) population has not been well studied. During LTx, hemodynamic stability and direct cardiac damage may confer a different value to hs-cTnI to that of non-cardiac surgery, but there is paucity of data regarding the elevation of hs-cTnI in the early postoperative course of LTx recipients, limited by sample size and lack of serial measurements [8, 9]. With the new hs-cTnI assays replacing the old ones, it remains crucial to examine the normal trend in hs-cTnI in LTx to guide decision-making.

This study sought was to evaluate the levels and trend of hs-cTnI during the first 72 h after LTx, factors impacting on hs-cTnI levels and the prognostic value of hs-cTnI in the intensive care unit (ICU) setting.

MATERIALS AND METHODS

Patient Population

Consecutive patients (≥18 years of age) who received a LTx from October 2015 to May 2017 who were admitted to Toronto General Hospital, University Health Network medical-surgical intensive care unit (MSICU) were included in this retrospective registry. The study protocol was approved by the local Research Ethics Board (CAPCR study #17-5633).

Data Collection and Measurement

Data was collected from the electronic medical record. Pre-operative demographics, co-morbid medical illnesses, medications, and cardiac testing was recorded. As part of their lung transplant workup, patients underwent extensive cardiac testing including electrocardiography, transthoracic echocardiography, non-invasive stress testing, coronary angiography and right heart catheterization. RV dysfunction was categorized as none, mild, moderate, or

TABLE 1 | Baseline characteristics of the cohort divided according to prolonged invasive mechanical ventilation (>3 days).

	Total (*n* = 206)	IMV for ≤3 days (*n* = 115)	IMV for >3 days (*n* = 91)	*p*-value
Age	58.2 (48.1–64.4)	59.9 (49.1–65.4)	57.8 (47.2–62.4)	0.19
Female sex	73 (35.4%)	39 (33.9%)	34 (37.4%)	0.61
BMI (kg/m^2)	24.7 (20.3–28.7)	24.9 (20.3–28.4)	24.1 (20.5–29.0)	0.92
Hypertension	39 (18.9%)	18 (15.7%)	21 (23.1%)	0.18
Etiology				0.45
COPD	41 (20.1%)	28 (24.6%)	13 (14.4%)	
Cystic fibrosis	24 (11.8%)	12 (10.5%)	12 (13.3%)	
Pulmonary hypertension	10 (4.9%)	3 (2.6%)	7 (7.8%)	
Sarcoid	2 (1.0%)	1 (0.9%)	1 (1.1%)	
Retransplant	2 (1.0%)	1 (0.9%)	1 (1.1%)	
Interstitial lung disease	106 (52.0%)	58 (50.9%)	48 (53.3%)	
Other	19 (9.3%)	11 (9.6%)	8 (8.9%)	
Dyslipidemia	42 (20.4%)	19 (16.5%)	23 (25.3%)	0.12
Diabetes	40 (19.4%)	17 (14.8%)	23 (25.3%)	0.059
Non-flow limiting CAD	149 (73.4%)	83 (73.5%)	66 (73.3%)	0.98
Previous MI	4 (2.0%)	3 (2.6%)	1 (1.1%)	0.43
History of PCI	19 (9.3%)	11 (9.6%)	8 (8.9%)	0.87
History of CABG	5 (2.4%)	3 (2.6%)	2 (2.2%)	0.85
Atrial fibrillation or flutter	4 (2.0%)	2 (1.8%)	2 (2.2%)	0.82
Chronic heart failure	1 (0.5%)	0 (0.0%)	1 (1.1%)	0.26
Right ventricular function				0.51
Normal	149 (72.3%)	85 (73.9%)	64 (70.3%)	
Mild	37 (18.0%)	22 (19.1%)	15 (16.5%)	
Moderate	10 (4.9%)	4 (3.5%)	6 (6.6%)	
Severe	10 (4.9%)	4 (3.5%)	6 (6.6%)	
Chronic kidney disease	2 (1.0%)	1 (0.9%)	1 (1.1%)	0.86
Cerebrovascular accident	4 (1.9%)	1 (0.9%)	3 (3.3%)	0.21
Hemoglobin (g/L)	142.0 (128.0–152.0)	144.0 (134.0–154.0)	137.0 (123.0–150.0)	0.006
Sodium (mmol/L)	140.0 (138.0–141.0)	140.0 (138.0–141.0)	140.0 (137.0–141.0)	0.84
Potassium (mmol/L)	4.0 (3.7–4.2)	4.0 (3.8–4.1)	4.0 (3.7–4.2)	0.55
eGFR (mL/m^2/1.73 m^2)	94.0 (79.0–105.0)	94.0 (78.0–104.0)	93.5 (79.0–107.0)	1.00
Bridged with VV-ECMO	4 (1.9%)	0 (0.0%)	4 (4.4%)	0.023
Bridged with VA-ECMO	15 (7.3%)	3 (2.6%)	12 (13.2%)	0.004
Bilateral lung transplant	164 (79.6%)	84 (73.0%)	80 (87.9%)	0.009
Use of EVLP	66 (32.0%)	32 (27.8%)	34 (37.4%)	0.15
ECMO intraoperatively	89 (43.2%)	38 (33.0%)	51 (56.0%)	<0.001
CPB intraoperatively	14 (6.8%)	5 (4.3%)	9 (9.9%)	0.12
Time on intraoperatively MCS	0.0 (0.0–199.0)	0.0 (0.0–123.0)	70.0 (0.0–243.0)	<0.001
Reperfusion PCO_2	0.4 (0.4–0.5)	0.5 (0.4–0.5)	0.4 (0.4–0.5)	0.070
Reperfusion O_2	1.0 (1.0–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.0)	0.95
Number of PRBCs				<0.001
No PRBC needed	92 (45.1%)	63 (55.3%)	29 (32.2%)	
1 PRBC used	69 (33.8%)	44 (38.6%)	25 (27.8%)	
>1 PRBC used	43 (21.1%)	7 (6.1%)	36 (40.0%)	
VV-ECMO after surgery	11 (5.3%)	1 (0.9%)	10 (11.0%)	0.001
VA-ECMO after surgery	10 (4.9%)	4 (3.5%)	6 (6.6%)	0.30
Ischemic time—left lung (min)	480.5 (409.5–609.5)	477.5 (402.0–626.0)	489.5 (419.0–589.0)	0.82
Ischemic time—right lung (min)	465.0 (373.0–629.0)	459.0 (361.0–573.0)	475.0 (394.0–652.0)	0.20
Troponin at 24 h (ng/mL)	3347.0 (1863.0–5823.0)	2749.5 (1556.0–4538.0)	4386.0 (2660.0–7716.0)	<0.001
Troponin at 48 h (ng/mL)	4339.0 (2615.0–6680.0)	3696.0 (2050.0–5999.0)	5314.0 (3594.0–8371.0)	<0.001
Troponin at 72 h (ng/mL)	3235.0 (2050.0–4800.0)	2875.0 (1824.0–3995.0)	3520.0 (2492.5–6321.5)	0.003
Peak troponin (ng/mL)	4820.0 (2894.0–7331.0)	3823.0 (2392.0–5992.0)	6429.0 (3873.0–9418.0)	<0.001

IMV, invasive mechanical ventilation; BMI, body mass index; COPD, chronic obstructive pulmonary disease; CAD, coronary artery disease; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; eGFR, estimated glomerular filtration rate; VV-ECMO, veno-venous extracorporeal membrane oxygenation; VA-ECMO, veno-arterial extracorporeal membrane oxygenation; EVLP, Ex-ViVo *Lung Perfusion; CPB, cardiopulmonary bypass; MCS, mechanical circulatory support; PRBC, packed red blood cells.*

severe based on the visual assessment on the echocardiography. Mild coronary artery disease (CAD) was defined as any non-obstructive coronary lesion <70% with <50% for left main disease. Any CAD more than mild was revascularized prior to listing.

Surgical interventions were performed by thoracic surgeons, along with a dedicated group of thoracic anesthesiologists, according to the Toronto Lung Transplant Program technique [10]. On a case-by-case basis, surgery was performed on cardiopulmonary bypass (CPB), veno-arterial extracorporeal

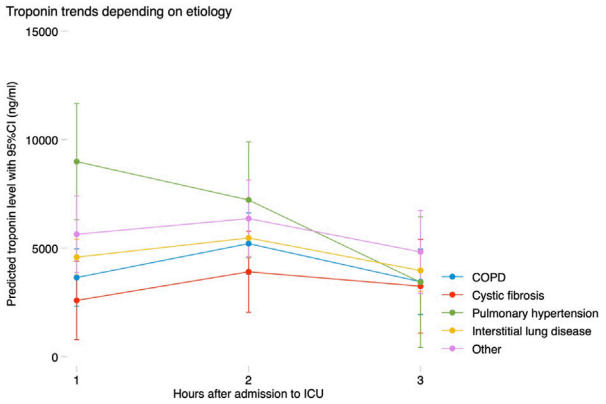


FIGURE 1 | Trends in troponin according to etiology of the underlying lung disease. COPD, chronic obstructive pulmonary disease; CI, confidence interval; ICU, intensive care unit.

membrane oxygenation (VA-ECMO) or without mechanical circulatory support (MCS). We collected the use of MCS before and after the surgery, and the length of MCS for the intraoperative period. Ischemic time and reperfusion arterial blood gas samples were also recorded.

Hs-cTnI was measured during the first 24 h, between 24 and 48 h and between 48 and 72 h after LTx on a routine basis. Peak hs-cTnI was considered the highest hs-cTnI among three measurements available. The hs-cTnI assay used at our institution was the Abbott Alinity high sensitivity troponin I assay, with a 99% upper limit of normal of 26 ng/L.

Definition of Endpoints

The primary endpoint was prolonged IMV defined as IMV for >3 days. Secondary endpoints included in-hospital mortality, postoperative atrial arrythmias (inclusive of atrial fibrillation, atrial flutter, or atrial tachycardia), length of stay in ICU and primary graft dysfunction (PGD) 72 h post LTx, defined by current guidelines based on a P/F ratio <300 72 h after LTx with lung infiltrates on a chest X ray [11]. All patients were followed up until death or hospital discharge, whichever occurred first, and there were no patients lost to follow-up.

Statistical Analysis

Mann-Whitney U test was used to assess differences in baseline characteristics at the time of admission, and median and interquartile range are provided. For categorical variables, proportions were compared using a chi-squared test, and counts and percentages are given.

To determine clinical factors determining peak hs-cTnI level, univariate analysis with linear regression for preoperative and surgical variables was done. Variables with missing in >25% were not used for multivariate analysis, and we performed complete case analysis. For the multivariate analysis, a backwards stepwise selection method ($p < 0.05$ for inclusion, $p > 0.10$ for exclusion) was conducted to include all relevant variables among those with a p-value <0.2 in univariate analysis. Collinearity within the final model was considered unacceptable if variance inflation factor was >4. The trends in hs-cTnI levels after LTx were assessed using a mixed-effects model for repeated measures, and both intercepts and slopes were compared between prespecified subgroups. Prespecified comparison groups to assess trends in postsurgical hs-cTnI trends were performed based on pre-existing non-flow limiting CAD, pulmonary hypertension, right ventricular (RV) dysfunction, use of preoperative ECMO, use of MCS during surgery, bilateral LTx, use of *ex vivo* lung perfusion system (EVLP) and number of blood transfusions required during surgery.

TABLE 2 | Effect on peak troponin within the initial 72 h after lung transplant of each predictor in univariate and multivariate linear regression analysis.

	Univariate		Multivariate	
	Peak troponin	***p*-value**	**Peak troponin**	***p*-value**
Hypertension	1,316	0.190	1,277	0.074
Dyslipidemia	2,245	0.021	—	—
Chronic kidney disease	19,063	0.001	—	—
Chronic heart failure	9,187	0.104	—	—
Mild coronary artery disease	1,407	0.123	—	—
Pulmonary hypertension	3,086	<0.001	—	—
Severe RV dysfunction	4,129	0.023	3,612	0.011
eGFR (per mL/min/1.73 m^2)	−55	0.007	−40	0.005
Bridged with VV-ECMO	4,167	0.143	—	—
Bridged with VA-ECMO	−1,977	0.191	—	—
Bilateral lung transplant	3,869	<0.001	3,288	<0.001
Use of EVLP	1,996	0.018	1,528	0.010
VA-ECMO during surgery	2,672	0.001	—	—
Time on MCS (per min)	16	<0.001	9	<0.001
>1 PRBC used	4,640	<0.001	1,827	0.011

The multivariate model was chosen based on a backwards stepwise regression.
RV, right ventricular; eGFR, estimated glomerular filtration rate; VV-ECMO, veno-venous extracorporeal membrane oxygenation; VA-ECMO, veno-arterial extracorporeal membrane oxygenation; CPB, cardiopulmonary bypass; MCS, mechanical circulatory support; PRBC, packed red blood cells.

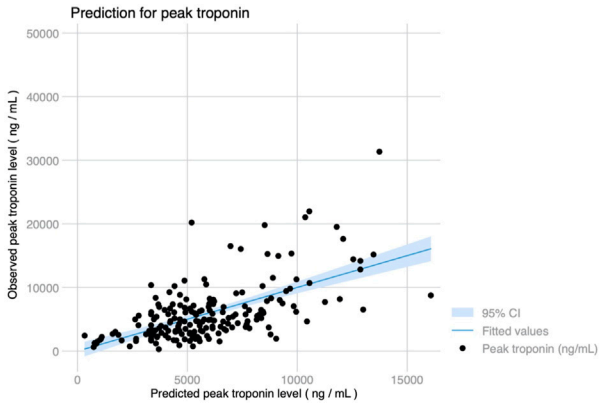


FIGURE 2 | Observed and predicted peak troponin levels using a 7-variable model including RV dysfunction, hypertension, eGFR, bilateral lung transplant, use of EVLP, length of intraoperative MCS and transfusion requirements (Pearson's r = 0.604, $p < 0.001$).

Peak hs-cTnI was tested as a predictor for the primary and secondary endpoints. Medians between patients with and without the endpoint were compared using U Mann Whitney test. Univariate analysis was performed using logistic regression and an optimal cutoff for each endpoint was selected using receiver-operator curve (ROC) analysis and Youden's index. Temporal trends were compared between groups in a mixed effects model for repeated measurements. Multivariate analysis for the primary endpoint was done by including in a backwards stepwise regression all clinically relevant predictors with a *p*-value <0.2. For in-hospital mortality, as there were a relatively low number of events, bivariate analyses for *a priori* clinically relevant covariates were done. To obtain the probability of the primary endpoint based on peak hs-cTnI levels, peak hs-cTnI was modelled as a restricted cubic spline with 3 knots. A two-tailed *p*-value <0.05 was considered significant for all comparisons. Analyses were performed using Stata 15.0 for Mac (StataCorp LLC, Texas, United States).

RESULTS

During the study period, 206 LTx patients were included (**Table 1**). Median age was 58 (48–64) years and 35.4% were women. Intestitial lung disease was the most common indication for LTx (52.0%), followed by chronic obstructive pulmonary disease (20.1%), with 79.6% of patients receiving a bilateral LTx. Though 73.4% of recipients had mild CAD following coronary angiography, only 9.3% received percutaneous coronary intervention prior to listing. RV dysfunction and atrial arrhythmias were infrequent at the time of surgery. Only 1.9% of patients were bridged to LTx with VV-ECMO, and 7.3% with VA-ECMO. However, VA-ECMO was applied intraoperatively in 43.2%, and CPB in 6.8% of cases. EVLP was used in 32% of cases.

All patients met the criteria for MINS, and 99.5% of them even reached a hs-cTnI 10 times above the upper limit of normal, which is considered the threshold to define a coronary artery bypass graft-related MI [12]. No patients suffered from a ST elevation MI. Hs-cTnI levels at 0–24 h, 24–48 h and 48–72 h were available in 207 (100%), 185 (89.4%) and 137 (66.2%) patients, respectively. Median hs-cTnI level in the first 24 h was 3,347 (1863–5,823) ng/mL, and maximum hs-cTnI in the first 72 h was 4,820 (2,894–7,331) ng/mL. After LTx, hs-cTnI progressively increased reaching a peak between 24 and 48 h, and then decreased between 48 and 72 h. This trend was similar in all the prespecified subgroups assessed, with higher baseline levels in patients with pulmonary hypertension, who experienced a steeper decline after LTx (**Figure 1**).

Peak hs-cTnI within the first 72 h was higher in patients with dyslipidemia, hypertension, previous heart failure, pulmonary hypertension, poor RV function, chronic kidney disease or mild CAD. Patients supported with either VV or VA-ECMO preoperatively, those receiving a bilateral LTx or patients in which EVLP was used had greater hs-cTnI levels postoperatively. The use of MCS (either CPB or VA-ECMO) during surgery and the time on support also were strongly associated with hs-cTnI rise, as well as the number of transfusions required. Independent predictors for peak hs-cTnI levels are shown in **Table 2**, and were severe RV dysfunction, eGFR at the time of LTx, use of EVLP, receiving a bilateral LTx, prolonged time on MCS intraoperatively and receiving >1 red blood cell transfusion. Predicted hs-cTnI levels using this model had a strong correlation with the observed peak hs-cTnI (Pearson's r = 0.604, $p < 0.001$) (**Figure 2**).

Median ICU stay was 4 (2–14) days, and median hospital stay was 23 (16–53) days. There were 91 (44.2%) patients who met the primary endpoint and were on IMV for >3 days. Peak hs-cTnI was significantly higher in patients that required IMV for >3 days compared to those that were weaned earlier (6,429 vs. 3,823 ng/mL, $p < 0.001$; **Figure 3**). Peak hs-cTnI alone displayed an AUC = 0.72 (0.65–0.79) to predict prolonged IMV. Peak hs-cTnI was associated with the primary endpoint in both unadjusted (OR per 100 ng/mL increase = 1.01, 95% CI 1.01–1.02) and adjusted analysis (OR per 100 ng/mL increase = 1.02, 95% CI 1.01–1.03). **Figure 4** shows how the probability of requiring prolonged IMV increases at higher peak hs-cTnI levels. **Table 3** shows univariate predictors for the primary endpoint. Multivariate analysis identified peak hs-cTnI, postoperative VV-ECMO, requirement of more than 1 red blood cell transfusion and pulmonary hypertension as the only independent predictors for prolonged mechanical ventilation.

There were 13 (6.3%) patients who died during hospital admission. Peak hs-cTnI was significantly higher in patients who died during admission (9,690 vs. 4,659 ng/mL, $p = 0.001$) (**Figure 5A**). Peak hs-cTnI had an AUC = 0.78 (0.63–0.93) for in-hospital mortality, with the best cut-off at 7,840 ng/mL. Death occurred in 9 (20.5%) patients with a hs-cTnI >7,840 ng/mL and in 4 (2.5%) with a peak hs-cTnI <7,840 ng/mL ($p < 0.001$, **Figure 5B**). Compared to

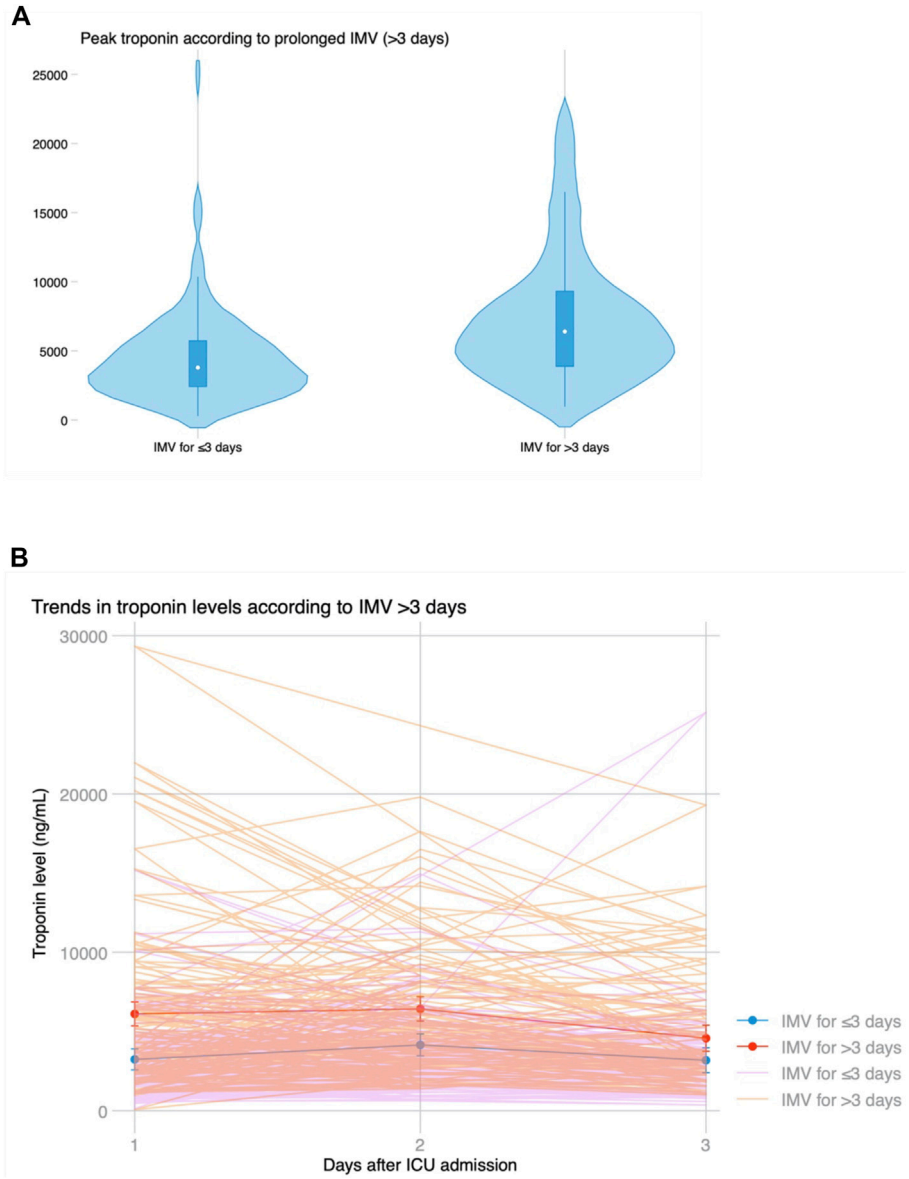


FIGURE 3 | (A) Violin plot showing peak troponin based on prolonged invasive mechanical ventilation for >3 days. **(B)** Temporal trends in troponin levels after LT based on prolonged ventilation, showing significantly higher initial troponin levels but similar kinetics and progressive decline on the third day in both groups.

survivors, patients experiencing in-hospital mortality had a continued hs-cTnI rise with failure to decrease the circulating hs-cTnI levels on the third day (**Figure 5C**). The association between peak hs-cTnI and mortality remained significant in all bivariate analysis (including MCS use before, during or after surgery, age, diabetes, pulmonary hypertension, or poor RV function).

Atrial arrhythmias were frequent and occurred in 88 (43.4%) patients after LTx. Peak hs-cTnI was associated with the occurrence of atrial arrhythmias (5,833 vs. 4,350 ng/mL, p = 0.008) (**Table 4**). There was no association between peak hs-cTnI and primary graft dysfunction at 72 h after LTx, suggesting that graft dysfunction does not mediate the association between peak hs-cTnI and prolonged IMV. Patients with a peak hs-cTnI above the median had a longer length of stay in ICU (median 8 vs. 3 days, $p < 0.001$), and a longer hospital length of stay (median 29 vs. 21 days, p = 0.002). After LTx, only six patients had a coronary angiogram performed as recommended by cardiology consultation based on hs-cTnI trends, and percutaneous coronary intervention was not necessary in any case. There was no correlation between peak hs-cTnI and left ventricular ejection fraction after LTx in those with an available measurement by echocardiography (n = 63, Pearson's r = −0.207, p = 0.10).

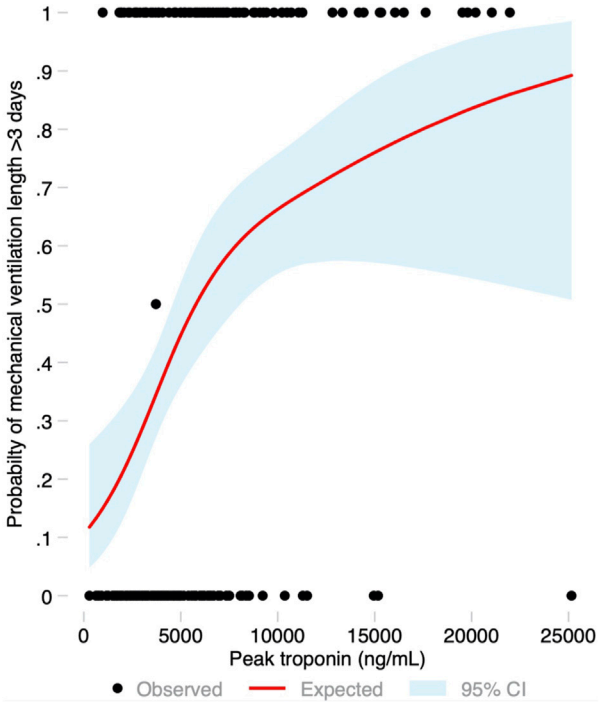


FIGURE 4 | Probability of the primary endpoint (invasive mechanical ventilation for more than 3 days) based on the peak troponin level modeled as a restricted cubic spline, with its 95% confidence interval.

TABLE 3 | Odds ratio and 95% confidence interval for the primary endpoint (invasive mechanical ventilation for >3 days) in univariate and multivariate logistic regression analysis.

	Univariate	Multivariate
	Peak troponin	Peak troponin
Hypertension	1.62 (0.80–3.26)	—
Dyslipidemia	1.71 (0.86–3.38)	—
Diabetes	1.95 (0.97–3.92)	—
Chronic heart failure	(collinear)	—
Pulmonary hypertension	2.60 (1.41–4.80)	2.25 (1.05–4.85)
Hemoglobin (per g/L)	0.98 (0.96–0.99)	—
Bridged with VV-ECMO	(collinear)	—
Bridged with VA-ECMO	5.67 (1.55–20.76)	—
Bilateral lung transplant	2.68 (1.26–5.70)	—
Use of EVLP	1.55 (0.86–2.79)	—
CPB during surgery	2.41 (0.78–7.47)	—
VA-ECMO during surgery	2.58 (1.46–4.56)	—
Time on MCS (per min)	1.00 (1.00–1.01)	—
>1 PRBC used	10.19 (4.26–24.40)	7.20 (2.45–21.16)
VV-ECMO after surgery	14.07 (1.77–112.12)	15.26 (1.68–138.51)
Peak troponin (per 100 ng/mL)	1.01 (1.01–1.02)	1.02 (1.01–1.03)

RV, right ventricular; eGFR, estimated glomerular filtration rate; VV-ECMO, veno-venous extracorporeal membrane oxygenation; VA-ECMO, veno-arterial extracorporeal membrane oxygenation; CPB, cardiopulmonary bypass; MCS, mechanical circulatory support; PRBC, packed red blood cells.
The table only shows predictors with a p-value <0.2 in univariate analysis. Chronic heart failure (n = 1) and preoperative support with VV-ECMO (n = 4) were perfect predictors of the endpoint and these patients were therefore excluded from multivariate analysis.

DISCUSSION

In this study, we measured serial hs-cTnI levels in consecutive LTx recipients upto 72 h after returning to the intensive care setting. We identified that hs-cTnI rise above the defined threshold for MINS was seen in all LTx recipients, and it was associated with perioperative risk factors and not with flow-limiting CAD. After LTx, high peak hs-cTnI is an independent predictor for prolonged IMV, postoperative atrial arrhythmias and in-hospital mortality, probably as a reflection of preoperative and perioperative cardiac stress.

The fourth universal definition of MI defines a type V MI as a CABG-related MI, leaving all other postoperative, non-revascularization related MI within a separate category poorly characterized [12]. Within 72 h after LTx, 99.5% of our population had an elevated hs-cTnI >10 times above the 99th percentile, as documented before [9]. There were no coronary plaque ruptures documented, no significant CAD before surgery, no need for revascularization during hospital admission and no correlation with left ventricular ejection fraction, suggesting that hs-cTnI rise is explained by factors related to the surgical intervention and the postoperative course, especially since the LTx operation necessitates cutting and sewing the atrial myocardium, and the rise in hs-cTnI does not reflect coronary artery disease. The independent predictors observed in our cohort for hs-cTnI rise can be explained by elevated end-diastolic pressures, such as an elevation of RV end-diastolic pressure in those with pre-existent RV dysfunction worsened with pulmonary artery cross-clamp, or elevation in left ventricular end-diastolic pressure with retrograde arterial flow from VA-ECMO. Longer time of surgical manipulation likely explains the association between bilateral LTx with peak hs-cTnI. The association between peak hs-cTnI, length of MCS support and requiring more than one transfusion may also be related to hemodynamic stability and supply-demand ischemia, whereas a worse preoperative eGFR may prevent hs-cTnI washout after LTx. The association with more extensive intreventions, length of cardiac manipulation and comorbidities have also been observed in other non-LT thoracic surgeries [13]. Therefore, our results support that coronary events are rare and peak hs-TnI levels correspond to either type 2 MI due to demand-supply mismatch related to preoperative factors, to the surgical intervention or more likely secondary to direct myocardial injury by cutting and suturing of the recipient and donor myocardium during LTx.

In our cohort, peak hs-cTnI was associated with prolonged IMV, atrial arrhythmias and death. There have been two other studies evaluating the role of hs-cTnI after LTx surgery. In one of them, including 95 patients, higher hs-cTnI measured on arrival to the ICU was found to be associated with mortality, but this was published only as an abstract with no more information being available [8]. Another study described the impact on 30 days and 1-year mortality of a single hs-cTnI measurement upon admission to the ICU. The authors also described an association between a higher troponin and mortality [9]. The association between peak hs-cTnI and worse outcomes is well described in both cardiac and non-cardiac surgery. A recent publication demonstrated a strong association between hs-

TABLE 4 | Peak troponin levels based on the occurrence of the primary and secondary endpoints.

	Number of events	Troponin in those with event	Troponin in those without event	*p*-value
IMV >3 days	91 (44.0%)	6,429 (3,873–9,418)	3,823 (2,392–5,992)	<0.001
In-hospital mortality	13 (6.3%)	11,081 (6,249–19,802)	4,675 (2,773–6,937)	<0.001
Atrial arrhythmias	88 (43.4%)	5,833 (3,334–8,107)	4,350 (2,499–6,428)	0.008
Primary graft dysfunction	48 (33.8%)	4,838 (3,035–7,405)	6,142 (3,749–8,262)	0.087

IMV, invasive mechanical ventilation.

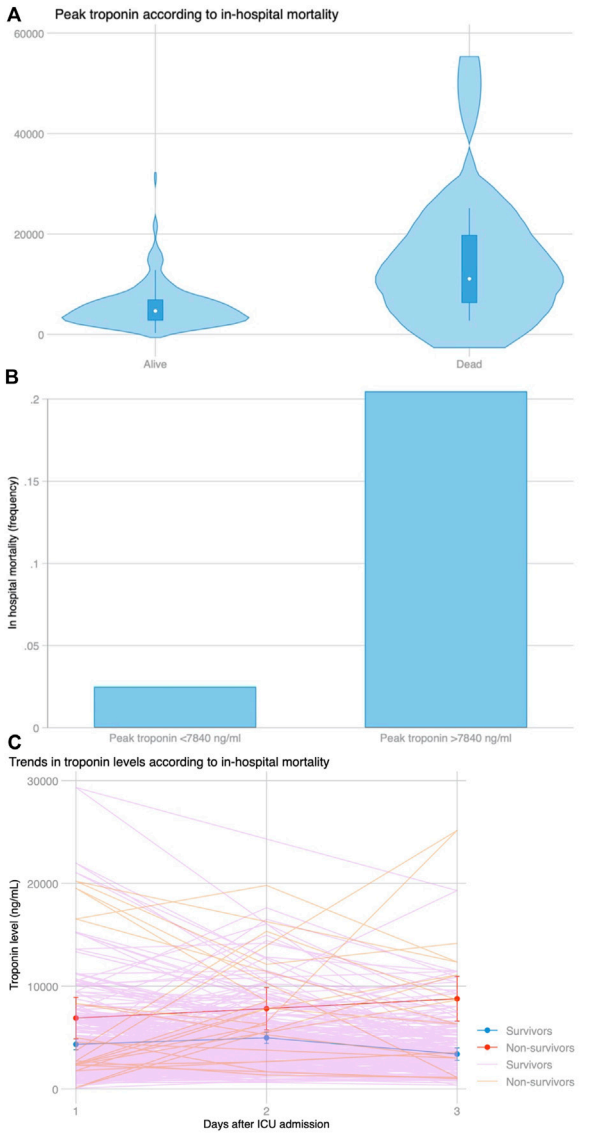


FIGURE 5 | (A) Distribution of peak troponin according to in-hospital mortality. **(B)** In-hospital mortality based on the best cut-off for peak troponin (7,840 ng/mL) selected by Youden's index using ROC curve analysis. **(C)** Trends in troponin levels among patients who survived during admission and those who did not survive the index admission. After a similar increase at day 2 compared to day 1 among both groups ($p = 0.778$), peak troponin continued to rise in patients who died, whereas it declined for patients who survived ($p = 0.008$ for the slope).

cTnI elevation and mortality with a similar cutoff to predict death (hs-cTnI >5,670 ng/mL), and a slightly lower cutoff for other complications, as observed in our study [14]. The most likely explanation for this association is that hs-cTnI probably captures several factors that confer a worse prognosis after LTx, as it reflects both direct and indirect cardiac damage, as demonstrated in our study.

Normal troponin kinetics after LTx consist of an early peak at 24–48 h and a progressive decline thereafter, as also noted in a study including only 10 LTx recipients [15]. However, we describe a novel association between failure to decrease hs-cTnI levels and in-hospital death, suggesting that hs-cTnI monitoring up to 72 h may be useful to identify these high-risk patients. We observed that a continued rise in hs-cTnI levels beyond 48 h is of concern, and this trend in hs-cTnI kinetics merits careful review from the clinician to identify alternative diagnoses which may explain the persistent rise in serum levels. If the hs-cTnI levels do not trend down beyond 48 h, this may suggest that hs-cTnI level may not be due to myocardial injury from the surgical intervention alone, although we did not observe in our cohort any significant coronary artery disease that could explain hs-cTnI rise, and the association could be explained by clinical worsening, oxygen supply-demand mismatch or decreased hs-cTnI clearance. A broad differential diagnosis exists and alternative etiologies of persistent hs-cTnI elevation in this population may include cardiac arrhythmias, renal failure, respiratory failure, or sepsis [16].

We must acknowledge the limitations of a single center retrospective study, and conclusions can only be hypothesis-generating. Despite having a relatively low number of deaths, it is the largest analysis of hs-cTnI trends after LTx. Unfortunately, hs-cTnI was not available in all LTx recipients at 48 and 72 h and selection bias cannot be completely excluded, but comparison of patients with missing and non-missing hs-cTnI did not reveal any major differences.

Overall, our study shows that, in LTx recipients, peak hs-cTnI within the initial 72 h after surgery is elevated ten times above the range of MINS in >99% of patients. Peak hs-cTnI is not related to coronary artery disease and is most likely related to surgical manipulation of the cardiac atrial tissue, recipient comorbidities and the clinical situation at the time of LTx, hemodynamic stability during the intervention and perioperative factors. An elevated peak and persistent elevation of hs-cTnI identifies patients with higher rates of prolonged ventilation, atrial arrhythmias, and in-hospital death, as it likely reflects a worsened preoperative status with a greater degree of oxygen supply-demand mismatch during surgery and in the early postoperative period.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by the University Health Network Research Ethics Board (CAPCR study #17-5633). The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because retrospective observational study.

AUTHOR CONTRIBUTIONS

AL, CO, and LD designed the study. AL, JG, AA, GM, CS, and KM collected the data. AL, JG, AA, GM, CS, KM, CO, MC, LS, JT, and SK provided care for the patients included in the study. ER-A did the statistical analysis and prepared the original draft. AL, JG, AA, GM, CS, KM, CO, MC, LS, JT, SK, and LD critically reviewed the manuscript. AL and LD supervised the project. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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REFERENCES

1. Heuts S, Gollmann-Tepeköylü C, Denessen EJS, Olsthoorn JR, Romeo JLR, Maessen JG, et al. Cardiac Troponin Release Following Coronary Artery Bypass Grafting: Mechanisms and Clinical Implications. *Eur Heart J* (2023) 44(2):100–12. doi:10.1093/eurheartj/ehac604
2. Erbel C, Taskin R, Doesch A, Dengler TJ, Wangler S, Akhavanpoor M, et al. High-Sensitive Troponin T Measurements Early After Heart Transplantation Predict Short and Long-Term Survival. *Transpl Int Off J Eur Soc Organ Transpl* (2013) 26(3):267–72. doi:10.1111/tri.12024
3. Devereaux PJ, Chan MTV, Alonso-Coello P, Walsh M, Berwanger O, Villar JC, et al. Association Between Postoperative Troponin Levels and 30-Day Mortality Among Patients Undergoing Noncardiac Surgery. *JAMA* (2012) 307(21):2295–304. doi:10.1001/jama.2012.5502
4. Lasocki S, Provenchère S, Bénessiano J, Vicaut E, Lecharny J-B, Desmonts J-M, et al. Cardiac Troponin I Is an Independent Predictor of In-Hospital Death After Adult Cardiac Surgery. *Anesthesiology* (2002) 97(2):405–11. doi:10.1097/00000542-200208000-00018
5. Ma Q-L, Wang H-J, Shi M-N, An J-H, Ma J, Yu D, et al. Serum Troponin I Concentrations Assessed 18-24 Hours After Coronary Artery Bypass Grafting Are Significant Predictors of Early Patient Prognosis. *Eur Rev Med Pharmacol Sci* (2016) 20(19):4129–35.
6. Botto F, Alonso-Coello P, Chan MTV, Villar JC, Xavier D, Srinathan S, et al. Myocardial Injury After Noncardiac Surgery: A Large, International, Prospective Cohort Study Establishing Diagnostic Criteria, Characteristics, Predictors, and 30-Day Outcomes. *Anesthesiology* (2014) 120(3):564–78. doi:10.1097/ALN.0000000000000113
7. Khan J, Alonso-Coello P, Devereaux PJ. Myocardial Injury After Noncardiac Surgery. *Curr Opin Cardiol* (2014) 29(4):307–11. doi:10.1097/HCO.0000000000000069
8. Celis R, Estep JD, Orrego C, Semones L, Kasevan R, Seethamraju H, et al. 29: Elevations in Troponin-I (Tn-I) Following Lung Transplantation Predict Survival. *J Hear Lung Transpl* (2010) 29(2):S16. doi:10.1016/j.healun.2009.11.036
9. Andrei S, Kantor E, Asssadi M, Boutten A, Pellenc Q, Jebrak G, et al. The Prognostic Role of Early Postoperative Troponin I in Lung Transplantation-A Retrospective 7-Year Analysis. *J Cardiothorac Vasc Anesth* (2022) 36(8):2328–34. doi:10.1053/j.jvca.2021.11.007
10. Boasquevisque CHR, Yildirim E, Waddel TK, Keshavjee S. Surgical Techniques: Lung Transplant and Lung Volume Reduction. *Proc Am Thorac Soc* (2009) 6(1):66–78. doi:10.1513/pats.200808-083GO
11. 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. *J Heart Lung Transplant* (2017) 36:1097–103. doi:10.1016/j.healun.2017.07.021
12. Jaffe AS, Chaitman BR, Morrow DA, Bax JJ, White HD, Alpert JS, et al. Fourth Universal Definition of Myocardial Infarction (2018). *Eur Heart J* (2018) 40(3):237–69. doi:10.1093/eurheartj/ehy462
13. Lucreziotti S, Conforti S, Carletti F, Santaguida G, Meda S, Raveglia F, et al. Cardiac Troponin-I Elevations After Thoracic Surgery. Incidence and Correlations With Baseline Clinical Characteristics, C-Reactive Protein and Perioperative Parameters. *Rev Española Cardiol* (2007) 60(11):1159–66. doi:10.1016/S1885-5857(08)60046-8
14. Devereaux PJ, Lamy A, Chan MTV, Allard RV, Lomivorotov VV, Landoni G, et al. High-Sensitivity Troponin I After Cardiac Surgery and 30-Day Mortality. *N Engl J Med* (2022) 386(9):827–36. doi:10.1056/NEJMoa2000803
15. Andrei S, Tran-Dinh A, Boutten A, Asssadi M, Tashk P, Castier Y, et al. The Perioperative Kinetics Profile of Troponin I in Lung Transplantation – A Pilot Observational Study. *J Cardiothorac Vasc Anesth* (2022) 36(8):2842–5. doi:10.1053/j.jvca.2022.03.026
16. Patil H, Vaidya O, Bogart D. A Review of Causes and Systemic Approach to Cardiac Troponin Elevation. *Clin Cardiol* (2011) 34(12):723–8. doi:10.1002/clc.20983

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ORIGINAL RESEARCH
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Impact of Everolimus Initiation and Corticosteroid Weaning During Acute Phase After Heart Transplantation on Clinical Outcome: Data from the Korean Organ Transplant Registry (KOTRY)

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The effect of changes in immunosuppressive therapy during the acute phase post-heart transplantation (HTx) on clinical outcomes remains unclear. This study aimed to investigate the effects of changes in immunosuppressive therapy by corticosteroid (CS) weaning and everolimus (EVR) initiation during the first year post-HTx on clinical outcomes. We analyzed 622 recipients registered in the Korean Organ Transplant Registry (KOTRY) between January 2014 and December 2021. The median age at HTx was 56 years (interquartile range [IQR], 45–62), and the median follow-up time was 3.9 years (IQR 2.0–5.1). The early EVR initiation within the first year post-HTx and maintenance during the follow-up is associated with reduced the risk of primary composite outcome (all-cause mortality or re-transplantation) (HR, 0.24; 95% CI 0.09–0.68; $p < 0.001$) and cardiac allograft vasculopathy (CAV) (HR, 0.39; 95% CI 0.19–0.79; $p = 0.009$) compared with EVR-free

Abbreviations: Anti-HLA, antibodies of human leukocyte antigen; CAV, cardiac allograft vasculopathy; CI, confidence interval; CNI, calcineurin inhibitor; CS, corticosteroid; HR, hazard ratio; HTx, heart transplantation; ISHLT, International Society for Heart and Lung Transplantation; KOTRY, Korean Organ Transplant Registry; LVEF, left ventricular ejection fraction; MMF, mycophenolate mofetil; EVR, everolimus; TAC, tacrolimus; VA-ECMO, venoarterial extracorporeal membrane oxygenation.

or EVR intermittent treatment regimen, regardless of CS weaning. However, the early EVR initiation tends to increase the risk of acute allograft rejection compared with EVR-free or EVR intermittent treatment.

Keywords: heart transplantation, mTOR inhibitor, Korean Organ Transplant Registry, steroid weaning, primary outcome, rejection, cardiac allograft vasculopathy

INTRODUCTION

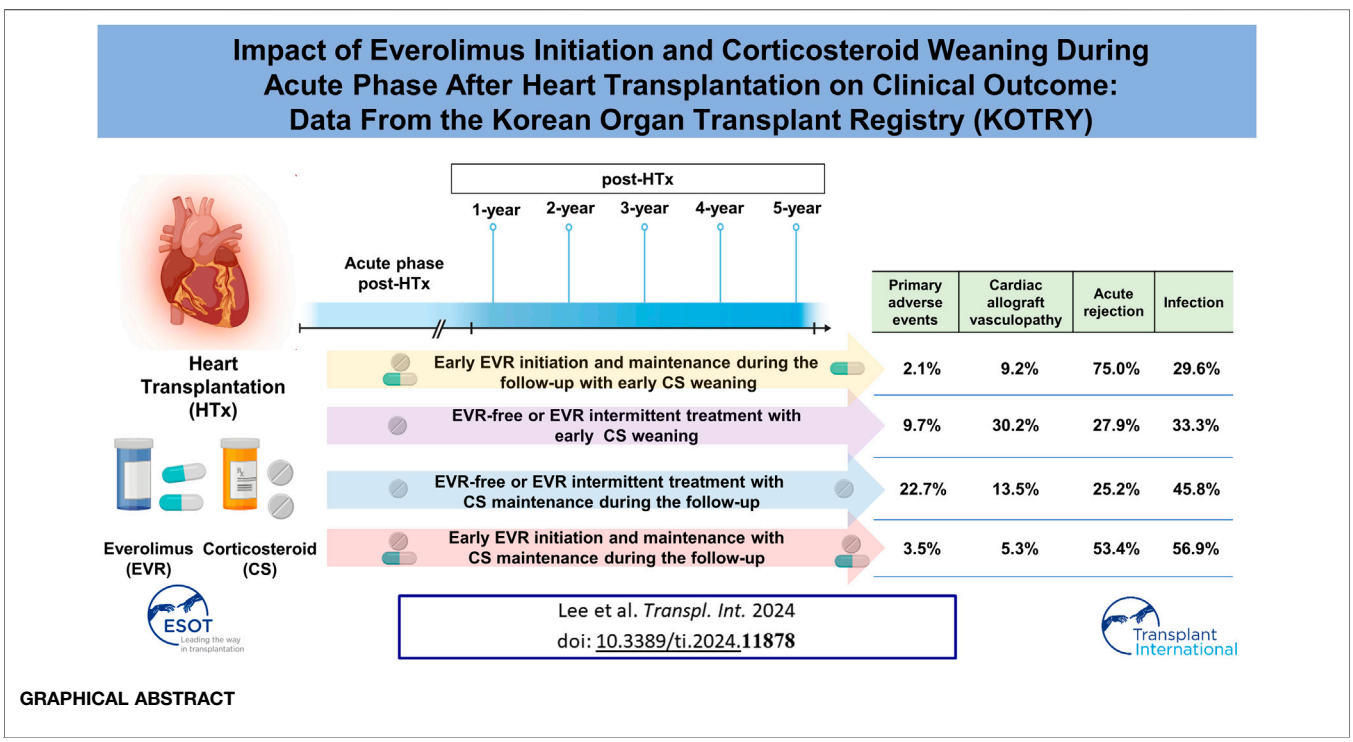
Calcineurin inhibitors (CNI), mycophenolic acid (MPA), mammalian target of rapamycin (mTOR) inhibitors, and corticosteroids (CS) are the main choices for immunosuppressive therapy after heart transplantation (HTx) [1, 2]. Advanced maintenance regimens consisting of immunosuppressive agents and therapeutic drug monitoring post-HTx contribute to the increased success of HTx by reducing the risk of rejection [3, 4]. However, temporal changes of regimens or dosages in immunosuppressive agents are still associated with a risk of acute rejection after transplantation, while inappropriate administration leads to adverse drug effects [5–7]. Therefore, the principal goal of immunosuppressive therapy is to balance the prevention of allograft rejection and adverse immunotherapeutic effects [8]. In this context, determining the optimal timing of the treatment initiation or change in immunosuppressant dosage is crucial to maximize efficacy and minimize adverse effects.

A previous study has reported the safety of tacrolimus (TAC) monotherapy compared with TAC and mycophenolate mofetil (MMF) therapy and CS withdrawal in the early phase post-transplantation [9]. Subsequently, recent studies have reported the safety and efficacy of mTOR inhibitor treatment initiation with CNI tapering or withdrawal in HTx recipients [10–15]. However, these studies have evaluated the efficacy and safety of single immunosuppressive agents. Initiation, adjustment, and changes in immunosuppressive agents are inevitable, depending on various factors, including drug adverse effects or tolerability during the acute phase post-transplantation. Against this background, the impact of concurrent changes in immunosuppressive agents with initiation, tapering, or withdrawal during the acute phase post-HTx on clinical outcomes remains to be determined. Therefore, this study aimed to evaluate whether temporal changes in the immunosuppressive agents during the acute phase post-HTx are associated with clinical outcomes in HTx recipients by using heart transplant cohort database of the Korean Organ Transplant Registry (KOTRY).

PATIENTS AND METHODS

Data Source and Collection

The KOTRY is the first nationwide prospective cohort study of solid organ transplantation launched in 2014 [16]. The KOTRY



GRAPHICAL ABSTRACT

consist of five organ-transplant cohorts (kidney, liver, lung, pancreas, and heart). Among cohorts, 7 hospitals (Seoul National University Hospital, Samsung Medical Center, Asan Medical Center, Seoul National University Bundang Hospital, Yonsei Severance Hospital, Keimyung University Dongsan Medical Center, Pusan National University Yangsan Hospital) are participating in the heart transplant cohort. After written informed consent was obtained from each recipient prior to HTx, HTx recipients from representative medical centers have been consecutively enrolled in the KOTRY[1] upon transplantation, and recipient-related data for study have been prospectively recorded. Detailed information regarding the collected data and the definition of comorbidities are described in the first and second reports of the Korean Heart Transplant Registry [17, 18]. Briefly, recipients enrolled in heart transplant cohort of the KOTRY are followed up at 1, 6, 12, 24, 36, 48, 60, and 120 months to monitor for rejection and screen for adverse events post-HTx according to the heart transplant cohort protocol. We annually collected the data including [1] the recipient's vital signs and comorbidities [2]; the information about prescribed medications and changes to medications including immunosuppressants [3]; a laboratory test [4]; PRA (panel reactive antibody) I & II [5]; DSA (donor specific antibodies) [6]; echocardiographic assessment [7]; recent events (death, rejection, cardiac allograft vasculopathy, renal replacement therapy or re-transplantation); and [8] post-transplantation complications (rejection, malignancy, diabetes mellitus, hypertension, stroke, infection, skeletal complication, and renal impairment).

Endomyocardial biopsy (EMB) was performed according to center-specific protocol biopsy for rejection surveillance. Typically, KOTRY protocol recommended EMBs within 30 days, 3, 6, 12, and 24 months after HTx. However, the specific timing and frequency of EMBs could vary slightly based on the center established protocol. After 24 months, routine EMBs were generally discontinued. Additional EMBs could be performed beyond 2 years if clinically indicated. The decision to perform an EMB was based on individual patient factors and clinical evaluation such as any suspicion of symptomatic allograft rejection, even if other tests are inconclusive. After all biopsies performed during the follow-up period were reviewed, only those cases where rejection was confirmed were recorded in the cohort. Further, if EMB was performed at a similar time as the protocol biopsy, the reason was clearly recorded at electronic case report form (eCRF).

Cardiac allograft vasculopathy (CAV) defined as abnormal coronary angiography findings diagnosed either by coronary angiography (CAG) with or without IVUS (adjunct intravascular imaging can be considered if expertise is available), or CT coronary angiography and graded using the international society of heart and lung transplantation (ISHLT) nomenclature [19, 20]. KOTRY heart transplant protocol recommended routine CAG at 12 months post-transplant. If CAG detected any abnormality, IVUS was further recommended for detailed assessment. Beyond 12 months, coronary evaluation was recommended annually through either CAG or CT-CAG. Additionally, coronary evaluation was performed (regardless of the one-year schedule) if there is clinical suspicion of cardiac allograft vasculopathy (CAV) during the follow-up period. This result was selected and recorded at eCRF during the follow-up period.

Data Quality

Data management of heart transplant cohort of the KOTRY was performed by using a web-based electronic case report form (eCRF) with Pharmacoepidemiology and Clinical Trial Applications X (PhactaX) system, which was developed by the Medical Research Collaborating Center of Seoul National University Hospital. All participating sites received multiple onsite monitoring visits to verify informed consent in all participants and to check key data in enrolled patients. All clinical data were collected and recorded in eCRF (version 2.7) compliant centralized online database. In addition to checking for outliers via automated computational methods, data quality was verified every 3 months by verifying values entered in the database against the primary source documents.

Study Population

From 2014 to 2021, 813 HTx recipients aged above 18 years were enrolled in the KOTRY HTx database. To evaluate the effect of the early EVR initiation and CS weaning within the first year post-HTx on clinical outcome, we excluded the following recipients from this study. 64 recipients were excluded due to insufficient follow-up (less than 1 month post-HTx). 56 recipients who died within the first year post-HTx were excluded. 73 recipients were excluded due to missing data on the presence of EVR and CS prescription, dose, or trough levels during the follow-up. Finally, 620 HTx recipients were included in this study. Written informed consent was obtained from each participant. The use of the registry data for this study was approved by the institutional review board of Seoul National University Hospital (IRB No. 1406-082-588).

Study Outcomes

The primary outcome was a composite of all-cause death or re-transplantation. Secondary outcomes were cardiac allograft vasculopathy (CAV), acute allograft rejection, infection, and malignancy during the follow-up period. The diagnosis of acute allograft rejection was based on endomyocardial biopsy (EMB) findings by an experienced pathologist following ISHLT guidelines [21, 22]. The acute allograft rejection was classified into acute cellular rejection (ACR) (Grade 1R, Grade 2R, Grade 3R, and Unspecified) and acute antibody-mediated rejection (AMR). The pathologic grading and reporting of AMR were as follows: pAMR 1 (pAMR 1-histopathologic, pAMR 1-immunopathologic), pAMR 2, pAMR 3, unspecified.

CAV was classified as insignificant (CAV 0), mild (CAV 1), moderate (CAV 2), or severe (CAV 3) according to the International Society for Heart and Lung Transplantation (ISHLT) CAV grading report [19]. The infection was defined as the cases requiring hospitalization due to pathogens such as viruses, bacteria, fungi, or parasites and was diagnosed by signs or

[1]https://www.kotry.org/ko/main.html

TABLE 1 | Baseline characteristics of HTx recipients and donors.

Variables	Overall (*N* = 622)
Recipient characteristics	
Age (years), median (IQR)	56.0 (45.0–62.0)
Sex (male), no. (%)	429 (69.2)
BMI (kg/m^2)	22.6 ± 3.5
Diabetes mellitus, no. (%)	169 (27.3)
Type 1 DM	4 (0.6)
Type 2 DM without insulin	138 (22.3)
Type 2 DM with insulin	27 (4.4)
Hypertension, no. (%)	196 (31.6)
Smoking status, no. (%)	
Never	360 (58.1)
Current	59 (9.5)
Former	197 (31.8)
Previous malignancy, no. (%)	49 (7.9)
Chronic kidney disease, no. (%)	100 (16.1)
CKD stage 3 (eGFR 30–59)	66 (10.6)
CKD stage 4 (eGFR 15–29)	8 (1.3)
CKD stage 5 (eGFR <15) with HD	26 (4.2)
Left ventricular ejection fraction (%)	27.1 ± 14.2
Lab findings at heart transplantation	
WBC (×10^3 μL/L)	6.8 (5.3–9.0)
Hemoglobin (g/dL)	11.0 (9.5–12.8)
Platelet (×10^3/μL)	161.0 (112.0–218.0)
BUN (mg/dL)	20.3 (15.1–29.3)
Creatinine (mg/dL)	1.0 (0.8–1.4)
Total cholesterol (mg/dL)	134.0 (108.0–163.0)
LDL-C (mg/dL)	76.5 (57.0–104.0)
HDL-C (mg/dL)	38.0 (29.0–46.0)
Causes of heart transplantation, no. (%)	
Ischemic	123 (19.8)
Cardiomyopathy	368 (59.4)
Valvular heart disease	25 (4.0)
Myocarditis	21 (3.4)
Infiltrative disease[a]	22 (3.5)
Congenital	21 (3.4)
Chemotherapy-induced	8 (1.3)
Panel-reactive antibody (PRA) > 50%	
Overall	124 (20.1)
Class-I	71 (11.6)
Class-II	85 (14.0)
Class-I & Class-II	37 (6.0)
Donor-specific antibodies (+)	74 (13.4)
Desensitization prior to HTx, no. (%)	50 (8.1)
Pre-operative support	
On IV inotropes	516 (83.2)
Mechanical support devices	
IABP	1 (0.2)
ECMO or PCPS	162 (26.1)
VAD	46 (7.4)
Ventilator	129 (20.8)
ECMO with ventilator	106 (17.1)
Operation time (min), median (IQR)	339.0 (286.0–405.0)
Cold ischemic time (min), median (IQR)	96.0 (65.0–169.0)
Warm ischemic time (min), median (IQR)	51.0 (38.0–75.0)
Post-op ECMO support, no. (%)	54 (8.7)
Post-op CRRT support, no. (%)	103 (16.6)
Donor characteristics	
Age (years), median (IQR)	43.0 (32.0–49.0)
Sex (male), no. (%)	442 (71.3)
BMI (kg/m^2)	23.5 ± 3.6
Diabetes mellitus, no. (%)	31 (5.0)
Hypertension	88 (13.2)
LVEF (%)	63.2 ± 9.4
Total CPR time (min)	15.2 ± 24.7
Donor cause of death	
Intracranial hemorrhage	264 (42.6)
Trauma	156 (25.2)
Hanging	121 (19.5)
Other	79 (12.7)

(Continued in next column)

TABLE 1 | (*Continued*) Baseline characteristics of HTx recipients and donors.

Abbreviations: BMI, body mass index; BUN, blood urea nitrogen; CKD, chronic kidney disease; CPR, cardiopulmonary resuscitation; CRRT, continuous renal replacement therapy; ECMO, extracorporeal membrane oxygenation; eGFR, estimated glomerular filtration rate; HD, hemodialysis; HDL-C, high-density lipoprotein cholesterol; HTx, heart transplantation; IABP, Intra-aortic balloon pump; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; VAD, ventricular assist device; PCPS; percutaneous cardiopulmonary support; WBC, white blood cell.

[a]*Infiltrative diseases including amyloidosis* n = *8 (1.1%) and sarcoidosis* n = *16 (2.1%).*

symptoms related to infection and detection of pathogen by laboratory tests. Malignancies included the following diseases diagnosed during the follow-up period: malignancy of skin, renal, urogenital, respiratory, upper/lower gastrointestinal, hepatobiliary-pancreas, gynecologic, breast, hematologic, intracranial, and thyroid.

Statistical Analysis

Descriptive statistics were used to describe and summarize patients' baseline characteristics and comorbidities. Categorical variables were compared using the chi-square or Fisher's exact test. Continuous variables are presented as mean ± standard deviation or medians (25th–75th percentiles), and group differences were compared using Student's *t* or the Mann-Whitney test.

The Cox proportional hazard regression model was used to calculate hazard ratios (HR) and 95% confidence intervals (CI). For the comparison of clinical outcomes regarding changes in immunosuppressive agents or regimens, a multivariate Cox proportional hazard regression model was used to calculate adjusted HR and its CI. The following variables were included for adjustment: age, sex, diabetes mellitus, hypertension, smoking, donor-specific antibody, and desensitization. The cumulative incidence of primary and secondary outcomes was estimated using the Kaplan–Meier method, and the log-rank test was used to evaluate differences between groups. Statistical significance was set at $p < 0.05$. SPSS Statistics version 25.0 (IBM, Chicago, IL, United States) was used for statistical analyses.

RESULTS

Recipient and Donor Characteristics

The baseline characteristics of recipients and donors are presented in **Table 1**. The median recipient age was 56 years,

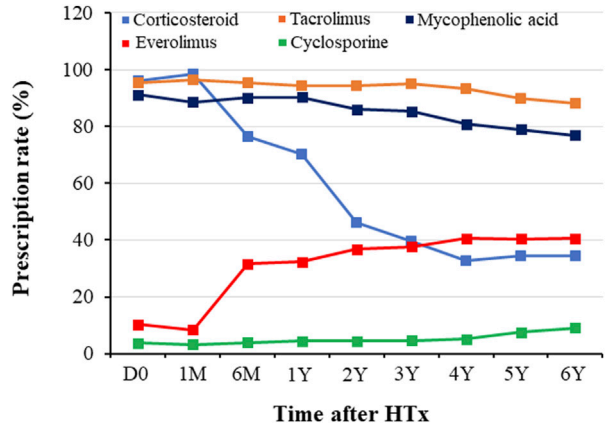


FIGURE 1 | Temporal trend of immunosuppressant prescription during follow-up periods. Prescription patterns for immunosuppressants during the follow-up period. D0, discharge; HTx, heart transplantation; M, month; Y, year.

and 69.2% were male. Of the recipients, 169 (27.3%) had diabetes mellitus, 196 (31.6%) had hypertension, and 100 (16.0%) had chronic kidney disease. The mean left ventricular ejection fraction (LVEF) at HTx was 27.1%. Cardiomyopathy (59.4%) was the most frequent cause of HTx, followed by ischemic heart disease (19.8%). In total, 516 (83.2%) patients required inotropic support to stabilize circulation. A total of 162 patients (26.1%) received venoarterial extracorporeal membrane oxygenation (VA-ECMO), and 46 (7.4%) received ventricular assist device therapy as a bridge to HTx. A total of 129 patients (20.8%) received mechanical ventilation, and 106 (17.1%) received mechanical ventilation in combination with VA-ECMO. In total, 124 (20.1%) patients exhibited class I and/or class II pre-transplantation panel reactive antibodies against human leukocyte antigen (anti-HLA) greater than 50%. 74 patients (13.4%) had donor-specific anti-HLA antibodies, and 50 (8.1%) were desensitized before transplantation. The median operative time was 339 min, and the median warm ischemic time was 51 min. A total of 54 patients (8.7%) received VA-ECMO, and 103 (16.6%) received continuous renal replacement therapy after transplantation.

The median donor age was 43.0 years (IQR 32.0–49.0), with males as the predominant sex (71.3%). The mean body mass index of the donors was 23.5 ± 3.6 kg/m^2, and the mean LVEF was 63.2%. Donors with diabetes mellitus and hypertension accounted for 5.0% and 13.2% of all donors, respectively. The leading causes of donor death were intracranial hemorrhage (42.6%), trauma (25.2%), and suicide by hanging (19.5%). The mean cardiopulmonary resuscitation time was 15.2 ± 24.7 min.

Immunosuppressive Agent Prescription Patterns

Prescription patterns for immunosuppressive agents during the follow-up period are shown in **Figure 1**. At discharge post-HTx, TAC, cyclosporine (CsA), MMF, CS, and everolimus (EVR) were prescribed to 95.5%, 3.8%, 91.1%, 96.2%, and 10.3% of recipients, respectively. The most frequently prescribed immunosuppressive agents were TAC (95.5% at discharge and 76.9% at the 6-year follow-up) and MMF (91.1% at discharge and 76.9% at 6-year follow-up). Notable changes in immunosuppressive agents were CS weaning (dose tapering or withdrawal) and EVR initiation. CS weaning attempts were initiated from the first month post-HTx, and the rate of CS prescription decreased from 96.2% at discharge to 34.3% at the 6-year follow-up. The prescription rate for EVR increased after the first month post-HTx, ranging from 10.3% at discharge to 31.6% at the 6-month follow-up and 40.6% at the 6-year follow-up. However, the CsA prescription rate was less than 5% during follow-up periods.

Changes in Prescribed Immunosuppressive Agents and Maintenance Regimens

The changes in prescribed immunosuppressive agent doses or in trough levels during the follow-up period are shown in **Figure 2**. The doses of immunosuppressive agents decreased rapidly in early post-HTx periods and remained constant throughout the follow-up period. The changes in maintenance regimens during follow-up period are shown in **Figure 3**. The most used maintenance regimen in the Korea during 2014–2021 was a triple therapy regimen consisting of TAC, MMF, and CS in the early phase post-HTx (**Supplementary Figure S1**). However, the prescription rate for TAC-based triple regimens gradually decreased from 76.2% at discharge to 16.1% at 6-year follow-up. The prescription rates for EVR-based regimens (from 8.6% at discharge to 40.6% at 6-year follow-up) and CS-free/TAC/MMF regimens (from 2.4% at discharge to 35.7% at 6-year follow-up) increased during follow-up periods (**Figure 3**). The EVR-based regimen consists of various combinations of immunosuppressive agents during the follow-up period. It consists of 4, 3, or 2 immunosuppressive agents including EVR (**Supplementary Figure S2**). Overall, the notable change in maintenance regimen was from TAC-based triple regimens to EVR-based or CS-free/TAC/MMF regimens via EVR initiation and CS weaning during follow-up periods.

Based on these findings, we hypothesized that temporal changes in immunosuppressive regimens and the prescribed doses or trough levels in immunosuppressive agents during early post-HTx periods affect the clinical outcomes of recipients. Thus, a receiver operating characteristic (ROC) curve was used to assess the prognostic value of each immunosuppressive agent's doses or trough levels during follow-up periods. We found that only CS and EVR doses or trough levels during the early post-HTx period (within the first year post-HTx) accurately predicted the primary outcome. ROC curves and optimal cutoff values of CS and EVR doses for the primary outcome are shown in **Supplementary Figure S3**. The CS dose at 1 year (AUC 0.72, sensitivity 64.7, specificity 58.3; $p < 0.001$) and EVR dose at 1 year (AUC 0.69, sensitivity 87.2, specificity 34.1; $p < 0.001$) showed good predictive ability for the primary composite outcome. The optimal cutoff values for predicting the primary outcome using the ROC curve and

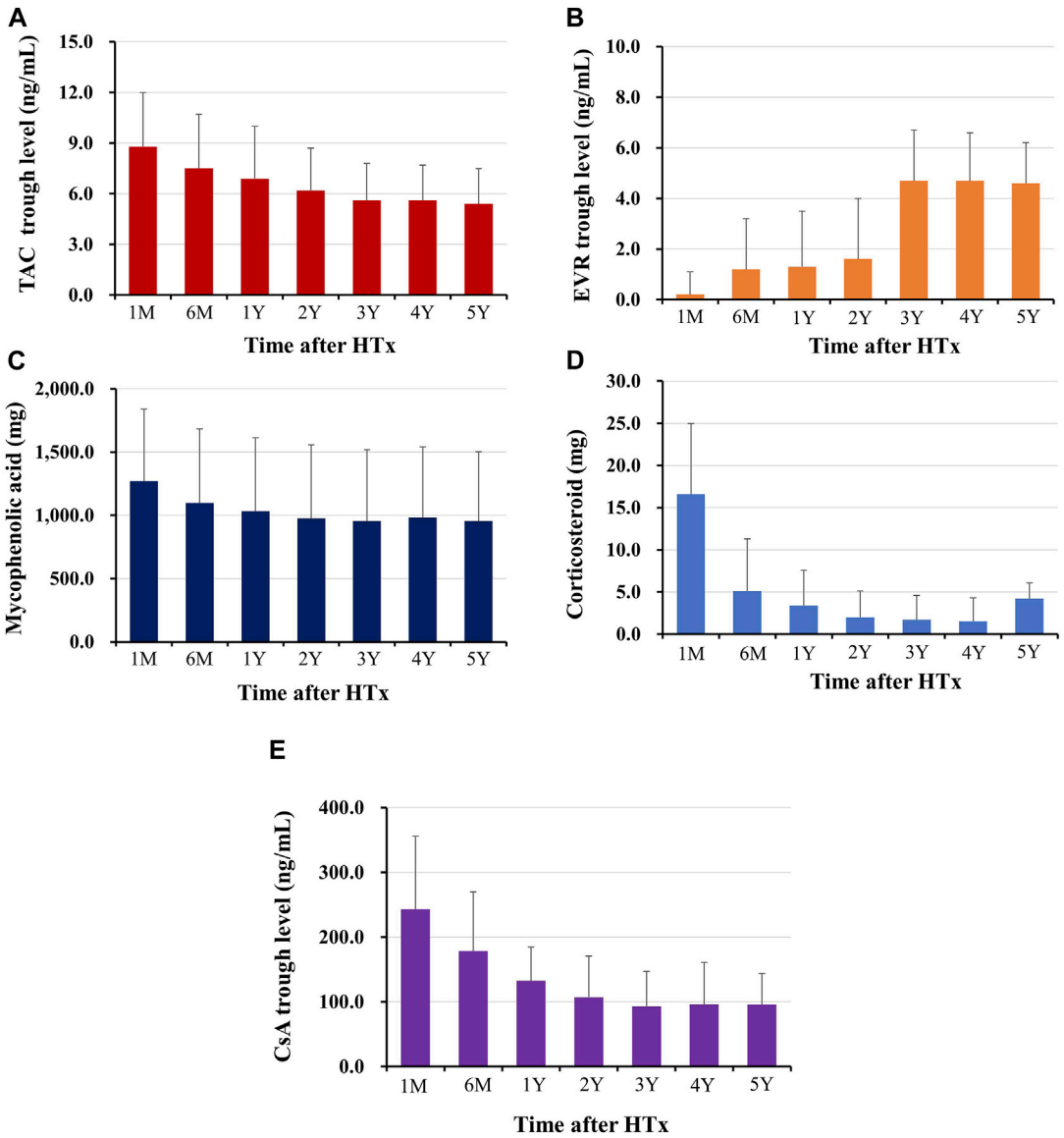


FIGURE 2 | Changes in immunosuppressant prescription doses or trough levels during follow-up periods The trough level of tacrolimus **(A)**, and everolimus **(B)**, the prescription dose of mycophenolic acid **(C)**, and corticosteroid **(D)**, the trough level of cyclosporine **(E)** during the follow-up periods are shown. HTx, heart transplantation; M, month; Y, year.

Youden index analyses were 3.5 mg (CS) and 0 mg (EVR). Based on these findings, HTx recipients (n = 620) were divided into CS weaning (CS withdrawal or tapered with less than 3.5 mg within the first year post-HTx) (n = 272) and CS maintenance (maintain CS more than 3.5 mg during the follow-up period) group (n = 346). In the case of EVR, the optimal cutoff value was 0 mg. For this reason, recipients were divided into EVR prescription and non-prescription groups. However, the treatment pattern of EVR in recipients was diverse in this study. Some patients were prescribed EVR intermittently, while others were continuously prescribed and taking EVR during the follow-up period. Therefore, HTx recipients were divided into three or two groups as follows: the EVR-free regimen group (n = 354), the EVR intermittent treatment regimen group (n = 100), and early EVR initiation/maintenance regimen group (n = 166) or EVR-free or EVR intermittent treatment regimen group (n = 454), and the early EVR initiation/maintenance regimen group (n = 166) (**Figure 4**).

Clinical Outcomes

Primary Outcome

To investigate the effects of early CS weaning during the first year post-HTx on clinical outcomes, we compared the clinical outcomes between the CS weaning within the first year post-HTx and CS dose maintenance (≥3.5 mg) during the follow-up. The early CS weaning within the first year post-HTx had

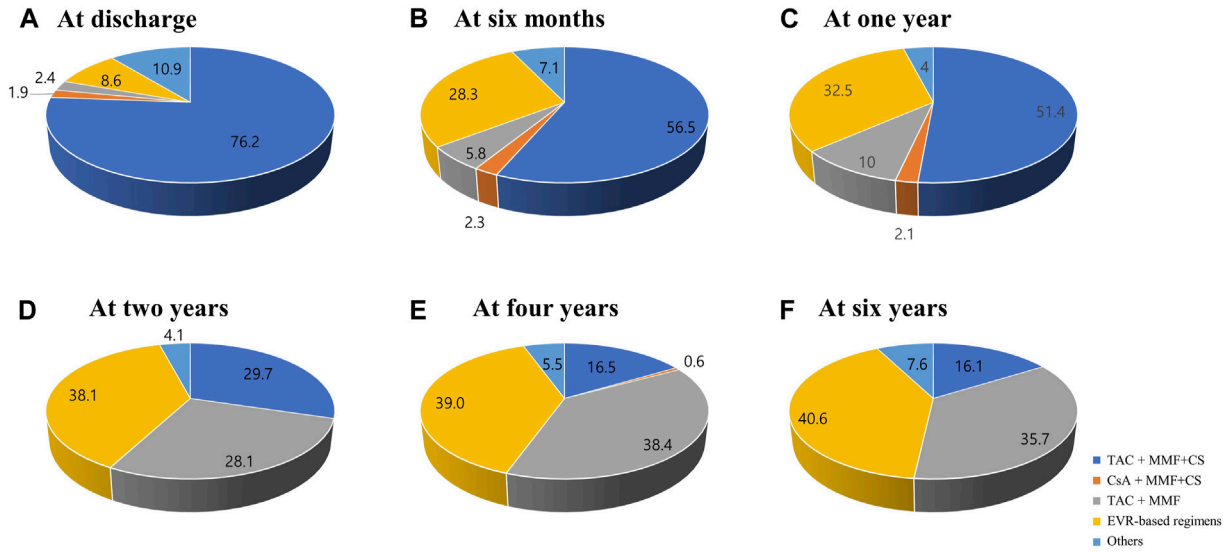


FIGURE 3 | Changes in maintenance regimens post-HTx. The Venn diagram shows the changes in maintenance regimens at discharge **(A)**, 6 months **(B)**, 1 year **(C)**, 2 years **(D)**, 4 years **(E)**, and 6 years **(F)** post-HTx. CS, corticosteroid; CsA, cyclosporine; EVR, everolimus; HTx, heart transplantation; MMF, mycophenolate mofetil; TAC, tacrolimus.

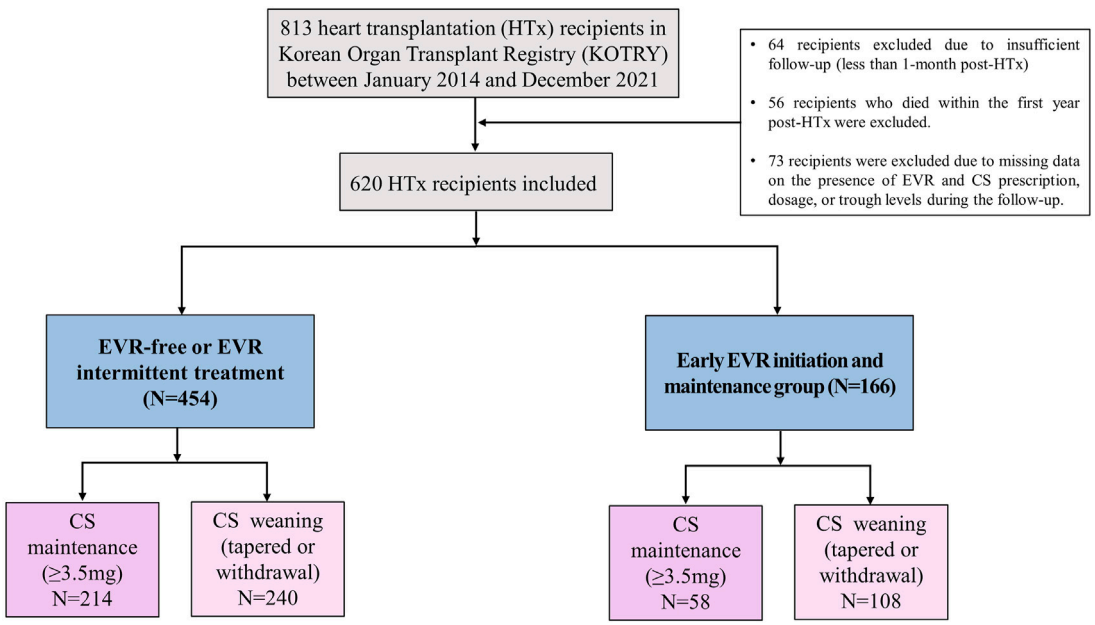


FIGURE 4 | Study flow A flow chart of the selection of eligible HTx recipients for this study. CS, corticosteroid; EVR, everolimus; HTx, heart transplantation.

reduced the risk of primary composite outcome (all-cause death or re-transplantation) compared with CS maintenance (≥3.5 mg) (7.2% vs. 17.7%; HR, 0.49; 95% CI 0.27–0.90, p = 0.022) (**Figure 5A**). Next, to investigate the effects of early EVR initiation within the first year post-HTx and continuously maintained EVR during the follow-up on clinical outcomes, we compared the clinical outcomes between the EVR-free or EVR intermittent treatment regimen group and the early EVR initiation/maintenance regimen group. The early EVR initiation during the first year post-HTx and continuously maintained EVR during the follow-up had reduced the risk of primary composite outcome compared with the EVR-free or EVR intermittent treatment regimen group (3.2% vs. 13.3% and 3.2% vs. 16.7%, log-rank p = 0.002 and p < 0.001, respectively). However, there was no significant difference in primary outcome

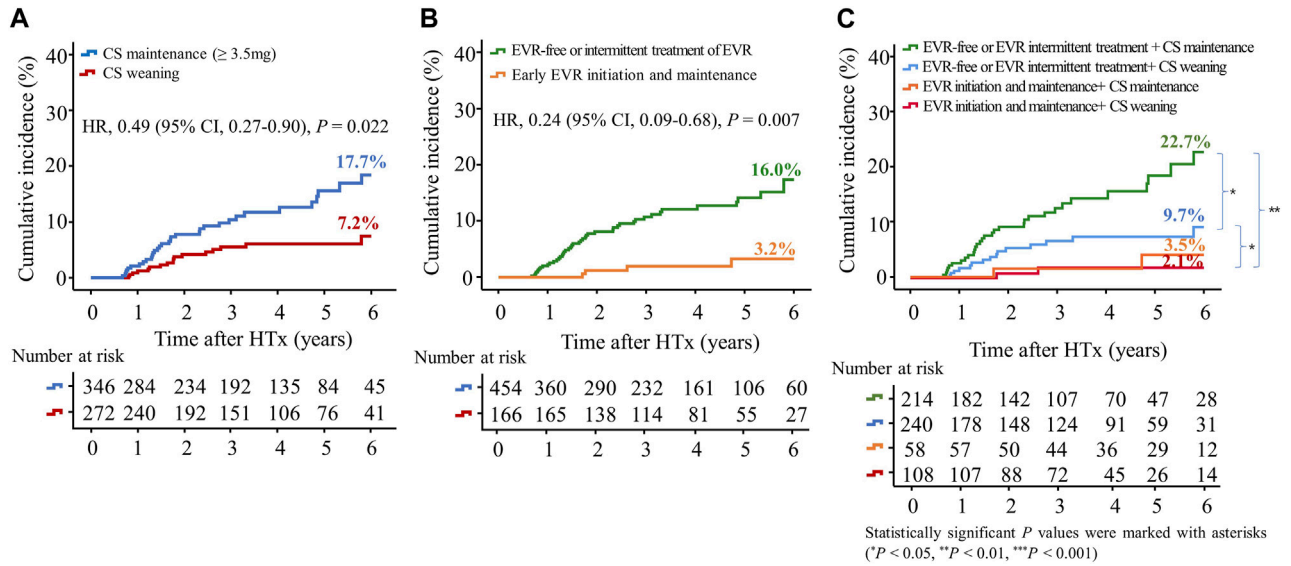


FIGURE 5 | Impact of everolimus initiation and corticosteroid weaning on the primary outcome. The primary outcome was a composite of all causes of death or re-transplantation. Kaplan–Meier curves comparing the risk of primary outcome between the CS weaning (tapering (<3.5 mg) or withdrawal) and CS maintenance (≥3.5 mg) regimens **(A)** and between early EVR initiation and EVR-free regimens **(B)**. Kaplan–Meier curves comparing the risk of primary outcome between four groups according to CS weaning and the presence of EVR initiation **(C)**. CI, confidence interval; CS, corticosteroid; EVR, everolimus; HR, hazard ratio; HTx, heart transplantation.

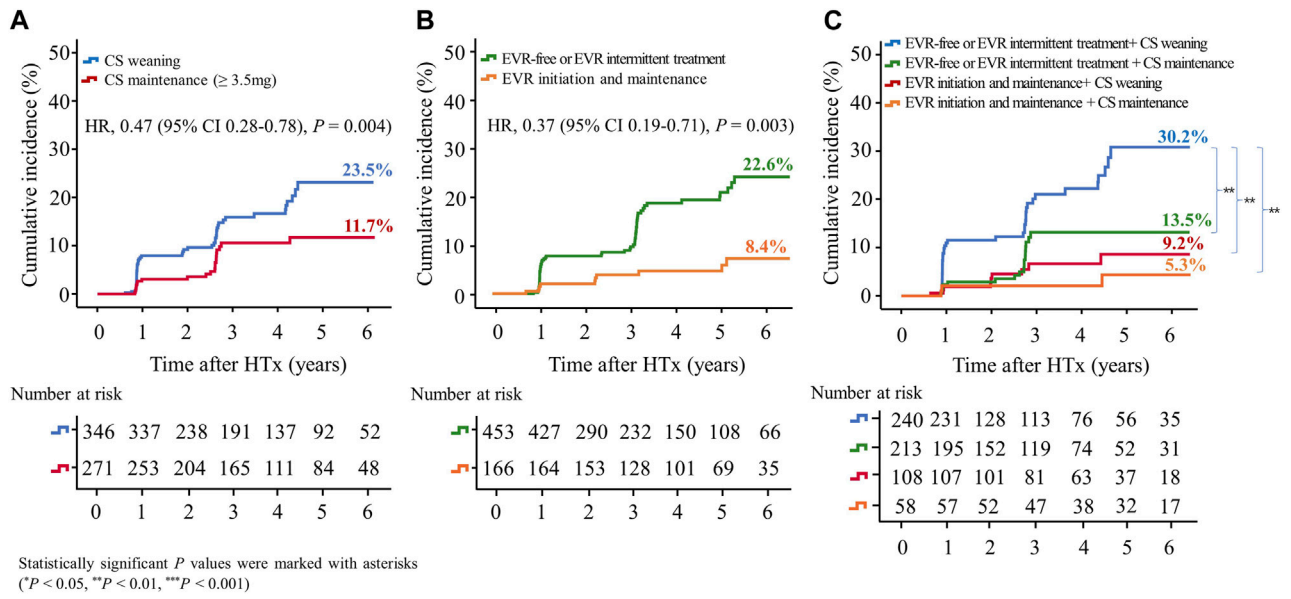


FIGURE 6 | Impact of everolimus initiation and corticosteroid tapering or withdrawal on CAV. The Kaplan–Meier curve shows the cumulative incidence of CAV. The cumulative incidence of CAV significantly decreased in HTx recipients undergoing CS maintenance (≥3.5 mg) compared with those undergoing CS tapering (<3.5 mg) or withdrawal regimens **(A)**, and in recipients undergoing early EVR initiation compared with those undergoing EVR-free regimens **(B)**. The cumulative incidence of CAV is the highest in HTx patients undergoing CS tapering (<3.5 mg) or withdrawal and EVR-free regimens compared with those undergoing other regimens **(C)**. CAV, cardiac allograft vasculopathy; CS, corticosteroid; EVR, everolimus; HR, hazard ratio; HTx, heart transplantation.

between EVR-free and EVR intermittent treatment (**Supplementary Figure S4**). For this reason, EVR-free and EVR intermittent treatment regimen groups were combined and defined as an EVR-free or EVR intermittent treatment regimen group (**Figure 4**). Ultimately, to investigate the effect of early CS weaning and early EVR initiation/maintenance on

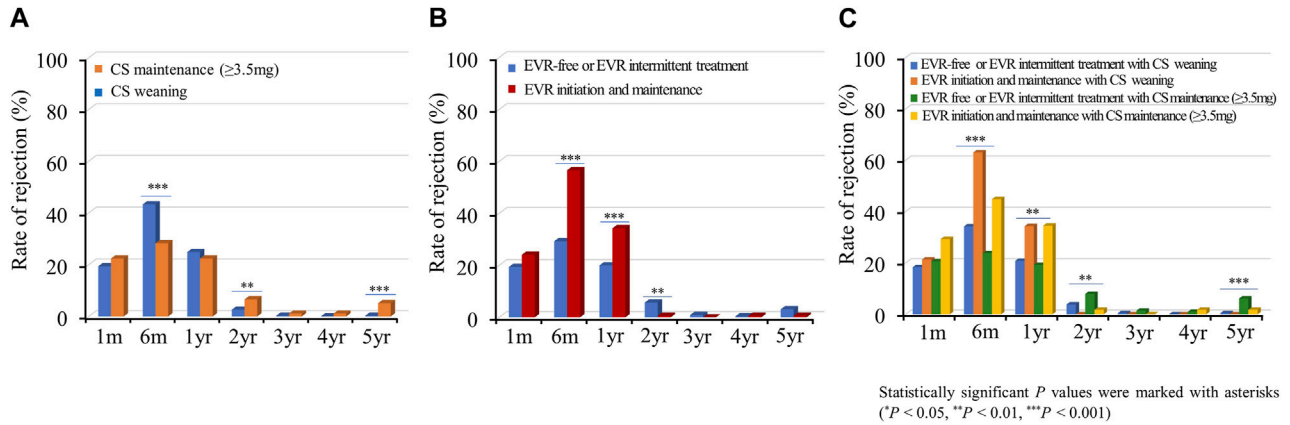


FIGURE 7 | Impact of everolimus initiation and corticosteroid weaning on acute cellular rejection. The rate of acute cellular rejection is shown according to the CS weaning **(A)**, EVR initiation **(B)**, and the combination of EVR initiation and CS weaning **(C)** regimens during the follow-up period. CS, corticosteroid; EVR, everolimus, HTx, heart transplantation.

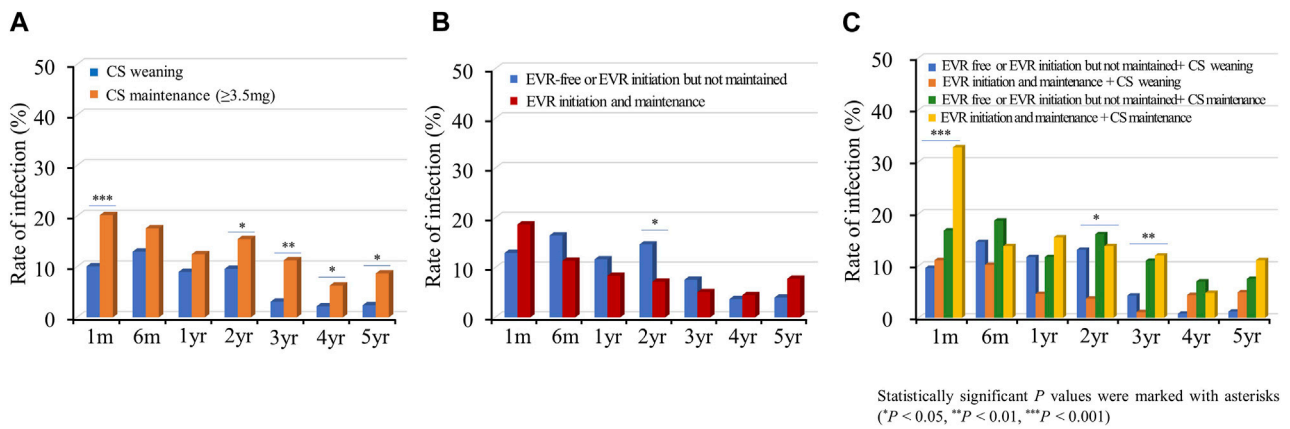


FIGURE 8 | Impact of everolimus initiation and corticosteroid weaning on infection. The incidence of infection requiring hospitalization is shown according to the CS weaning **(A)**, EVR initiation **(B)**, and the combination of EVR initiation and CS weaning **(C)** regimens during the follow-up period. CS, corticosteroid; EVR, everolimus; HTx, heart transplantation.

clinical outcomes, 622 HTx recipients were divided into four subgroups as follows: early EVR initiation and maintenance with CS weaning regimen (n = 108), EVR-free or EVR intermittent treatment with CS weaning regimen (n = 240), early EVR initiation and maintenance with CS maintenance (≥3.5 mg) regimen (n = 58), and EVR-free or EVR intermittent treatment with CS maintenance regimen (n = 214).

The early EVR initiation during the first year post-HTx and continuously maintained EVR during the follow-up had reduced the risk of primary composite outcome compared with EVR-free or EVR intermittent treatment regimen group (3.2% vs. 16.0%; HR, 0.24; 95% CI 0.09–0.68, p = 0.007) regardless of CS weaning (**Figures 5B, C**). However, CS weaning within the first year post-HTx had reduced the risk of primary composite outcome compared with CS maintenance (≥3.5 mg) in EVR-free or EVR intermittent treatment regimen (9.7% vs. 22.7%; HR, 0.51; 95% CI 0.27–0.97, p = 0.042) (**Figure 5C**). An EVR-based regimen had reduced the risk of primary composite outcome compared with the TAC + MMF + CS regimen (HR, 0.41; 95% CI 0.17–0.99, p = 0.048) and other regimens (HR, 0.21; 95% CI 0.08–0.56, p = 0.002) (**Supplementary Figure S5A**).

Cardiac Allograft Vasculopathy

In this study, CAV events were identified in the first year after HTx, and CAV grades were mostly mild to moderate (**Supplementary Table S1**). The cumulative incidences of CAV according to CS weaning and the presence of EVR initiation are shown in **Figure 6**. The incidence of CAV decreased in the CS maintenance group compared with in the CS weaning group (11.7% vs. 23.5%, HR 0.47; 95% CI 0.28–0.78, p = 0.004) and in the EVR initiation/maintenance regimen group

TABLE 2 | Changes in immunosuppressive agent dosages or trough levels according to the presence of everolimus prescription post-HTx.

	EVR-free or EVR intermittent treatment	Early EVR initiation and maintenance	*p*-Value
Discharge after HTx			
TAC (ng/mL)[a]	5.1 ± 3.6	5.4 ± 3.7	0.665
CsA (ng/mL)[a]	196.7 ± 89.6	203.3 ± 116.0	0.889
MMF (mg)	1,278.3 ± 559.0	943.1 ± 519.4	<0.001
CS (mg)	18.2 ± 12.8	11.0 ± 7.2	<0.001
One month after HTx			
TAC (ng/mL)	9.2 ± 3.3	8.1 ± 2.6	<0.001
CsA (ng/mL)	232.9 ± 100.8	227.7 ± 121.4	0.915
MMF (mg)	1,309.6 ± 663.3	797.9 ± 435.3	<0.001
CS (mg)	16.9 ± 8.4	13.6 ± 7.5	0.004
Six months after HTx			
TAC (ng/mL)	8.2 ± 3.1	5.6 ± 2.5	<0.001
CsA (ng/mL)	192.4 ± 67.6	147.0 ± 67.6	0.182
MMF (mg)	1,285.1 ± 562.8	612.6 ± 289.6	<0.001
CS (mg)	6.2 ± 6.8	2.7 ± 3.5	<0.001
One year after HTx			
TAC (ng/mL)	7.7 ± 3.2	4.8 ± 1.7	<0.001
CsA (ng/mL)	141.2 ± 56.9	119.1 ± 42.7	0.280
MMF (mg)	1,207.7 ± 579.0	606.1 ± 294.6	<0.001
CS (mg)	4.0 ± 4.5	2.1 ± 3.1	<0.001
Two years after HTx			
TAC (ng/mL)	7.4 ± 1.9	4.5 ± 1.6	<0.001
CsA (ng/mL)	121.3 ± 71.9	84.7 ± 42.6	0.070
MMF (mg)	1,134.7 ± 582.4	597.5 ± 371.0	<0.001
CS (mg)	2.1 ± 2.8	1.7 ± 3.6	0.126
Three years after HTx			
TAC (ng/mL)	6.4 ± 2.1	4.0 ± 1.3	<0.001
CsA (ng/mL	117.9 ± 56.5	65.7 ± 35.7	0.029
MMF (mg)	1,097.8 ± 576.2	607.8 ± 351.9	<0.001
CS (mg)	1.9 ± 3.1	1.4 ± 2.3	0.098
Four years after HTx			
TAC (ng/mL)	6.4 ± 2.0	4.0 ± 1.1	<0.001
CsA (ng/mL	127.1 ± 76.0	61.3 ± 21.9	0.032
MMF (mg)	1,139.2 ± 571.3	617.6 ± 299.6	<0.001
CS (mg)	1.6 ± 3.1	1.3 ± 2.3	0.380

Abbreviations: CS, corticosteroid; CsA, cyclosporine; EVR, everolimus; HTx, heart transplantation; MMF, mycophenolic mofetil; TAC, tacrolimus.
[a]*TAC and CsA were represented to trough level.*

compared with in the EVR-free or intermittent EVR treatment regimen group (8.4% vs. 22.6%, HR 0.39; 95% CI 0.19–0.79, p = 0.009), respectively (**Figures 6A,B**). Furthermore, CS maintenance (≥3.5 mg) reduced the risk of CAV events compared with CS weaning in the EVR-free or intermittent treatment regimen group (13.5% vs. 30.2%, HR 0.44; 95% CI 0.25–0.77, p = 0.004) (**Figure 6C**).

The cumulative Incidence of CAV decreased in the EVR-based regimen group (7.8% vs. 24.2%, HR, 0.34; 95% CI, 0.17–0.68, p = 0.002) compared with the TAC + MMF + CS regimen group (**Supplementary Figure S5B**).

Acute Rejection

Acute allograft rejection occurred most frequently during the first 6 months post-HTx, and the number of rejections decreased during the follow-up periods (**Figures 7A–C**; **Supplementary Figure S6**). Rates of biopsy-proven acute cellular rejection (ACR) and acute antibody-mediated rejection (AMR) were higher in the CS weaning group at 6 months and at 1 month post-HTx than in the CS maintenance group (**Figure 7A**; **Supplementary Figure S6A**). Furthermore, biopsy-proven ACR and AMR were higher in the early EVR initiation/maintenance regimen group during the first year post-HTx than in the EVR-free or EVR intermittent treatment regimen group (**Figures 7B, C**; **Supplementary Figure S6**).

Infection and Malignancy

Subsequently, we investigated the rate of infections requiring hospitalization according to the immunosuppressive regimen. Infection events frequently occurred in the acute phase post-HTx, and the infection rate decreased during the follow-up period (**Figure 8**). The CS maintenance (≥3.5 mg) significantly increased

the incidence of infection during the follow-up period (**Figure 8A**). No significant difference was found in rates of infections requiring hospitalization between the EVR initiation/maintenance regimen group and the EVR-free or EVR intermittent treatment regimen group except for the 2-year follow-up period (**Figure 8B**). However, the CS maintenance (≥3.5 mg) or EVR initiation/maintenance with CS maintenance (≥3.5 mg) regimens were associated with higher risks of infection compared with other regimens during the follow-up period (**Figure 8C**).

The incidence of malignancies tends to slightly increase during the follow-up period. The CS maintenance (≥3.5 mg) slightly increased the incidence of malignancy during the follow-up period compared with CS weaning (8.5% vs. 4.7%; OR, 1.7; 95% CI 0.9–3.4, $p = 0.125$). However, there was no significant difference in the incidence of malignancy between the early EVR initiation/maintenance and EVR-free or intermittent treatment (6.6% vs. 6.4%; OR, 1.0; 95% CI 0.5–2.1, $P = 0.973$). The EVR initiation and maintenance with CS maintenance regimen tend to increase the risk of malignancy compared to EVR initiation and maintenance with CS weaning regimen (12.1% vs. 3.7%; OR, 3.0; 95% CI 0.8–11.0, $p = 0.105$) (**Supplementary Table S2**; **Supplementary Figure S7–S9**).

DISCUSSION

This study was the first to investigate changes in the immunosuppressive agents and maintenance regimens and evaluated the effects of these temporal changes during the acute phase on the clinical outcomes in Korean HTx recipients. The major characteristics of the immunosuppressive agents for HTx recipients enrolled in the KOTRY were as follows: First, the initial backbone of immunosuppressive maintenance regimens was TAC and MMF. Second, notable changes in the prescription of CS and EVR were found. CS weaning attempts were initiated after one-month post-HTx, and the rate of CS prescription and dose gradually decreased during the follow-up period. However, the prescription of EVR increased after one-month post-HTx. Third, temporal dose changes in immunosuppressive agents mainly occurred during the acute phase (within 1 year post-HTx). Fourth, the maintenance immunosuppressive therapy was changed from a TAC-based triple therapy (TAC + MMF + CS) to EVR-based and CS-free/TAC/MMF therapy during the follow-up period.

CS are important components of induction, maintenance, and rejection regimens post-HTx [2, 6]; however, CS administration was associated with the highest number of long-term adverse effects. Therefore, attempts at CS withdrawal or dose tapering are continuously being made in the HTx field. Delgado et al. [23] reported that the use of CS for more than 1 year post-HTx is unlikely to provide clinical benefits. Furthermore, the ISHLT guidelines recommend that CS withdrawal can be achieved within 3–12 months post-HTx in low-rejection risk patients to minimize CS adverse effects [21]. Consistent with other studies, our study showed that CS weaning within the first year post-HTx was associated with a reduced risk of the primary outcome. However, the effects of CS weaning on the primary outcome differed according to the presence of early EVR initiation. In EVR-free or EVR intermittent treatment regimens, CS dose maintenance (≥3.5 mg) had a higher risk of the primary composite outcome than CS weaning. However, no significant difference was observed in the primary composite outcome between the two groups in the early EVR initiation/maintenance regimen. This may be explained by the effect of EVR. Recipients receiving EVR during the follow-up period had a lower mean CS dose compared with recipients who were not administered EVR (**Table 2**). For this reason, adverse effects due to CS dose maintenance during the follow-up period would have been minimized.

However, it is possible that the initiation of EVR and CS weaning attempts were preferentially considered in recipients at low risk of rejection. Conversely, recipients at high risk of rejection may have been maintained on a higher dose of CS. Therefore, considering the confounding and selection bias, we should be cautious in extrapolating the current results to all HTx recipients who may have a different immunosuppressant regimen. This study showed that various EVR-based regimens are being applied in HTx recipients. Furthermore, the EVR initiation is associated with changes in prescription rate or dose of other immunosuppressive agents including TAC, MMF, or CS. Because the initiation of EVR indirectly affects changes in the prescription rate or dosage of other immunosuppressive agents, further research is needed to confirm whether our results are a direct effect on EVR or an effect due to changes in the prescription rate or dose of other immunosuppressive agents.

The safety and efficacy of mTOR inhibitor treatment have been reported [11, 12]. The SCHEDULE study showed that the EVR initiation with cyclosporine withdrawal 7–11 weeks after HTx reduced CAV progression at 12 months than standard cyclosporine-based immunosuppression [11]. Furthermore, early conversion (median time of 1.1 years [IQR 0.6–3.0 years]) to sirolimus is associated with attenuated CAV progression, lower long-term mortality, and fewer CVA-related events than continued CNI use [12]. However, the EVERHEART study reported that the initiation of mTOR inhibitors immediately (≤144 h post-HTx) post-transplantation is associated with a poor safety profile, driven primarily by a higher rate of pericardial effusions compared with delayed (4–6 weeks post-transplantation) mTOR inhibitor treatment initiation [13]. These varying results suggest that the optimal timing of mTOR inhibitor treatment initiation to maintain an adequate balance between drug efficacy and safety remains unclear.

In this study, the early (within the first year post-HTx) EVR initiation and maintenance during the follow-up period is associated with reduced risk of the primary composite outcome and CAV events in recipients compared with the EVR-free or intermittent treatment regimens. Furthermore, compared with the TAC-based triple regimen (TAC + MMF + CS), the EVR-based regimen is associated with reduced risk of the primary composite outcome and CAV events. These results suggest that the early EVR initiation-based regimen can be an alternative treatment option for HTx recipients to improve clinical outcomes. The EVR-based regimen largely consists of

a combination of 4, 3, or 2 immunosuppressants including EVR during the follow-up period. In clinical settings, EVR is used for HTx recipients for a variety of reasons. Although the reason for switching or adding EVR from other immunosuppressants is not clearly described in the KOTRY heart transplant cohort, the use of EVR is primarily [1] to increase immunosuppression in the early phase after HTx [2], for minimization of other immunosuppressants (TAC, MMF or CS) or for CNI-free regimen, and [3] when CAV is suspected. In our study, the prescription rates, doses, or trough levels of TAC, CsA (cyclosporine A), MMF, or CS are lower in the early EVR initiation and maintenance regimen than in the EVR-free or EVR intermittent treatment regimen (**Table 2**; **Supplementary Table S3**). Furthermore, the TAC trough level is significantly lower in the early EVR initiation and maintenance regimen than in the EVR-free or EVR intermittent treatment regimen (**Supplementary Table S4**). These findings suggest that the use of EVR is associated with the minimization of other immunosuppressive agents or conversion to a CNI-free regimen.

In the EVR-free or EVR intermittent treatment regimen, TAC trough levels are lower in the third (Q3) or fourth (Q4) quartiles than in the lower quartiles (Q1 and Q2) of the serum creatinine during the follow-up period. However, there was no significant difference in TAC trough levels between lower quartiles (Q1 and Q2) and the third (Q3) or fourth (Q4) quartiles of the serum creatinine in the early EVR initiation and maintenance regimen (**Supplementary Table S4**). Furthermore, TAC trough levels and serum creatinine levels were lower in the early EVR initiation and maintenance regimen than in the EVR-free or EVR intermittent treatment regimen (**Supplementary Tables S4, S5**). These findings suggest that the initiation of EVR may not affected by serum creatinine levels. However, the early initiation of EVR is associated with a reduced risk of CNI-related nephrotoxicity by minimizing CNI exposure during the follow-up period.

EVR is a mammalian target of rapamycin (mTOR) inhibitor/proliferation-signal inhibitor with potent immunosuppressive and anti-proliferative effects. Several studies have demonstrated the efficacy of EVR in reducing acute rejection, progression, and development of CAV [11, 24]. Furthermore, EVR has the potential to facilitate the reduction of CNI therapy and preserved renal function [10, 15]. The current study's findings on the efficacy of EVR initiation were consistent with previous studies. However, tolerability and safety of EVR remain a concern. EVR-related pneumonitis, pericardial effusion, mouth ulcers, and impaired wound healing were associated with morbidity and mortality. Another issue is which immunosuppressive agents should be used in combination with EVR in HTx recipient during the long-term period. The combination of EVR and CS may be associated with a reduced risk of rejection and the progression or development of CAV by enhancing immunosuppression in HTx recipients. Although there are limitations in drawing conclusions due to the number of subjects in this study being relatively small, long-term treatment of EVR and CS combination therapy may have increased the incidence of infection or malignancy compared to EVR with CS weaning therapy in our study (**Supplementary Table S2**; **Figure 8**; **Supplementary Figure S9**). Considering that our study is an observational study, and the sample size is small, further studies are needed to verify the safety of long-term treatment of EVR and CS combination therapy.

CAV remains a long-term complication of HTx and is the major cause of death in patients surviving 1 year after transplantation [3, 25, 26]. According to a previous study, the prevalence of CAV is 3.3%, 5.1%, and 9.7% at one, two, and 5 years after transplantation, respectively [27]. The occurrence of CAV in our study was confirmed in the first year after transplantation, and the incidence rates were 5.5%, 6.1%, 12.3%, 13.7%, 15.4%, and 14.7% at one, two, three, four, five, and 6 years after transplantation, respectively. Although the grade of CAV was mostly mild (CAV 1) to moderate (CAV 2), an early EVR initiation-based regimen effectively prevented CAV progression. Furthermore, CS prevented CAV progression in recipients receiving EVR-free or EVR intermittent treatment regimens in our study. CS and EVR had a synergistic effect in preventing CAV. CAV incidence was the highest in EVR-free or EVR intermittent treatment with CS weaning regimen, whereas CAV incidence was the lowest in the early EVR initiation/maintenance with CS maintenance regimen (30.2% vs. 5.3%, $p = 0.002$). However, even if CS prevents CAV progression, CS is not effective in terms of CAV prevention considering the adverse effects that may occur due to long-term CS administration.

These findings suggest that the early initiation of EVR and maintenance therapy post-HTx may be reasonable, considering the efficacy of EVR. However, although the intention of the early initiation of EVR during the first year post-HTx is to effectively suppress immunity in recipients at high risk of rejection, the early EVR initiation may increase the risk of acute rejection due to reduced prescribed doses or trough levels of other immunosuppressive agents, including TAC, MMF, or CS. This finding suggests that changes in regimen, dose, or trough level of immunosuppressive agents during the first year post-HTx, when the risk of acute allograft rejection is the highest, may increase the risk of acute rejection. Therefore, these changes can increase the risk of acute rejection by destabilizing the patient's immunosuppressive state during the first year post-HTx. The KOTRY data revealed that the prescription rates for TAC and MMF were consistently higher than those for other immunosuppressive agents during the follow-up period. At 6 years post-HTx, TAC and MMF prescription rates were 88.1% and 76.9%, respectively, with 35.7% of patients prescribed a TAC/MMF regimen and 16.1% of patients prescribed a TAC + MMF + CS regimen. Despite the TAC-based regimen increasing the risk of primary composite outcome and the incidence of CAV compared with the EVR-based regimen, 51.8% of recipients in Korea were still prescribed a TAC/MMF-based regimen. CS withdrawal was 65.6% at the 6-year follow-up post-HTx, whereas the prescription rate for EVR rapidly increased from 8.1% to 31.6% between one and 6 months but slightly increased thereafter to 40.6% at the 6-year follow-up. Although the excellent efficacy of EVR has been demonstrated in trials, several possible reasons exist for the low prevalence of early EVR-based regimens in Korea. First, the adverse effects and lower tolerability of EVR may affect their early or long-term use in HTx recipients. Second, adherence to traditional TAC-based regimens

limits the use of EVR. Additional clinical studies are needed to investigate the use of early EVR-based maintenance regimens as an effective treatment strategy for HTx recipients.

This study has several limitations. First, this study was a retrospective observational study, and the analysis was based on a heart transplant cohort in KOTRY which has not been externally validated. Therefore, the results should be generalized with caution. Second, potential confounding and selection bias regarding CS weaning and EVR initiation may exist in a selected group of recipients. Further, we excluded 56 patients that died within the first year post-HTx due to evaluate the effect of CS weaning and EVR initiation during the first year post-HTx on long term clinical outcome. However, this exclusion may influence outcomes. Third, the indication and timing of CS weaning and EVR initiation differed per patient in the KOTRY. This is likely influenced by center-specific protocols and physician expertise or recipient characteristics and tolerability. This raises a very important bias (confounding by indication). Fourth, some information on the prescription status, dose, or trough level of immunosuppressive agents is missing during the follow-up. Finally, this study was conducted on an Asian population. Therefore, caution should be exercised when extrapolating these results to non-Asian HTx recipients.

In conclusion, the early EVR initiation within the first year post-HTx and maintenance during the follow-up period is associated with reduced risk of primary composite outcome and CAV events in HTx recipients. However, changes in the prescription rate, dose, or trough level of TAC, MMF, or CS due to early EVR initiation may increase the risk of acute allograft rejection during the first year post-HTx.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The use of the registry data for this study was approved by the institutional review board of Seoul National University Hospital (IRB No. 1406-082-588). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

The authors confirm contribution to the paper as follows: study conception and design: H-JC. Data collection: all authors were equally contributed analysis and interpretation of results: K-SL, HK, SeL, D-JC, MY, E-SJ, J-OC, JK, H-YL, S-HJ, JO, S-MK, SoL, MJ, J-JK, and MK. Draft manuscript preparation: K-SL. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.11878/full#supplementary-material

REFERENCES

1. Stehlik J, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dipchand AI, et al. The Registry of the International Society for Heart and Lung Transplantation: 29th Official Adult Heart Transplant Report--2012. *J Heart Lung Transpl* (2012) 31:1052–64. doi:10.1016/j.healun.2012.08.002
2. Baraldo M, Gregoraci G, Livi U. Steroid-Free and Steroid Withdrawal Protocols in Heart Transplantation: The Review of Literature. *Transpl Int* (2014) 27:515–29. doi:10.1111/tri.12309
3. Kim IC, Youn JC, Kobashigawa JA. The Past, Present and Future of Heart Transplantation. *Korean Circ J* (2018) 48:565–90. doi:10.4070/kcj.2018.0189
4. Stehlik J, Kobashigawa J, Hunt SA, Reichenspurner H, Kirklin JK. Honoring 50 Years of Clinical Heart Transplantation in Circulation: In-Depth State-Of-The-Art Review. *Circulation* (2018) 137:71–87. doi:10.1161/CIRCULATIONAHA.117.029753
5. Ruiz R, Kirk AD. Long-Term Toxicity of Immunosuppressive Therapy. In: Busuttil RW, Klintmalm GBG, editors. *Transplantation of the Liver (3rd Edition)*. Philadelphia, PA: Saunders (2015). p. 1354–63.
6. Lindenfeld J, Miller GG, Shakar SF, Zolty R, Lowes BD, Wolfel EE, et al. Drug Therapy in the Heart Transplant Recipient: Part II: Immunosuppressive Drugs. *Circulation* (2004) 110:3858–65. doi:10.1161/01.CIR.0000150332.42276.69
7. Heegaard B, Nelson LM, Gustafsson F. Steroid Withdrawal After Heart Transplantation in Adults. *Transpl Int* (2021) 34:2469–82. doi:10.1111/tri.14142
8. Chang DH, Youn JC, Dilibero D, Patel JK, Kobashigawa JA. Heart Transplant Immunosuppression Strategies at Cedars-Sinai Medical Center. *Int J Heart Fail* (2021) 3:15–30. doi:10.36628/ijhf.2020.0034
9. Baran DA, Zucker MJ, Arroyo LH, Camacho M, Goldschmidt ME, Nicholls SJ, et al. A Prospective, Randomized Trial of Single-Drug Versus Dual-Drug Immunosuppression in Heart Transplantation: The Tacrolimus in

Combination, Tacrolimus Alone Compared (TICTAC) Trial. *Circ Heart Fail* (2011) 4:129–37. doi:10.1161/CIRCHEARTFAILURE.110.958520

10. Barten MJ, Hirt SW, Garbade J, Bara C, Doesch AO, Knosalla C, et al. Comparing Everolimus-Based Immunosuppression With Reduction or Withdrawal of Calcineurin Inhibitor Reduction From Six Months After Heart Transplantation: The Randomized MANDELA Study. *Am J Transpl* (2019) 18. doi:10.1111/ajt.15361
11. Arora S, Andreassen AK, Karason K, Gustafsson F, Eiskjaer H, Botker HE, et al. Effect of Everolimus Initiation and Calcineurin Inhibitor Elimination on Cardiac Allograft Vasculopathy in De Novo Heart Transplant Recipients. *Circ Heart Fail* (2018) 11:e004050. doi:10.1161/CIRCHEARTFAILURE.117.004050
12. Asleh R, Briasoulis A, Kremers WK, Adigun R, Boilson BA, Pereira NL, et al. Long-Term Sirolimus for Primary Immunosuppression in Heart Transplant Recipients. *J Am Coll Cardiol* (2018) 71:636–50. doi:10.1016/j.jacc.2017.12.005
13. Potena L, Pellegrini C, Grigioni F, Amarelli C, Livi U, Maccherini M, et al. Optimizing the Safety Profile of Everolimus by Delayed Initiation in De Novo Heart Transplant Recipients: Results of the Prospective Randomized Study EVERHEART. *Transplantation* (2018) 102:493–501. doi:10.1097/TP.0000000000001945
14. Fine NM, Kushwaha SS. Recent Advances in Mammalian Target of Rapamycin Inhibitor Use in Heart and Lung Transplantation. *Transplantation* (2016) 100:2558–68. doi:10.1097/TP.0000000000001432
15. Nelson LM, Andreassen AK, Andersson B, Gude E, Eiskjaer H, Radegran G, et al. Effect of Calcineurin Inhibitor-Free, Everolimus-Based Immunosuppressive Regimen on Albuminuria and Glomerular Filtration Rate After Heart Transplantation. *Transplantation* (2017) 101:2793–800. doi:10.1097/TP.0000000000001706
16. Yang J, Jeong JC, Lee J, Kim YH, Paik HC, Kim JJ, et al. Design and Methods of the Korean Organ Transplantation Registry. *Transpl Direct* (2017) 3:e191. doi:10.1097/TXD.0000000000000678
17. Lee HY, Jeon ES, Kang SM, Kim JJ. Initial Report of the Korean Organ Transplant Registry (KOTRY): Heart Transplantation. *Korean Circ J* (2017) 47:868–76. doi:10.4070/kcj.2016.0403
18. Kim D, Choi JO, Oh J, Cho HJ, Jung SH, Lee HY, et al. The Korean Organ Transplant Registry (KOTRY): Second Official Adult Heart Transplant Report. *Korean Circ J* (2019) 49:724–37. doi:10.4070/kcj.2018.0392
19. Mehra MR, Crespo-Leiro MG, Dipchand A, Ensminger SM, Hiemann NE, Kobashigawa JA, et al. International Society for Heart and Lung Transplantation Working Formulation of a Standardized Nomenclature for Cardiac Allograft Vasculopathy-2010. *J Heart Lung Transpl* (2010) 29:717–27. doi:10.1016/j.healun.2010.05.017
20. Velleca A, Shullo MA, Dhital K, Azeka E, Colvin M, DePasquale E, et al. The International Society for Heart and Lung Transplantation (ISHLT) Guidelines for the Care of Heart Transplant Recipients. *J Heart Lung Transpl* (2023) 42:e1–e141. doi:10.1016/j.healun.2022.10.015
21. Stewart S, Winters GL, Fishbein MC, Tazelaar HD, Kobashigawa J, Abrams J, et al. Revision of the 1990 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Heart Rejection. *J Heart Lung Transpl* (2005) 24(11):1710–20. doi:10.1016/j.healun.2005.03.019
22. Berry GJ, Burke MM, Andersen C, Bruneval P, Fedrigo M, Fishbein MC, et al. The 2013 International Society for Heart and Lung Transplantation Working Formulation for the Standardization of Nomenclature in the Pathologic Diagnosis of Antibody-Mediated Rejection in Heart Transplantation. *J Heart Lung Transpl* (2013) 32(12):1147–62. doi:10.1016/j.healun.2013.08.011
23. Delgado D, Arazi HC, Sellanes M, Cáceres M, Cárdenas C, Morales C, et al. Study of Early Corticosteroid Withdrawal in Cardiac Transplantation. *Transpl Proc* (1999) 31:2524–5. doi:10.1016/s0041-1345(99)00446-7
24. Eisen HJ, Tuzcu EM, Dorent R, Kobashigawa J, Mancini D, Valantine-von Kaeppler HA, et al. Everolimus for the Prevention of Allograft Rejection and Vasculopathy in Cardiac-Transplant Recipients. *N Engl J Med* (2003) 349(9):847–58. doi:10.1056/NEJMoa022171
25. Weis M, von Scheidt W. Cardiac Allograft Vasculopathy: A Review. *Circulation* (1997) 96:2069–77. doi:10.1161/01.cir.96.6.2069
26. Sharples LD, Jackson CH, Parameshwar J, Wallwork J, Large SR. Diagnostic Accuracy of Coronary Angiography and Risk Factors for Post-Heart-Transplant Cardiac Allograft Vasculopathy. *Transplantation* (2003) 76:679–82. doi:10.1097/01.TP.0000071200.37399.1D
27. Picão S, Oliveira-Santos M, Batista M, Prieto D, Antunes MJ, Pego M, et al. Cardiac Allograft Vasculopathy: Incidence and Predictors in a Single-Center Cohort. *Rev Port Cardiol Engl Ed* (2020) 39:205–12. doi:10.1016/j.repc.2019.10.007

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ORIGINAL RESEARCH
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European Survey on Clinical Practice of Detecting and Treating T-Cell Mediated Kidney Transplant Rejection

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The KDIGO guideline for acute rejection treatment recommends use of corticosteroids and suggests using lymphocyte-depleting agents as second line treatment. Aim of the study was to determine the current practices of detection and treatment of TCMR of kidney allografts amongst European kidney transplant centres. An invitation was sent through ESOT/EKITA newsletters and through social media to transplant professionals in Europe for taking part in the survey. A total of 129 transplant professionals responded to the survey. There was equal representation of small and large sized transplant centres. The majority of centres treat borderline changes (BL) and TCMR (Grade IA-B, IIA-B) in indication biopsies and protocol biopsies with corticosteroids as first line treatment. Thymoglobulin is used mainly as second line treatment for TCMR Grade IA-B (80%) and TCMR IIA-B (85%). Treatment success is most often evaluated within one month of therapy. There were no differences observed between the large and small centres for the management of TCMR. This survey highlights the common practices and diversity in clinics for the management of TCMR in Europe. Testing new therapies for TCMR should be in comparison to the current standard of care in Europe. Better consensus on treatment success is crucial for robust study designs.

Keywords: survey, clinical practice, therapy, diagnostics, TCMR

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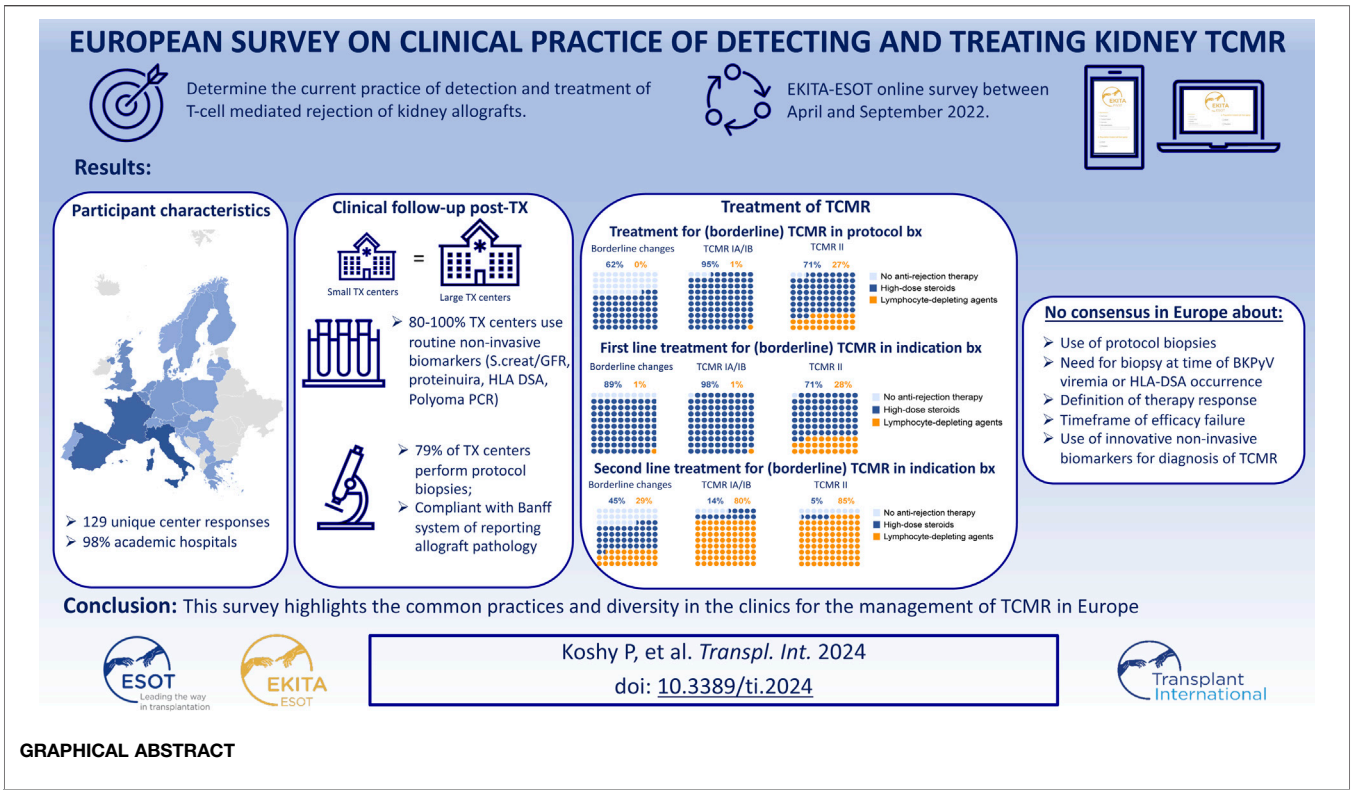
INTRODUCTION

One of the major causes of graft failure is alloimmune rejection, either T cell-mediated, antibody-mediated, or mixed [1, 2]. The histopathological diagnosis of allograft rejection is established by following the Banff working scheme [3–5], which has undergone periodic revisions, based on immunological and clinical insights, clinical and epidemiological studies, and emerging trends of molecular diagnostics.

Despite the progress in precision diagnostics of allograft rejection, very little progress has been made in therapeutics. While the past two decades have seen several attempts to establish the treatment for antibody-mediated rejection (AMR) [6], lesser studies have evaluated treatment



GRAPHICAL ABSTRACT

TABLE 1 | Participant characteristics.

Question	Multiple choices	Number of centres (*N*)	Percentages (%)
Specialization	Nephrologist	100	78.1%
(*n* = 128)	Transplant surgeon	21	16.4%
[1 participant did not respond to this question]	Pathologist	3	2.3%
	Others (transplant coordinator, immunologist, intensivist)	4	3.1%
Population treated	Adult	110	85.9%
(*n* = 128)	Paediatric	5	3.9%
[1 participant did not respond to this question]	Adult and paediatric	13	10.2%
Years in practice	Still in training	6	4.7%
(*n* = 127)	<5 years	16	12.6%
[2 participants did not respond to this question]	5–10 years	19	15.0%
	11–20 years	39	30.7%
	>20 years	47	37.0%
Type of centre	Academic	125	97.7%
(*n* = 128)	Private	1	0.8%
[1 participant did not respond to this question]	Others (public hospital, non-benefit pvt hospital)	2	1.6%
Size of centre	<50 kidney transplantations/year	25	19.4%
(*n* = 129)	50–100 kidney transplantations/year	44	34.1%
	100–150 kidney transplantations/year	32	24.8%
	150–250 kidney transplantations/year	23	17.8%
	>250 kidney transplantations/year	5	3.9%
Living donor %	<10%	30	23.3%
(*n* = 129)	10–<25%	58	45.0%
	>25%	41	31.8%
Repeat transplants %	<10%	24	18.8%
(*n* = 128)	11%–25%	89	69.5%
[1 participant did not respond to this question]	25%–50%	15	11.7%
	>50%	0	0

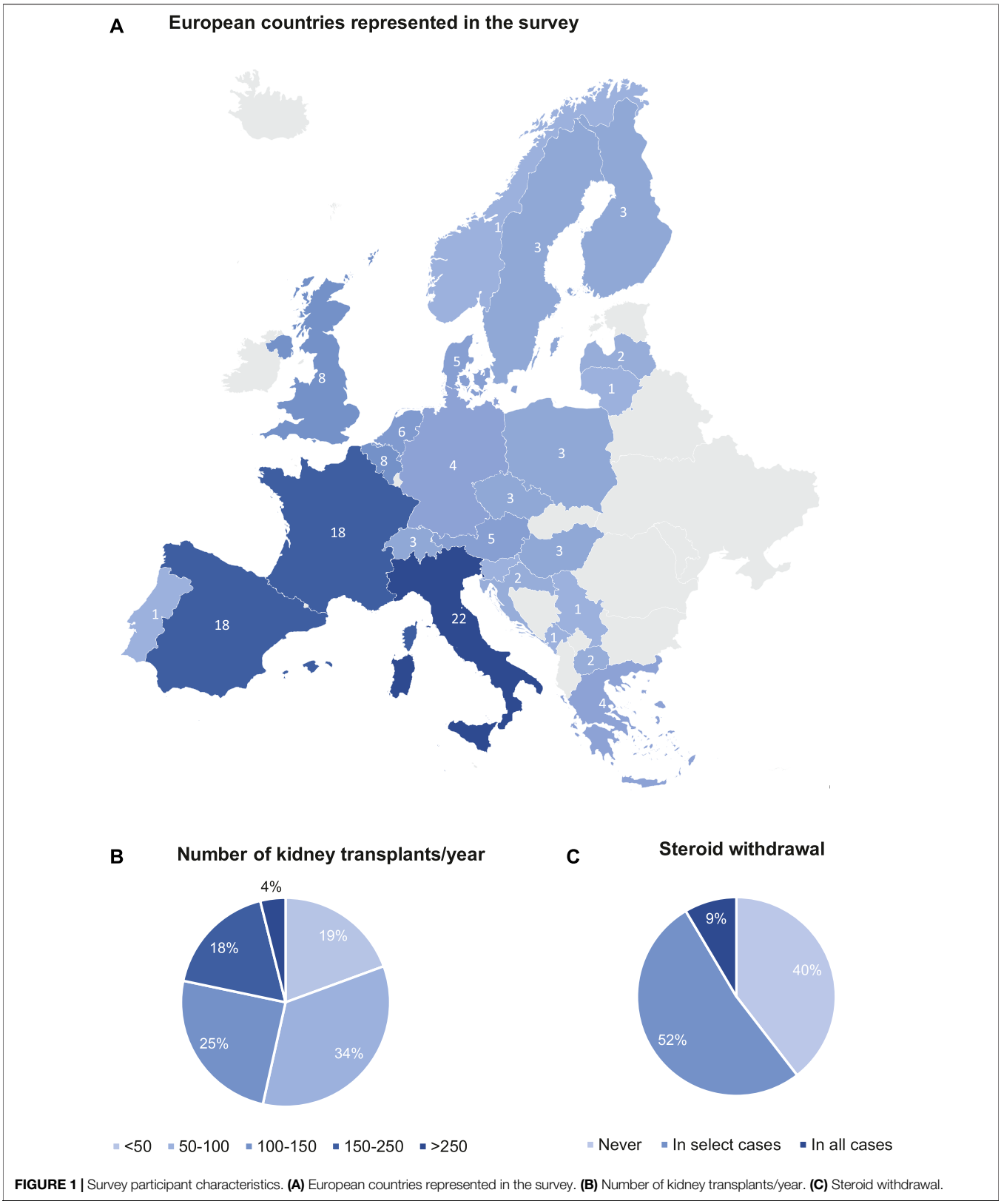


FIGURE 1 | Survey participant characteristics. **(A)** European countries represented in the survey. **(B)** Number of kidney transplants/year. **(C)** Steroid withdrawal.

options for T cell-mediated rejection (TCMR) [7]. Thymoglobulin, the last drug approved for treatment of TCMR, was approved in 1998. A systematic review indicated that antibody therapy was probably better than steroids in reversing acute cellular rejection and in preventing subsequent rejection, and also in preventing graft loss. T cell depleting

TABLE 2 | Standard of care therapy for kidney transplantation—induction and treatment for TCMR other than steroids.

Question	Multiple choices	Number of centres (*N*)	Percentages (%)
Type of induction therapy used at the time of transplantation (*n* = 125) [4 participants did not respond to this question]	Basiliximab	20	16.0
	Thymoglobulin/ATG	5	4.0
	Alemtuzumab	1	0.8
	Basiliximab or Thymoglobulin/ATG	90	72.0
	Basiliximab or Alemtuzumab	7	5.6
	Basiliximab or Thymoglobulin/ATG or Alemtuzumab	1	0.8
	Thymoglobulin/ATG or Alemtuzumab	1	0.8
Steroid withdrawal within the first months after transplantation (*n* = 129)	Yes, in all cases	11	8.5
	Yes, in select cases	67	51.9
	No	51	39.5
Authority approval of thymoglobulin/ATG in kidney transplantation—all that apply (*n* = 120) [9 participants did not respond to this question]	For treatment of rejection, without specification, to be decided by the treating physician	89	74.2
	Only for treatment of steroid-resistant rejection	32	26.7
	Only in case of rejection at time of graft dysfunction (indication biopsies)	6	5.0
	Only as induction therapy	77	64.2
	There is no reimbursement	1	0.8
	Other (desensitization, as primary treatment for TCMR - Grade 2a upward, steroid resistant rejection, v > 0)	3	2.5
Availability of alemtuzumab for treatment of rejection (*n* = 112) [17 participants did not respond to this question]	For treatment of rejection, without specification, to be decided by the treating physician	19	17.0
	For treatment of steroid-resistant rejection	7	6.2
	Not available for treatment of rejection	86	76.8

antibodies are efficacious but associated with a much greater risk for adverse effects [8, 9]. However, no information is available on rejection grades or clinical context; most studies were performed only with rejection in indication biopsies. Few clinical trials on treatment for subclinical TCMR with steroids showed mixed results [10–12]. Since the T-cell depleting agents were approved, no new drugs were studied for this indication, despite the high unmet need for effective treatment of TCMR, with less therapeutic side effects.

The 2009 KDIGO guideline for treatment of acute TCMR recommends the use of corticosteroids as the initial treatment and suggests using lymphocyte-depleting antibodies (ATG or thymoglobulin; OKT3 is no longer available) if the patient is non-responsive to corticosteroids or if there is recurrence of acute cellular rejection. It was also suggested that subclinical and borderline TCMR should be treated and background immunosuppression optimized [13]. More recent guidelines echo these recommendations, without further evidence supporting them, also acknowledging that the use of protocol biopsies to detect and treat subclinical rejection is not built on strong evidence [14].

Because of both the lack of strong evidence for treatment choices in subtypes or different grades of (borderline) TCMR and the absence of international consensus guidelines on this topic, transplantation centre practices differ substantially. Not only do transplantation centre practices differ in the performance of protocol biopsies [6], but also in the treatment approaches for patients with rejection as reported in study reports on this topic [7]. Surveys in the United States and Canada confirmed this heterogeneity and indicate also differences between countries [15, 16]. Recent reports, on the background of tacrolimus-mycophenolate based therapy, document a high rate of persistent rejection following anti-rejection therapy for both clinical and subclinical rejection, which is associated with poor long-term outcomes (i.e., *de novo* anti-HLA donor-specific antibodies, AMR graft loss) [17, 18].

The last consensus forum defining efficacy endpoints for the assessment of anti-rejection therapy was in 1995 and relied primarily on renal functional criteria [19]. The definitions of rejection, insights in pathophysiology and outcome, and treatment options have changed significantly over the past 25 years. Therefore, a new consensus on more recent data is needed. However, European data on the current clinical practice of detection, treatment, and follow-up after rejection are lacking.

As the clinical practice in Europe is likely different from that in Canada and the United States, enriching the debate and adapting

TABLE 3 | Clinical follow-up post-transplant.

Question	Multiple choices	Number of centres (*N*)	Percentages (%)
By whom	Nephrologist	117	92.1
(*n* = 127)	Transplant surgeon	4	3.1
[2 participants did not respond to this question]	Others (both)	6	4.7
Where	Transplant centre	79	62.2
(*n* = 127)	Referring centre	5	3.9
[2 participants did not respond to this question]	Mixed/Hybrid	40	31.5
	Others	3	2.4
Protocol biopsies performed	Never	54	42.5
(*n* = 127)	Always	46	36.2
[2 participants did not respond to this question]	In specific groups	27	21.3
Definition of protocol biopsies	Prescheduled, irrespective of kidney function	87	81.3
(*n* = 107)	Defined based on stable kidney function	20	18.7
[22 participants did not respond to this question]			
Timing of protocol biopsies—all that apply	1 week	1	1.4
(*n* = 73)	2 weeks	1	1.4
	1 month	5	6.8
	3 months	48	65.8
	6 months	8	11.0
	1 year	45	61.6
	2 years	9	12.3
	5 years	2	2.7
	10 years	1	1.4
	Others (3 years)	3	4.1
Standard biopsy procedure	Hospitalization	71	55.9
(*n* = 127)	Outpatient based	56	44.1
[2 participants did not respond to this question]			
Indications for "for-cause" biopsies—all that apply	Slow recovery of graft function	117	92.1
(*n* = 127)	Deterioration of eGFR	126	99.2
[2 participants did not respond to this question]	Proteinuria	117	92.1
	Polyomavirus replication	76	59.8
	HLA-DSA occurrence	76	59.8
	Others	7	5.5
Routine non-invasive testing to guide kidney transplant biopsies—all that apply		127	100
(*n* = 127)	Serum creatinine/eGFR		
[2 participants did not respond to this question]	Proteinuria	123	96.9
	Cystatin C	12	9.4
	Polyomavirus PCR in urine	28	22.0
	Polyomavirus PCR in blood	105	82.7
	Urinary chemokines	4	3.1
	Donor-derived cell-free DNA testing	8	6.3
	Monitoring for *de novo* HLA-DSA occurrence	102	80.3
	Other tests (CMV, non-HLA antibody testing, MAG3 at DGF, DSA for high risk cases only)	4	3.1

consensus to the current European reality is necessary. Charting the standard of care in clinical practice is essential in designing innovator drug trials, which need a well-defined comparator group. Insight in current routine practice of TCMR diagnosis and treatment could pave the way to new trials heavily needed in the field.

Here, we report on a survey conducted to determine the current practices of detection and treatment of TCMR of kidney allografts amongst European kidney transplant centres, and compare these practices with previous reports from the United States and Canada [15, 16].

METHODS

A survey was drafted by all co-authors and transferred to a SurveyMonkey (Momentive Global Inc., San Mateo, California, United States) web-based platform, which was tested by all co-authors. An invitation to participate in the survey was sent through the European Society for Organ Transplantation (ESOT) and European Kidney Transplant Association (EKITA) newsletters and through a social media campaign to transplant professionals in Europe for taking part in the survey. Several reminders were sent. Also, an individual email campaign was

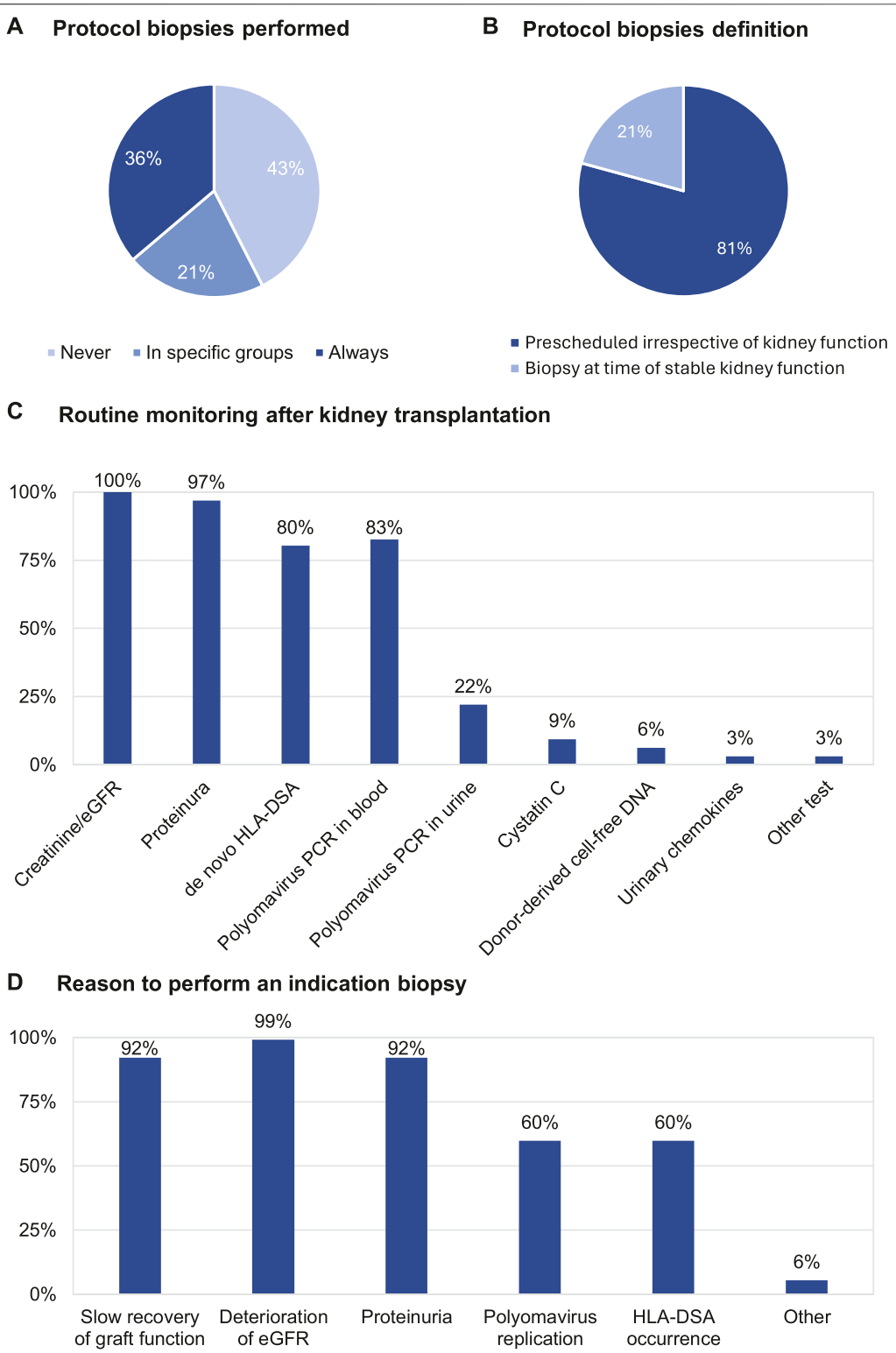


FIGURE 2 | Clinical follow-up post-transplant: **(A)** Protocol biopsies performed. **(B)** Protocol biopsies definition. **(C)** Routine monitoring after kidney transplantation. **(D)** Reason to perform an indication biopsy.

TABLE 4 | Diagnosis of rejection.

Question	Multiple choices	Number of centres (*N*)	Percentages (%)
Biopsy results evaluated by	Nephropathologist	111	91.7
(*n* = 121)	General pathologist	7	5.8
[8 participants did not respond to this question]	Nephrologist	3	2.5
Pathology report—definition of TCMR	According to the most recent Banff 2019 classification	117	96.7
(*n* = 121)	According to older versions of Banff classification	2	1.7
[8 participants did not respond to this question]	Not according to Banff classification	2	1.7
Pathology report—individual lesion scores	Individual Banff lesion scores are routinely reported	109	90.8
(*n* = 120)	Individual Banff lesion scores are not routinely reported	11	9.2
[9 participants did not respond to this question]			
Diagnosis of rejection without performing a kidney transplant biopsy	Never	78	64.5
(*n* = 121)	Based on non-invasive markers but not always confirmed by biopsy	29	24.0
[8 participants did not respond to this question]	We do not do biopsies to confirm rejection	1	0.8
	Others (in patients with high risk/contraindication)	13	10.7
Molecular microscope for diagnosis of rejection in routine clinic	Never	113	93.4
(*n* = 121)	Always	1	0.8
[8 participants did not respond to this question]	In specific cases (mainly for clinical trials/research)	7	5.8
Rate of clinical TCMR (in indication biopsies)	<5%	15	12.8
(*n* = 117)	5-<11%	39	33.3
[12 participants did not respond to this question]	11–<16%	30	25.6
	16-<26%	23	19.7
	>26%	10	8.5
Definition of borderline changes	t ≥ 1, i ≥ 1 threshold	73	60.3
(*n* = 121)	t 1/2/3 with i0 considered as borderline changes	21	17.4
[8 participants did not respond to this question]	Other (t1 or t0 with i1 or i0)	1	0.8
	Unknown	26	21.5

launched to reach as many centres as possible in Europe. The survey was conducted in 2022.

The survey questionnaire was divided into two parts.

Part 1 consisted of four categories:

- Category 1—Survey participant characteristics—questions regarding specialization, population treated, years in practice, type of transplantation centre, size of centre, induction therapy at time of transplantation, time period of steroid withdrawal, percentage of living donors and percentage of repeat transplants.
- Category 2—Clinical follow-up post-transplant—questions regarding clinical follow-up by whom, where, performance of protocol biopsies, indications for for-cause biopsies and about non-invasive testing to guide kidney transplant biopsies.
- Category 3—Diagnosis of rejection—questions regarding reporting of allograft biopsies, use of Banff lesion scores, diagnosis of rejection without performing kidney biopsy, use of molecular microscope for diagnosis of rejection in routine clinic, rate of clinical TCMR, definition of borderline rejection, authority approval of thymoglobulin and alemtuzumab.
- Category 4—Definition of successful rejection treatment of TCMR.

Part 2 consisted of questions on treatment of subclinical and clinical TCMR.

Descriptive statistical analyses were performed in Prism 9 for macOS (GraphPad Prism version 9.5.0, GraphPad Software, San Diego, California United States[1]).

RESULTS

Survey Participant Characteristics

Survey participant characteristics are detailed in **Table 1**. A total of 129 European transplant professionals representing 25 European countries responded to the survey (**Figure 1A**). Most of the participants were transplant nephrologists (78.1%) treating the adult population with more than 11 years of experience. 94 (72.9%) participants volunteered to mention their affiliation, and they represent 92 major university hospitals in Europe. 85.9% of centres perform uniquely adult kidney transplants, 10.2% both adults and paediatric transplants, and 3.9% in children/adolescents only. 69 (53.5%) transplant centres perform <100 kidney transplantations per year, while

[1]www.graphpad.com

TABLE 5 | Definition of successful rejection treatment of TCMR.

Question	Multiple choices	Number of centres (*N*)	Percentages (%)
Definition of "therapy resistant TCMR"—all that apply (*n* = 117) [12 participants did not respond to this question]	When creatinine/eGFR does not completely return to baseline	35	29.9
	When creatinine/eGFR recovers not at all or at best partly	59	50.4
	When creatinine/eGFR does not improve anything	44	37.6
	Based on follow-up biopsy histology	62	53.0
	Others	4	3.4
Definition of "therapy resistant TCMR" (*n* = 117) [12 participants did not respond to this question]	Based on graft functional evolution	55	47.0
	Based on follow-up biopsy histology	19	16.2
	Based on combination of functional evolution and follow-up biopsy histology	43	36.8
Definition of "steroid-resistant TCMR" (*n* = 116) [13 participants did not respond to this question]	When creatinine/eGFR does not completely return to baseline after high-dose steroid treatment	29	25.0
	When creatinine/eGFR recovers not at all or at best partly after highdose steroid treatment	42	36.2
	When creatinine/eGFR does not improve anything	10	8.6
	Based on follow-up biopsy histology	28	24.1
	When second-line therapy is initiated, irrespective of kidney function or histology	4	3.4
	Other	3	2.6
Definition of "return to baseline kidney transplant function" (*n* = 117) [12 participants did not respond to this question]	Based on whole eGFR/creatinine trajectory	66	56.4
	Based on best value of eGFR/creatinine	19	16.2
	Based on graft function prior to the diagnostic biopsy	31	26.5
	Other	1	0.8
Timeframe of efficacy failure of antirejection treatment (*n* = 113) [16 participants did not respond to this question]	At 1 week	30	26.5
	At 14 days	37	32.7
	Within 1 month	33	29.2
	Within 3 months	8	7.1
	Within 6 months	0	0
	Others	5	4.4
Performance of a control/follow-up biopsy after rejection treatment to see disease resolution (*n* = 117) [12 participants did not respond to this question]	After every antirejection treatment, also when diagnosed in protocol biopsies	8	6.8
	After every treatment for clinical TCMR, also when kidney function improved	7	6.0
	When kidney function did not completely recover to baseline	29	24.8
	When renal function did not improve sufficiently upon treatment	61	52.1
	In selected cases	5	4.3
	(Almost) never	7	6.0
If control biopsies are performed, when are they planned (*n* = 99) [30 participants did not respond to this question]	After 14 days	29	29.3
	After 1 month	23	23.2
	After 3 months	18	18.2
	After 6 months	3	3.0
	Others	26	26.3

60 (46.5%) transplant centres perform >100 kidney transplantations per year on average (**Figure 1B**). Living donation rates vary greatly between centres and countries. The majority (69.5%) perform 11%–25% repeat transplantations. It has not been surveyed whether induction therapy is used in all or selected patients. There is a heterogeneity in the drugs used for induction therapy at the time of transplantation (**Table 2**): 72% of the respondents use either basiliximab or thymoglobulin; 8% of the respondents include alemtuzumab in their armamentarium for induction. Many respondents (51.9%) stop administering steroids within the first months after transplantation in selected cases (not further specified), while other respondents (39.5%) do not have steroid withdrawal protocols. Only few respondents (8.5%) systematically discontinue steroids in all cases within the first months after transplantation (**Figure 1C**).

Clinical Follow-Up Post-Transplant

Table 3 summarizes the standard practices for post-transplant follow-up by the respondents included in the survey. The clinical follow-up post-transplant is conducted mainly by the transplant nephrologists (92.1%) in the transplant centre (62.2%) but hybrid follow-up in collaboration with the referring centre is also common (31.5%). Protocol biopsies are performed in the centres of 57.5% of respondents (**Figure 2A**), but only 36.2% of respondents always perform a protocol biopsy. 21.3% of respondents perform protocol biopsies in specific subgroups of

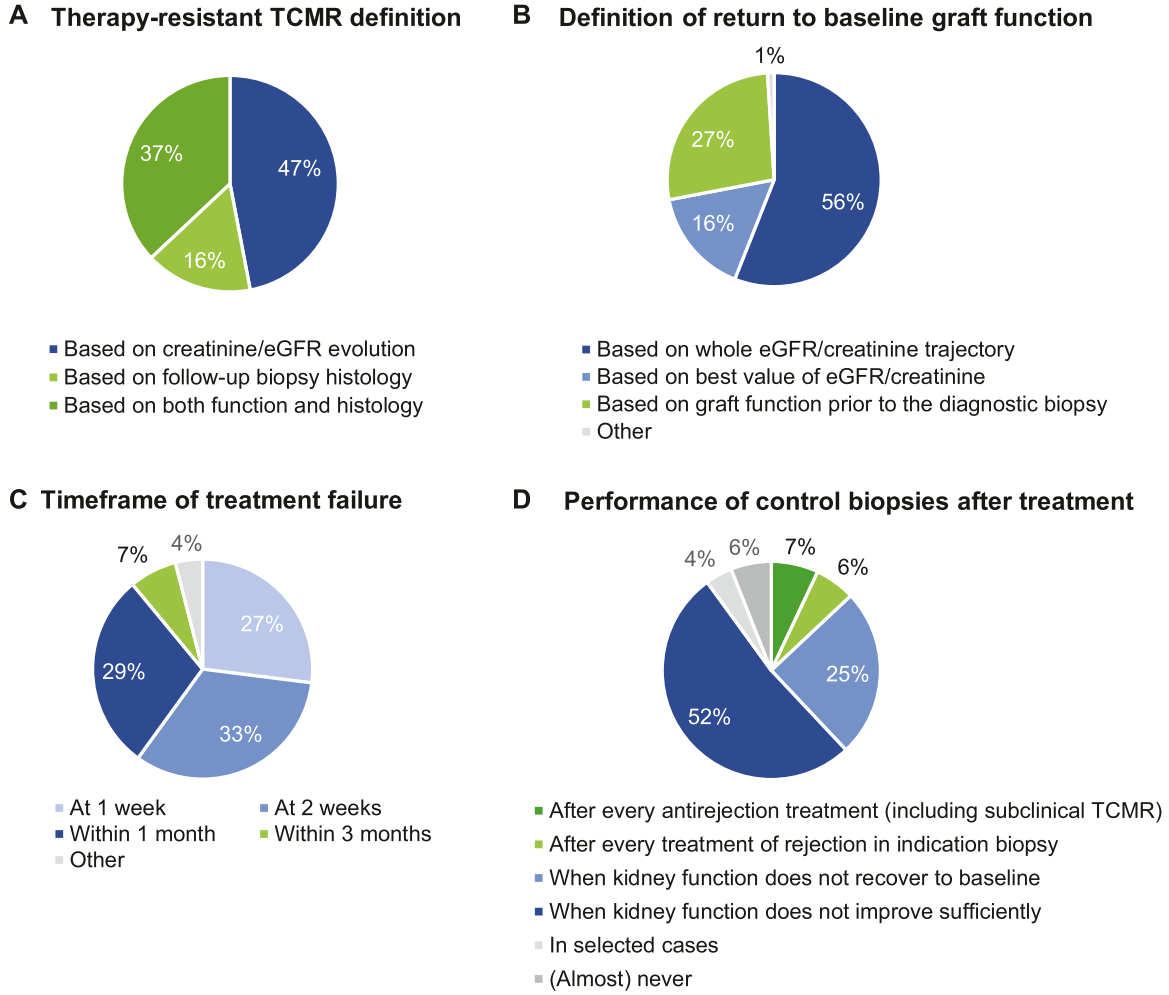


FIGURE 3 | Definition of successful rejection treatment of TCMR. **(A)** Therapy-resistant TCMR definition. **(B)** Definition of return to baseline graft function. **(C)** Timeframe of treatment failure. **(D)** Performance of control biopsies after treatment.

patients, for example, in highly sensitized/immunized patients; in patients with positive donor-specific anti-HLA antibodies (HLA-DSA); in patients participating in clinical trials; and depending on the primary native kidney disease. Protocol biopsies are mainly conducted at 3 months and 1 year post-transplant; very few respondents perform protocol biopsies later after transplantation. There is no difference in performing protocol biopsies between the respondents performing <100 renal transplantations/year and the respondents performing >100 renal transplantations/year (**Supplementary Table S1**). Most of the respondents performing protocol biopsies (81.3%), defined protocol biopsies as "prescheduled, irrespective of kidney function (**Figure 2B**)." 55.9% of respondents perform kidney biopsies after hospitalization of patients and 44.1% respondents perform kidney biopsies as outpatient procedure. The routine non-invasive testing to guide kidney transplant biopsies are serum creatinine/eGFR (100%), proteinuria (96.9%), polyomavirus PCR in blood (82.7%), monitoring for *de novo* HLA-DSA (80.3%) and polyomavirus PCR in urine (22%). Only a few respondents (<10%) also monitor cystatin C (9.4%), urinary chemokines (3.1%), and donor derived cell-free DNA (6.3%) (**Figure 2C**). The common indications for "for-cause" biopsies are slow recovery of graft function (92.1%), deterioration of eGFR (99.2%), and proteinuria (92.1%). There is less concordance about performing an indication biopsy at the time of polyomavirus replication (59.8%) or with HLA-DSA occurrence (59.8%) (**Figure 2D**).

Diagnosis of TCMR

In Europe, the kidney transplant biopsies are mostly evaluated by renal pathologists (91.7%), who are considered to follow the most recent Banff 2019 classification (**Table 4**). Most of the pathology reports (90.8%) include the individual Banff lesion scores routinely. Many respondents (64.5%) never diagnose rejection without performing a kidney biopsy, but this is not universal and 24% of respondents diagnose rejection based on non-invasive markers

TABLE 6 | Treatment of TCMR.

First-line therapy	Protocol biopsies			Indication biopsies		
	Borderline changes	TCMR grade IA/IB	TCMR grade II	Borderline changes	TCMR grade IA/IB	TCMR grade II
Number of respondents	85	85	85	108	108	107
Anti-rejection therapy	53 (62.4%)	82 (96.5%)	83 (97.6%)	97 (89.8%)	107 (99.1%)	106 (99.1%)
Thymoglobulin/ATG/alemtuzumab	0 (0%)	1 (1.2%)	23 (27.1%)	1 (0.9%)	1 (0.9%)	30 (28.0%)
High-dose steroids	53 (62.4%)	81 (95.3%)	60 (70.6%)	96 (88.9%)	106 (98.1%)	76 (71.0%)
- High-dose IV steroids followed by PO taper	7 (8.2%)	23 (27.1%)	24 (28.2%)	16 (14.8%)	28 (25.9%)	32 (29.9%)
- High-dose IV steroids	44 (51.8%	55 (64.7%)	35 (41.2%)	76 (70.4%)	78 (72.2%)	44 (41.1%)
- Steroid taper PO	2 (2.4%)	3 (3.5%)	1 (1.2%)	4 (3.7%)	0 (0%)	0 (0%)
Increased baseline immunosuppression	20 (23.5%)	1 (1.2%)	0 (0%)	11 (10.2%)	1 (0.9%)	1 (0.9%)
No change	12 (14.1%)	2 (2.4%)	2 (2.4%)	0 (0%)	0 (0%)	0 (0%)
Second-line therapy	**Borderline changes**	**TCMR grade IA/IB**	**TCMR grade II**	**Borderline changes**	**TCMR grade IA/IB**	**TCMR grade II**
Number of respondents	—	—	—	98	106	106
Anti-rejection therapy	—	—	—	72 (73.5%)	100 (94.3%)	95 (89.6%)
Thymoglobulin/ATG/alemtuzumab	—	—	—	28 (28.6%)	85 (80.2%)	90 (84.9%)
High-dose steroids	—	—	—	44 (44.9%)	15 (14.2%)	5 (4.7%)
- High-dose IV steroids followed by PO taper	—	—	—	11 (11.2)	6 (5.7%)	1 (0.9%)
- High-dose IV steroids	—	—	—	30 (30.6%)	9 (8.5%)	4 (3.8%)
- Steroid taper PO	—	—	—	3 (3.1%)	0 (0%)	0 (0%)
Increased baseline immunosuppression	—	—	—	17 (17.3%)	4 (3.8%)	8 (7.5%)
No change	—	—	—	9 (9.2%)	2 (1.9%)	3 (2.8%)

not always confirmed by tissue biopsy. Most respondents (93.4%) do not use biopsy-based molecular diagnostics for the diagnosis of rejection in routine clinical practice.

The rate of clinical TCMR in indication biopsies reported by the respondents is highly variable, and significantly relates to the rate of repeat transplantations (**Supplementary Table S2**). Most respondents (60.3%) report using the Banff 2019 ($t \geq 1$, $i \geq 1$) threshold for the definition of borderline changes in their centre, but 26 respondents (21.5%) do not know the threshold used at their centre for defining borderline changes.

Definition of Successful Rejection Treatment of TCMR

We next evaluated the definitions of "successful rejection treatment." The definition of therapy resistant TCMR is highly heterogeneous (**Table 5**). The question asked to the participants ("all that apply") lead to redundancy in the responses, as several respondents ticked multiple choices—"When creatinine/eGFR does not completely return to baseline"; "When creatinine/eGFR recovers not at all or at best partly" and "When creatinine/eGFR does not improve anything." This indicates that the definitions of complete return to baseline, partial recovery or "any improvement" are unclear to the respondents. Therefore, we reformatted the responses to evaluate whether creatinine/eGFR vs. histological evaluations was considered for the definition of therapy resistant TCMR. This indicates high heterogeneity in this definition, with 47% of respondents using creatinine/eGFR evolution, 16% pure biopsy histology, and 37% integration of information from biopsies and from creatinine/eGFR for the definition of therapy resistance; 53% of respondents integrate the use of a repeat biopsy in the definition of therapy resistance (**Figure 3A**).

The majority of respondents define "steroid resistant TCMR" based on graft functional characteristics (36.2% when creatinine/eGFR recovers not at all or at best partly; 25.0% when creatinine/eGFR does not completely return to baseline; 8.6% when creatinine/eGFR does not improve anything), but 24.1% indicate defining this based on follow-up biopsy histology; combinations between graft functional and histological definition were not allowed for this question (**Table 5**).

The majority of respondents define "return to baseline kidney transplant function" by assessing the whole eGFR/creatinine trajectory (56.4%), while others base this evaluation on graft function prior to the diagnostic biopsy (26.5%) and based on the best value of eGFR/creatinine (16.2%), again indicating lack of consensus in these responses (**Table 5**; **Figure 3B**).

Next, we surveyed the timeframe of efficacy failure of antirejection treatment. Most respondents (88.5%) consider therapy failure "within 1 month" as the period of efficacy failure of antirejection treatment. Only 7.1% of respondents consider therapy failure at 3 months or later (**Table 5**; **Figure 3C**). Many respondents (76.9%) perform a control/follow-up biopsy after rejection treatment for assessment of disease resolution only when the renal function does not improve sufficiently upon treatment; systematic control biopsies are performed in only 12.8% (**Table 5**; **Figure 3D**). If control biopsies are performed

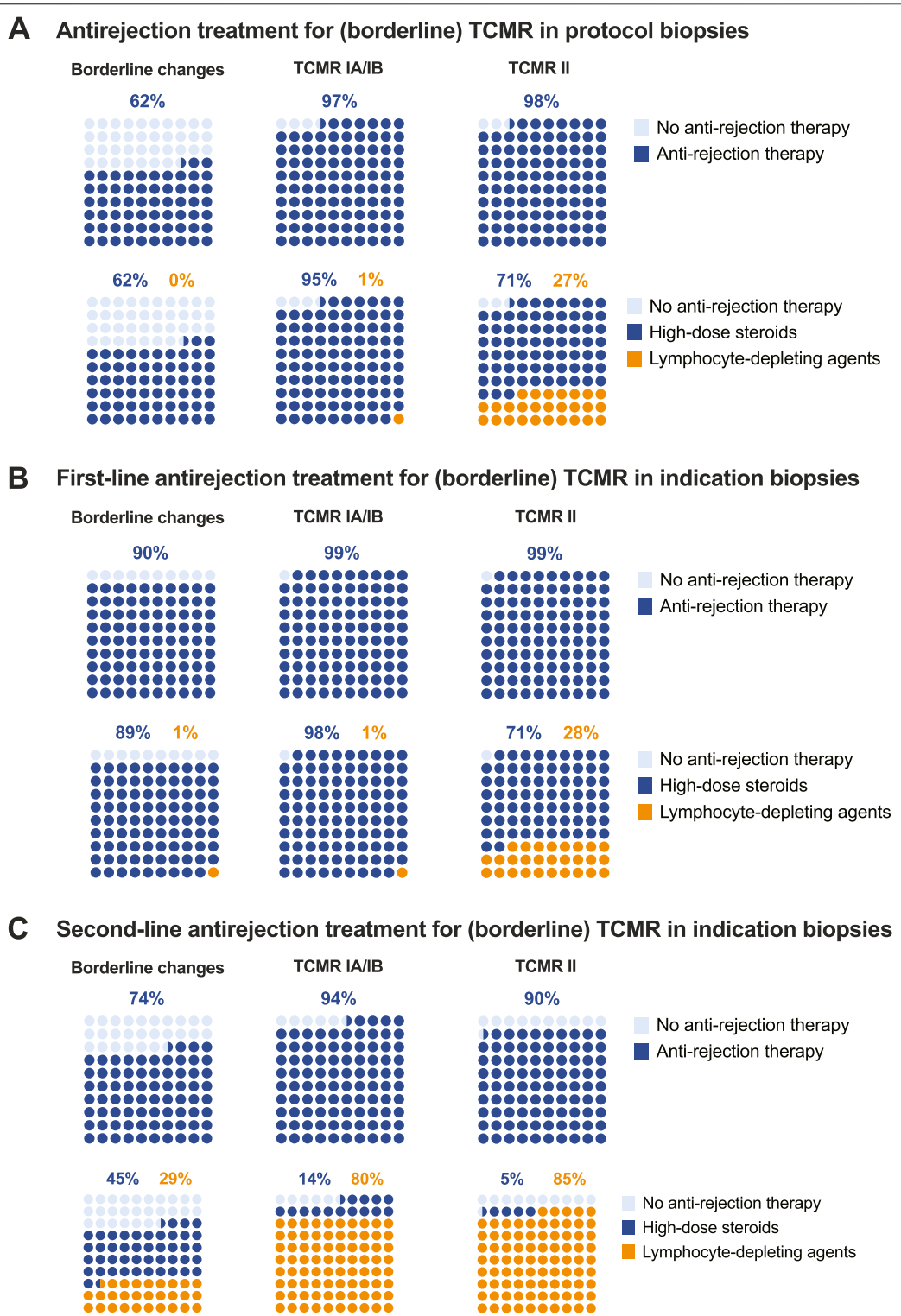


FIGURE 4 | Treatment of TCMR. **(A)** Antirejection treatment for (borderline) TCMR in protocol biopsies. **(B)** First-line antirejection treatment for (borderline) TCMR in indication biopsies. **(C)** Second-line antirejection treatment for (borderline) TCMR in indication biopsies.

after rejection treatment, their timing is very variable between respondents; either after 14 days (29.3%), after 1 month (23.2%), or after 3 months (18.2%); others responded that this timing depends on kidney functional evolution. Altogether, this indicates that there is no consensus on the best timing for performing a control biopsy (**Table 5**).

Treatment of TCMR

The responses to the questions about first-line and second-line treatment for TCMR were highly variable between respondents. The granular responses are summarized by counting the strongest therapy indicated by the respondent for each rejection type (Thymoglobulin/ATG/alemtuzumab > IV steroids with PO taper > high-dose IV steroids > PO steroid taper > increase baseline immunosuppression > no change). Several centres report, e.g., combinations of ATG with IV steroids and increase baseline immunosuppression. Doses of IV corticosteroids range between 250, 500 and 1,000 mg for 3 days. PO steroid taper was not further specified.

Most centres (74.2%) report having authority approval for using thymoglobulin/ATG at the physician's discretion, while others (23.7%) can use thymoglobulin/ATG only for treatment of steroid-resistant rejection. Alemtuzumab is not widely available in Europe; only 23.2% of centres report having access for anti-rejection treatment (**Table 2**).

Subclinical (Borderline) TCMR in Protocol Biopsies

Not all centres perform protocol biopsies. Per definition, centres not performing protocol biopsies do not diagnose and do not treat subclinical rejection. Upon detection of subclinical borderline changes, 62.4% of respondents report treating such cases with high-dose steroids, but never with lymphocyte-depleting agents. Other respondents just optimize baseline immunosuppression. Only a small minority reports not changing therapy after the detection of subclinical borderline changes. Most centres treat subclinical TCMR. Treatment of subclinical TCMR consists mainly of high-dose IV steroids, although 27% of respondents report using lymphocyte-depleting agents for treatment of subclinical TCMR grade II (**Table 6**; **Figure 4A**).

(Borderline) TCMR in Indication Biopsies

Borderline changes are almost universally treated when diagnosed at the time of graft dysfunction (in indication biopsies). Even more so for TCMR grade I-II, which is universally treated. Lymphocyte-depleting agents are not used as first-line therapy for borderline changes or TCMR grade I, but 28% of respondents report treating TCMR grade II with thymoglobulin, ATG or alemtuzumab in the first line (**Table 6**; **Figure 4B**).

Second-line treatment of (borderline) TCMR, after the failure of first-line treatment (with varying definitions), is less universally applied than could be anticipated. This relates especially to borderline changes, where second-line antirejection therapy is not considered in 26.5% of cases, and to TCMR grade II, where 10.4% of respondents would not treat, likely because they already treat these patients with strong therapies (including lymphocyte depleting agents) in first line (**Table 6**; **Figure 4C**). Of the 39 respondents proposing lymphocyte-depleting agents as first-line therapy for TCMR grade II, 4 (10.3%) propose alemtuzumab as second-line therapy (after thymoglobulin/ATG); 15 (38.5%) do not propose second-line therapy but just increase baseline immunosuppression after failure of first-line therapy. The other respondents (N = 20; 51.2%) repeat the same therapy with lymphocyte-depleting agents despite the lack of success in first-line treatment.

DISCUSSION

This survey assesses the clinical practices in the transplant centres across Europe for detecting and treating TCMR. A total of 129 participants took part in the survey, wherein the majority were transplant nephrologists with over 11 years of clinical experience, covering the routine clinical practice across all European areas. There were almost equal numbers of small sized transplant centres (centres performing less than 100 kidney transplantations per year) and large sized transplant centres (centres performing 100 to 250 kidney transplantations per year). All conclusions made are against the background of relatively low numbers of centres systematically withdrawing corticosteroids after transplantation, and with a lack of access to, e.g., alemtuzumab in a majority of centres.

The main conclusions of the survey are:

1) Protocol biopsies to detect subclinical rejection are not universally performed, not different between small and larger transplant centres. Some centres always perform protocol biopsies, others never, and still some others only in specific patient populations.
2) The definition of a protocol biopsy is not standardized.
3) The large majority of European centres use classic biomarkers for follow-up after transplantation; donor-derived cell-free DNA assessment or other biomarkers are not used to non-invasively assess the probability of ongoing or future rejection. Sixty percent of centres see BKPyV replication in plasma and *de novo* occurrence of HLA-DSA as indications for a biopsy, but this is also not universal.
4) The most updated Banff Classification is considered as the gold standard for diagnosis of TCMR with also individual Banff lesion scores given, although many respondents are not aware of the detailed thresholds for borderline changes applicable.
5) Biopsy based molecular diagnostics are not commonly used in Europe.
6) There is great heterogeneity in the definition of anti-rejection treatment success. Therapy resistance is sometimes defined based on graft functional evolution, sometimes on histological evaluation of a follow-up biopsy, and often on both together. Systematic control or follow-up biopsies are not common though (and less common than in the US where 40% perform follow-up biopsies [15]); subclinical disease continuation would thus be missed by most European centres.
7) The lack of standardized definition of "baseline graft function" complicates the definition of treatment success,

which is often estimated by the total eGFR/creatinine trajectory and not based on a single measurement.
8) There is quite consensus that treatment success or failure is evaluated on a short term, within the first month.
9) Transplant centres consider borderline changes often as indication for therapy, even when diagnosed in protocol biopsies, although not all centres perform such biopsies systematically and subclinical rejection is per definition missed in those centres. Certainly in indication biopsies, borderline changes are deemed clinically meaningful, leading to treatment with high-dose steroids and the related treatment burden/risk.
10) Full TCMR is almost universally treated, with some difference in the approach to TCMR grade I vs. grade II, the latter being treated sometimes with lymphocyte-depleting agents in the first line, although this is the case in only a minority of the centres.
11) Second-line therapy of TCMR consists of a step-up approach towards almost universal use of lymphocyte-depleting agents, if not already used in first line. Centres using lymphocyte-depleting agents in first line (for grade II TCMR) lack efficacious second-line therapies, clear indication of the great unmet need.

Our results about the heterogeneity in the implementation of protocol biopsies are in line with other recent reports [6, 15, 20]. In our survey, respondents indicate that subclinical (borderline) TCMR is treated very similarly to clinical (borderline) TCMR. In Europe, subclinical borderline changes are treated with high-dose steroids in 62% of cases, similar to the 64% reported in Canada [16]. This phenotype is even more often treated in the US with high-dose IV/PO steroids (50%/33%), and even thymoglobulin. Only 22% of subclinical borderline rejections are not being treated in the United States [15], despite lack of evidence of effects on outcome. In case of subclinical TCMR IA and IB, all US centres performing protocol biopsies reported treating this entity, which is comparable to our European survey results and previous Canadian results [15, 16]. Like in Canada, thymoglobulin is virtually not used in Europe for subclinical TCMR grade IA/IB. However, quite some respondents (27%) in Europe propose lymphocyte-depleting agents for subclinical TCMR grade II, again like the practice in the United States [15]. Although performing a biopsy and treating subclinical (borderline) TCMR is not based on strong evidence [10, 12, 21, 22], this indicates that subclinical rejection, when detected and subsequently treated, is a clinically meaningful event, as was also concluded recently by a working group of ESOT [23].

Our survey illustrates that, in Europe, very few centres use innovative non-invasive markers for kidney transplant rejection, and most rely solely on eGFR/creatinine and proteinuria as clinical indication for performing biopsies, while some also see HLA-DSA occurrence and BKPyV replication in plasma as indications for performing a biopsy [24]. At time of graft dysfunction, in indication biopsies, borderline changes is routinely treated in Europe by 90% of respondents using high-dose steroids, even slightly higher than the 81% of the respondents in the US survey who treat this entity using high-dose steroids [15]. This strongly confirms that borderline changes diagnosed at time of graft dysfunction is a clinically meaningful event, potentially suitable as an endpoint for clinical trials [23].

For clinical TCMR IA and IB, all US centres treat with either IV steroids (91%, 71%), PO steroids (21%), or thymoglobulin (13%) [15]. In contrast, thymoglobulin is not often used for this type of rejection in Europe and corticosteroids remain the European mainstay as first-line therapy for this entity, as was also reported for Canada [16]. A final major difference between EU and US is that TCMR grade II is treated with thymoglobulin in 98% of cases in the United States [15], while this is the case for only 28% of respondents in Europe; no data are available for Canada for this rejection type.

Finally, we assessed the definition of successful anti-rejection treatment. The lack of international standardization/consensus on primary definitions hampers the field. Previously, the Canadian survey [16] and an older multicentre survey from 1998 [19], indicated that therapy success is typically measured against the prerejection creatinine level. Our survey adds to this by indicating that most respondents evaluate the overall trajectory of eGFR/creatinine (no single values), and often also integrate information from follow-up biopsies in this evaluation. However, the latter is not at all standardized. Likewise, the Canadian survey indicated that 30% of respondents assessed histological response to treatment independent of changes in kidney function [16]. More systematic study of post-treatment follow-up biopsies would be needed to understand the rate of disease persistence/recurrence despite treatment, which is very likely underestimated according to single-centre data [17, 18].

Notwithstanding the important conclusions of this survey, some limitations are worth mentioning. Not all responses were easily interpretable, especially when "all that apply" multiple choices were allowed (e.g., for definition of steroid/therapy-resistant rejection). We did not assess the baseline immunosuppression or standard induction therapy used by the centres. This study focused on (borderline) TCMR; it remains unclear whether, e.g., repeat biopsies, definition of treatment success/failure, etc. can be generalized also to, e.g., AMR or mixed TCMR-AMR phenotypes. Data analysis remains largely descriptive, and potential relationships between different answers were not systematically assessed.

CONCLUSION

In conclusion, our survey indicates that the treatment of TCMR is a great unmet clinical need. Current TCMR treatment is still primarily based on high dose corticosteroids, resembling early transplantation practices. Testing new therapies for TCMR should be in comparison to the current standard of care for TCMR, which differs between the United States and Europe/Canada. Better consensus on treatment success is crucial for robust study designs. However, there is good consensus that treatment success is a short-term outcome parameter, achieved within the first few weeks of/after antirejection treatment. Borderline changes are typically treated like full TCMR, and are thus clinically meaningful when diagnosed in indication biopsies. Subclinical rejections, even borderline changes, diagnosed by some centres performing protocol biopsies, are

also often treated despite a lack of robust scientific evidence. The field should investigate innovative treatment options for TCMR after kidney 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

MN, LF, PN, GZ, MH, and AdV drafted the questionnaire and tested the survey. MN and PK analysed the data and wrote the manuscript. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.12283/full#supplementary-material

REFERENCES

1. Van Loon E, Senev A, Lerut E, Coemans M, Callemeyn J, Van Keer JM, et al. Assessing the Complex Causes of Kidney Allograft Loss. *Transplantation* (2020) 104:2557–66. doi:10.1097/TP.0000000000003192
2. Mayrdorfer M, Liefeldt L, Wu K, Rudolph B, Zhang Q, Friedersdorff F, et al. Exploring the Complexity of Death-Censored Kidney Allograft Failure. *J Am Soc Nephrol* (2021) 32:1513–26. doi:10.1681/ASN.2020081215
3. Roufosse C, Simmonds N, Clahsen-van Groningen M, Haas M, Henriksen KJ, Horsfield C, et al. A 2018 Reference Guide to the Banff Classification of Renal Allograft Pathology. *Transplantation* (2018) 102:1795–814. doi:10.1097/TP.0000000000002366
4. Naesens M, Roufosse C, Colvin RB, Haas M, Lefaucheur C, Adam B, et al. *The Banff 2022 Kidney Meeting Report: Re-Appraisal of Microvascular Inflammation and the Role of Biopsy-Based Transcript Diagnostics* (2023). doi:10.1016/j.ajt.2023.10.016
5. Roufosse C, Naesens M, Colvin RB, Haas M, Lefaucheur C, Aubert O, et al. *The Banff 2022 Kidney Meeting Work Plan: Data-Driven Refinement of the Banff Classification for Renal Allografts* (2023). doi:10.1016/j.ajt.2023.10.031
6. Schinstock CA, Askar M, Bagnasco SM, Batal I, Bow L, Budde K, et al. Banff Antibody-Mediated Injury Working Group Examination of International Practices for Diagnosing Antibody-Mediated Rejection in Kidney Transplantation – A Cohort Study. *Transpl Int* (2020) 34:488–98. doi:10.1111/tri.13813
7. Ho J, Okoli GN, Rabbani R, Lam OLT, Reddy VK, Askin N, et al. Effectiveness of T Cell–Mediated Rejection Therapy: A Systematic Review and Meta-Analysis. *Am J Transplant* (2022) 22:772–85. doi:10.1111/ajt.16907
8. Webster AC, Wu S, Tallapragada K, Park MY, Chapman JR, Carr SJ. Polyclonal and Monoclonal Antibodies for Treating Acute Rejection Episodes in Kidney Transplant Recipients. *Cochrane Database Syst Rev* (2017) 7:CD004756. doi:10.1002/14651858.CD004756.pub4
9. Nikolova A, Patel JK. Induction Therapy and Therapeutic Antibodies. *Handbook Exp Pharmacol* (2022) 272:85–116. doi:10.1007/164_2021_570
10. Rush D, Nickerson P, Gough J, McKenna R, Grimm P, Cheang M, et al. Beneficial Effects of Treatment of Early Subclinical Rejection: A Randomized Study. *J Am Soc Nephrob* (1998) 9:2129–34. doi:10.1681/ASN.V9112129
11. Rush D, Arlen D, Boucher A, Busque S, Cockfield SM, Girardin C, et al. Lack of Benefit of Early Protocol Biopsies in Renal Transplant Patients Receiving TAC and MMF: A Randomized Study. *Am J Transplant* (2007) 7:2538–45. doi:10.1111/j.1600-6143.2007.01979.x
12. Szederkényi E, Iványi B, Morvay Z, Szenohradszki P, Borda B, Marofka F, et al. Treatment of Subclinical Injuries Detected by Protocol Biopsy Improves the Long-Term Kidney Allograft Function: A Single Center Prospective Randomized Clinical Trial. *Transplant Proc* (2011) 43:1239–43. doi:10.1016/j.transproceed.2011.03.078
13. Kasiske BL, Zeier MG, Chapman JR, Craig JC, Ekberg H, Garvey CA, et al. KDIGO Clinical Practice Guideline for the Care of Kidney Transplant Recipients: A Summary. *Kidney Int* (2010) 77:299–311. doi:10.1038/ki.2009.377
14. Baker RJ, Mark PB, Patel RK, Stevens KK, Palmer N. Renal Association Clinical Practice Guideline in Post-Operative Care in the Kidney Transplant Recipient. *BMC Nephrol* (2017) 18:174. doi:10.1186/s12882-017-0553-2
15. Sood P, Cherikh WS, Toll AE, Mehta RB, Hariharan S. Kidney Allograft Rejection: Diagnosis and Treatment Practices in USA- A UNOS Survey. *Clin Transpl* (2021) 35:e14225. doi:10.1111/ctr.14225
16. Leblanc J, Subrt P, Paré M, Hartell D, Sénécal L, Blydt-Hansen T, et al. Practice Patterns in the Treatment and Monitoring of Acute T Cell-Mediated Kidney Graft Rejection in Canada. *Can J Kidney Health Dis* (2018) 5:2054358117753616. doi:10.1177/2054358117753616
17. Rampersad C, Balshaw R, Gibson IW, Ho J, Shaw J, Karpinski M, et al. The Negative Impact of T Cell–Mediated Rejection on Renal Allograft Survival in the Modern Era. *Am J Transplant* (2022) 22:761–71. doi:10.1111/ajt.16883
18. Nankivell BJ, Agrawal N, Sharma A, Taverniti A, P'Ng CH, Shingde M, et al. The Clinical and Pathological Significance of Borderline T Cell–Mediated Rejection. *Am J Transplant* (2019) 19:1452–63. doi:10.1111/ajt.15197
19. Guttmann RD, Soulillou JP, Moore LW, First MR, Gaber AO, Pouletty P, et al. Proposed Consensus for Definitions and Endpoints for Clinical Trials of Acute Kidney Transplant Rejection. *Am J Kidney Dis* (1998) 31(6):S40–6. doi:10.1053/ajkd.1998.v31.pm9631863
20. Mehta R, Bhusal S, Randhawa P, Sood P, Cherukuri A, Wu C, et al. Short-Term Adverse Effects of Early Subclinical Allograft Inflammation in Kidney Transplant Recipients With a Rapid Steroid Withdrawal Protocol. *Am J Transplant* (2018) 18:1710–7. doi:10.1111/ajt.14627
21. Kurtkoti J, Sakhuja V, Sud K, Minz M, Nada R, Kohli HS, et al. The Utility of 1- and 3-Month Protocol Biopsies on Renal Allograft Function: A Randomized Controlled Study. *Am J Transplant* (2008) 8:317–23. doi:10.1111/j.1600-6143.2007.02049.x
22. Rush DN, Gibson IW. Subclinical Inflammation in Renal Transplantation. *Transplantation* (2019) 103:E139–E145. doi:10.1097/TP.0000000000002682
23. Seron D, Rabant M, Becker JU, Roufosse C, Bellini MI, Böhmig GA, et al. Proposed Definitions of T Cell-Mediated Rejection and Tubulointerstitial Inflammation as Clinical Trial Endpoints in Kidney Transplantation. *Transpl Int* (2022) 35:10135. doi:10.3389/ti.2022.10135
24. van den Broek DAJ, Meziyerh S, Budde K, Lefaucheur C, Cozzi E, Bertrand D, et al. The Clinical Utility of Post-Transplant Monitoring of Donor-Specific Antibodies in Stable Renal Transplant Recipients: A Consensus Report With Guideline Statements for Clinical Practice. *Transpl Int* (2023) 36:11321. doi:10.3389/ti.2023.11321

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Tacrolimus's Time Below Therapeutic Range Is Associated With Acute Pancreatic Graft Rejection and the Development of *De Novo* Donor-specific Antibodies

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Tacrolimus is pivotal in pancreas transplants but poses challenges in maintaining optimal levels due to recipient differences. This study aimed to explore the utility of time spent below the therapeutic range and intrapatient variability in predicting rejection and *de novo* donor-specific antibody (dnDSA) development in pancreas graft recipients. This retrospective unicentric study included adult pancreas transplant recipients between January 2006 and July 2020. Recorded variables included demographics, immunosuppression details, HLA matching, biopsy results, dnDSA development, and clinical parameters. Statistical analysis included ROC curves, sensitivity, specificity, and predictive values. A total of 131 patients were included. Those with biopsy-proven acute rejection (BPAR, 12.2%) had more time (39.9% ± 24% vs. 25.72% ± 21.57%, $p = 0.016$) and tests (41.95% ± 13.57% vs. 29.96% ± 17.33%, $p = 0.009$) below therapeutic range. Specific cutoffs of 31.5% for time and 34% for tests below the therapeutic range showed a high negative predictive value for BPAR (93.98% and 93.1%, respectively). Similarly, patients with more than 34% of tests below the therapeutic range were associated with dnDSA appearance (38.9% vs. 9.4%, $p = 0.012$; OR 6.135, 1.346–27.78). In pancreas transplantation, maintaining optimal tacrolimus levels is crucial. Suboptimal test percentages below the therapeutic range prove valuable in identifying acute graft rejection risk.

Keywords: pancreas transplantation, tacrolimus, FK, time in therapeutic range, TTR

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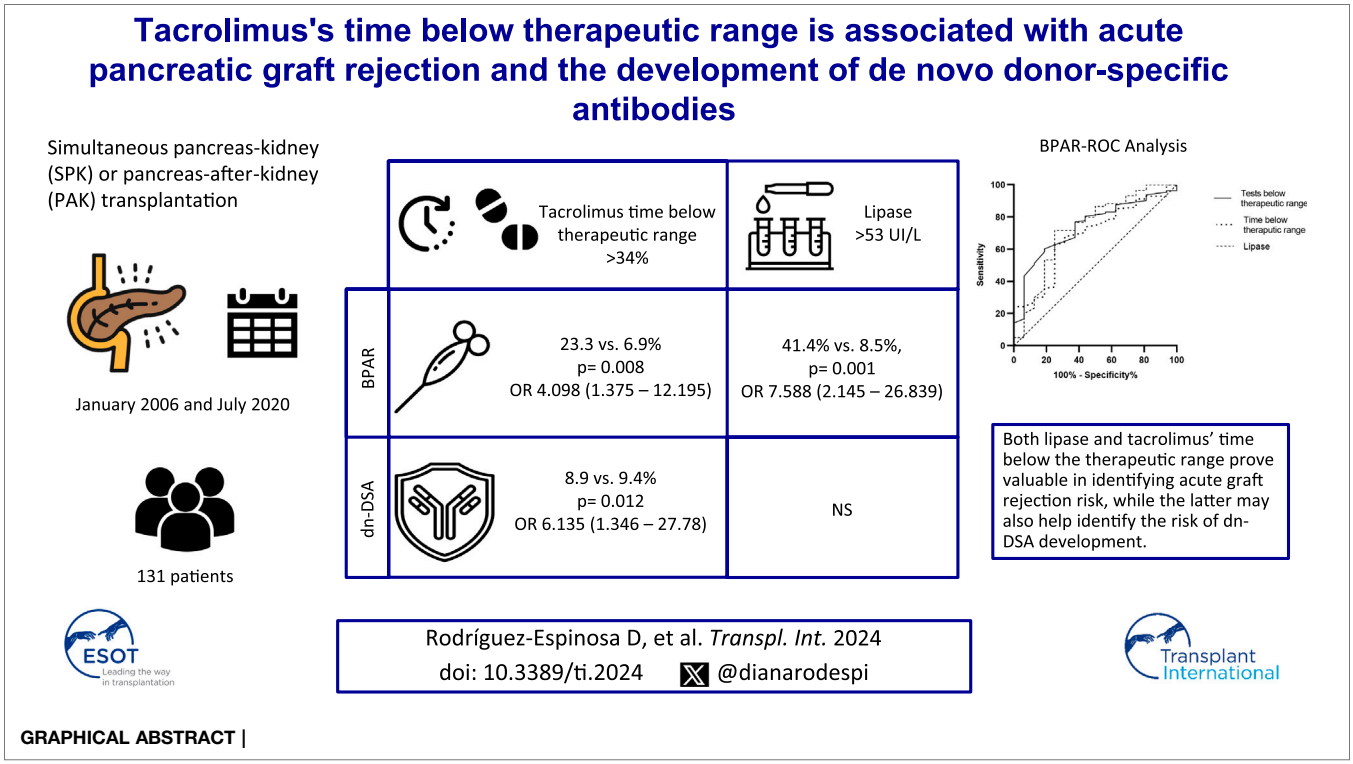
Abbreviations: antiGAD, glutamic acid decarboxylase antibodies; AUC, area under the curve; BPAR, biopsy-proven acute rejection; dnDSA, *de novo* donor-specific antibody; HbA1c, hemoglobin A1C; HLA, human leukocyte antigen; INR, international normalized ratio; IPV, intrapatient variability; MFI, mean fluorescence intensity; NPV, negative predictive value; PAK, pancreas after kidney; PPV, positive predictive value; rATG, rabbit antithymocyte globulin; ROC, receiver operating characteristic; SPK, simultaneous pancreas-kidney; TTR, time in therapeutic range.



GRAPHICAL ABSTRACT |

INTRODUCTION

Tacrolimus has been the mainstay immunosuppressive agent in pancreas transplantation in the last two decades [1–3], given its effectiveness in preventing rejections and increasing graft survival [4]. It presents a narrow therapeutic window, requiring strict monitoring and constant dosing modification [5]. Differences in tacrolimus absorption [6, 7], metabolism [8, 9], and drug interactions [6, 10, 11] often lead to either sub- or supratherapeutic trough levels [12, 13]. Above-target trough levels are associated with adverse effects, whereas those below target are associated with an increased risk of rejection and development of *de novo* donor-specific antibody (dnDSA) [14, 15].

Given the pharmacokinetics and pharmacodynamics of tacrolimus [6, 8–11], several formulas have been developed to explore the correlation of tacrolimus trough levels with graft outcomes. Intrapatient variability (IPV) calculates the variability coefficient by dividing the standard deviation of tacrolimus samples by their mean [16, 17]. A high IPV has been associated with an increased risk of rejection, development of dnDSA, and graft failure in kidney transplantation [16–20] and with rejections in heart [21] and lung [22], though not in liver transplantation [23]. Time in therapeutic range (TTR), first developed by Rosendaal et al. to monitor anticoagulation time in patients on warfarin [24], has been recently used to explore the correlation of the time of tacrolimus within the therapeutic window and its correlation with graft outcomes. In lung, heart, and kidney transplantation, a lower TTR is associated with dnDSA development [25], acute rejection, and graft survival [26–29]. However, there are many concerns about the accuracy of this formula, as it assumes tacrolimus will change linearly from test to test [30]. The method used for managing warfarin assumes a linear increase or decrease between two consecutive INR (International Normalized Ratio) determinations [30, 31]. Therefore, we propose using the formula that calculates the ratio of samples within the therapeutic range to the total number of samples, also from Rosendaal et al. [24]. Additionally, if the primary study outcome is immunological, it may be more useful to only determine the time below the therapeutic range [25]. To date, the ability of these formulas to predict pancreas graft outcomes has yet to be explored.

In this study, we aimed to determine the utility of tacrolimus IPV and the time and test results below the therapeutic range in identifying the risks of rejection and dnDSA development for pancreatic grafts in recipients of pancreas transplants.

MATERIALS AND METHODS

Patient Population

We conducted a retrospective unicentric study including all adult pancreas transplant recipients between January 2006 and July 2020 from Hospital Clínic of Barcelona. Both simultaneous pancreas-kidney (SPK) and pancreas after kidney (PAK) were analyzed. We excluded patients in whom TTR was not possible to calculate; those who had a primary non-function pancreas graft, those lost to follow-up, and those who died due to transplantation

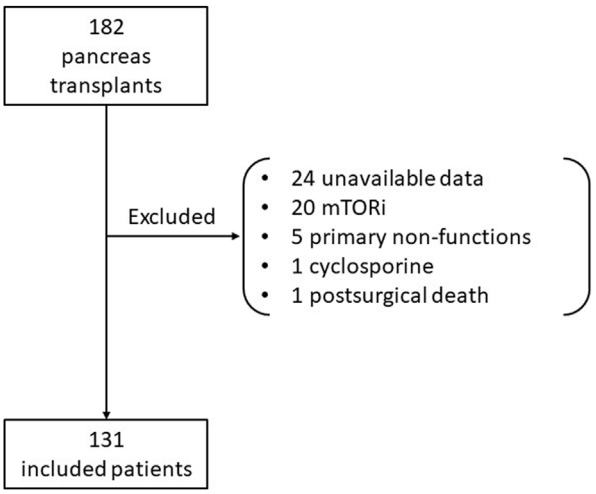


FIGURE 1 | Flowchart of included patients.

surgery. One-hundred and eighty-two pancreas transplants were performed during the study period; fifty-one were excluded (**Figure 1**). Data was gathered from electronic clinical records. This study was conducted in accordance with the Declaration of Helsinki.

Variables

Demographic data such as age, weight, body mass index, sex, and race at the time of transplantation were recorded for donors and recipients. Induction immunosuppression was performed with rabbit antithymocyte globulin (rATG) or basiliximab. Maintenance immunosuppression consisted of tacrolimus in combination with mycophenolic acid and prednisone. The human leukocyte antigen (HLA) A, B, and DR for both recipient and donor and the number of HLA mismatches were registered. Other variables recorded were amylase, lipase, blood glucose, hemoglobin A1C (HbA1C), c-peptide, glutamic acid decarboxylase antibodies (antiGAD), blood type, surgical technique (duo-duodenal or duo-jejunal anastomosis), diabetes mellitus type and vintage, renal replacement therapy at time of transplantation (peritoneal dialysis, hemodialysis, non-dialysis dependent chronic kidney disease), dialysis vintage, graft perfusion solution, and post-transplant surgical reintervention.

Time and Tests Below the Therapeutic Range

The first tacrolimus dose was administered pre-transplantation as part of the induction immunosuppression protocol, and the first levels were drawn 48 h after surgery. The minimum tacrolimus levels targeted were 10 ng/mL during the first 3 months, 8 ng/mL between the third and sixth month, and 6 ng/mL afterward. The percentage of time below the tacrolimus therapeutic range was calculated by adding the number of days below the target and dividing them by the total number of monitored days. Likewise, the percentage of the number of tests below the therapeutic range was calculated by adding the number of test results below the target and dividing them by the total number of tests performed respectively [24].

Biopsy-Proven Rejection and DSA Determination

Pancreatic graft biopsies were conducted per protocol (3 weeks and 12 months after transplantation) or per cause. According to the center's guidelines, biopsies prompted per cause were indicated when patients exhibited a consistent rise (on at least two occasions, with a gap of more than 48 h) in pancreatic enzymes (amylase and/or lipase) exceeding three times the upper normal levels, developed dnDSA, or persistent hyperglycemia (fasting blood glucose >120 mg/dL on more than two determinations). Tissue samples were collected using a percutaneous needle puncture guided by ultrasound, and their histological categorization followed the criteria outlined in the 2011 Banff classification [32]. Tacrolimus trough levels, amylase, lipase, c-peptide, HbA1C, and anti-HLA and antiGAD antibodies were determined at the time of biopsy. *De novo* DSAs were defined as HLA antibodies against the donor that were absent before transplantation. DSAs were characterized by having a mean fluorescence intensity (MFI) that was more than double the negative control's value and an absolute MFI exceeding 500 [33]. The MFIs were adjusted based on the manufacturer's guidelines by comparing them to the negative control beads.

Statistical Methods

For data following normal distribution, quantitative variables are presented as mean and standard deviation; otherwise, they are presented as median and interquartile range. Categorical variables are described in terms of absolute and relative frequencies. The normality of quantitative variables was assessed using the Shapiro-Wilk test and Q-Q plots. When the data was not normally distributed, a U-Mann Whitney test was employed for the quantitative variables' comparison between the two groups; for normally distributed data, an independent Student's t-test was used instead. Disparities in categorical variables were evaluated using the $\chi 2$ test, while Fisher's exact test was utilized when a category contained fewer than five occurrences. Receiver Operating Characteristic (ROC) curves were generated, and metrics such as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. A p-value of less than 0.05 was considered statistically significant. All analyses were conducted using IBM SPSS® Statistics version 26.

RESULTS

Participants

One hundred and thirty-one patients were included; sixty-nine (52.7%) were male, 122 (93.1%) were SPK, and nine were PAK. One hundred and twenty-nine (98.5%) had type 1 diabetes, one had type 2 diabetes, and one had diabetes after a necrotizing hemorrhagic pancreatitis. Seventy (53.4%) of the donors were

TABLE 1 | Differences in clinical and analytical variables between groups.

Variable	Biopsy-proven rejection	No rejection	*p*-value
	N = 16	N = 115	
Pancreas transplantation type, n (%)			0.045
SPK	13 (10.66)	109 (89.34)	
PAK	3 (33.33)	6 (66.67)	
Indication for pancreas transplantation, n (%)			0.868
Type 1 diabetes	16 (12.4)	113 (87.6)	
Type 2 diabetes	0	1	
Hemorrhagic pancreatitis	0	1	
Male donor, n (%)	9 (12.85)	61 (87.14)	0.810
Lipase, U/L, median (IQR)	96 (161)	41 (39.75)	0.078
Amylase U/L, median (IQR)	111.5 (99.5)	88.5 (49.75)	0.111
Glucose mg/dL, median (IQR)	92.5 (38.25)	87.5 (14)	0.284
HbA1C (%), median (IQR)	5.35 (0.93)	5.5 (0.65)	0.894
antiGAD U/mL, median (IQR)	0.55 (15.73)	0.6 (3.72)	0.692
C-peptide ng/mL, mean ± SD	3.4 ± 1.81	3.52 ± 2.53	0.807
Recipient age, mean ± SD	39.66 ± 9.41	41.35 ± 7.53	0.281
Donor age, median (IQR)	36 (23)	36 (14.5)	0.430
BMI, kg/m^2	23.9 ± 2.6	23.35 ± 3.03	0.488
Diabetes vintage (months), mean ± SD	25.7 ± 9.59	27.19 ± 8.21	0.982
Intrapatient variability, median (IQR)	52.18 (0.32)	43.3 (0.13)	0.809
Time BTR, median (IQR)	37.98 (0.37)	20.45 (0.26)	0.028
Tests BTR, mean ± SD	42.43 ± 13.81	32.5 ± 17.07	0.013

antiGAD, Anti-glutamic acid decarboxylase antibody; BMI, body mass index; BTR, below therapeutic range; HbA1C, hemoglobin A1C; PAK, pancreas after kidney; SPK, simultaneous pancreas kidney.

male. Sixty-four patients (48.9%) received basiliximab, and 65 (49.6%) received rATG as induction immunosuppression. All patients were on mycophenolate and tacrolimus as maintenance treatment. The median follow-up was 104 (45.5–139) months. Recipient and donor characteristics are detailed in **Table 1**.

Clinical Outcomes

The median time and tests below the therapeutic range for the entire group were 24.44% (8.58%–40.52%) and 28.6% (20.26%–42.45%), respectively, with a median IPV of 45.15% (38.87%–55.56%). Overall, 32.8% and 35.1% of patients had tacrolimus levels below the therapeutic range in more than 36% of the tests performed and more than 31.5% of the time. Eighteen (13.74%) patients died, nine (50%) of them with a functioning graft. Thirty patients lost their pancreatic graft function (22.9%), with a median survival time of 101.8 (67.6–119.9) months.

Out of the 16 instances of pancreas BPAR (12.2%), two occurred in the same patient. Two were antibody-mediated rejections, while the remaining 14 were T-cell-mediated, with 11 being grade I, 2 grade II, and 1 grade III. The mean BPAR-free survival time was 85.24 ± 17.38 months. Lipase levels were higher in patients with BPAR (185.25 ± 264.37 vs. 59.77 ± 47.92 U/L in those without rejection, $p = 0.001$). There was a trend towards a higher incidence of rejection between PAKs and SPKs (3, 50% vs. 13, 11.9%; OR 4.19, 0.935–18.798; $p = 0.045$).

Patients with BPAR had a significantly higher time (39.9% ± 24% vs. 25.72 %± 21.57%, $p = 0.016$) and number of tests (41.95% ± 13.57% vs. 29.96% ± 17.33%, $p = 0.009$) below therapeutic range compared to those without rejection. There was no association between tacrolimus' IPV or amylase with pancreas BPAR incidence (48.7% vs. 49.9%, $p = 0.81$; 125.63 vs. 98.9, $p = 0.11$).

The area under the curve (AUC) for time and tests below the therapeutic range and BPAR incidence were 70.5% and 73.2%, respectively, and 72.9% for lipase and 63.2% for amylase (**Figure 2**). Based on the highest sensitivity and specificity coordinates, we evaluated the former in two categories: 31.5% for time and 34% for tests. Patients who maintained tacrolimus levels more than 31.5% of the time below the therapeutic range until the moment of the biopsy had a significantly higher probability of having a BPAR (22.1% vs. 6%, $p = 0.004$; OR 4.629, 1.502–14.286) than those who did not. This test had a sensitivity of 68.75%, a specificity of 67.83%, a PPV of 22.92%, and an NPV of 93.98%. Similarly, patients with 36% or more tacrolimus tests below the therapeutic range had a higher probability of pancreas BPAR (23.3% vs. 6.9%, $p = 0.008$; OR 4.098, 1.375–12.195). This test had a sensitivity of 62.5%, a specificity of 71.05%, a PPV of 23.26%, and an NPV of 93.1%. On the other hand, lipase had a specificity of 98.33% and a similar NPV of 89.70%. In this case, also based on the ROC coordinates, we divided the set with a lipase cutoff of 53 U/L. A lipase higher than this correlated with an increased risk of pancreas BPAR (41.4% vs. 8.5%, $p = 0.001$; OR 7.588, 2.145–26.839) with a sensibility of 68.75%, a specificity of 67.83%, a PPV of 22.92%, and an NPV of 93.98%.

Eleven (8.4%) patients developed dnDSAs. Among them, eight recipients had dnDSAs from class II, two from class I, and one from both class I and II. Of these antibodies, 6 (46.1%) were HLA-DQ, 4 (30.78%) were HLA-DR, and 3 (23.08%) were HLA-A. There was a non-significant difference with tacrolimus' IPV (47.9% ± 14.44% vs. 69.27% ± 44.76%, $p = 0.193$), amylase

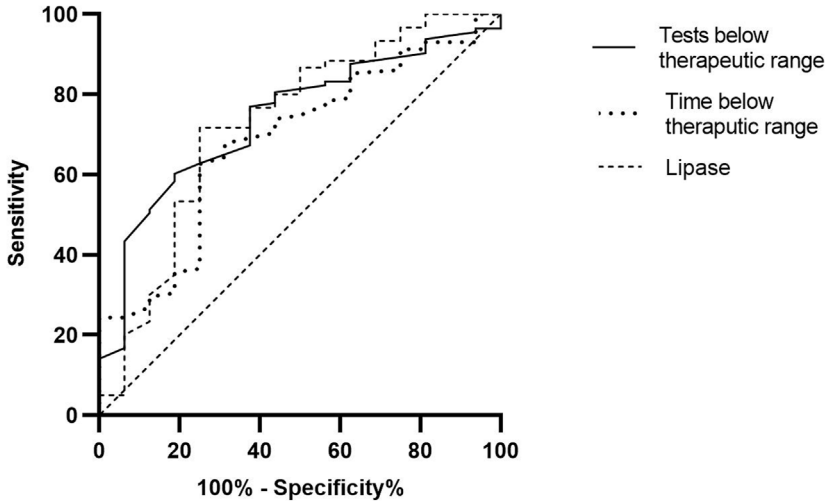


FIGURE 2 | Receiver operating characteristic (ROC) curves of evaluated tests.

(116 ± 42.54 vs. 108 ± 50.23 U/L, $p = 0.68$), and lipase (234.67 ± 344.53 vs. 83.83 ± 71.46 U/L, $p = 0.23$).

The time and tests below the therapeutic range were associated with an increased incidence of dnDSA development (30.18% vs. 43.93%, $p = 0.017$ and 40.57% vs. 71.26%, $p = 0.048$, respectively). However, the AUC for the time was smaller than for the number of tests below the therapeutic range (66.2% vs. 71.3%). When analyzing both variables as dichotomic based on the cutoff values established previously, only the number of tests remained statistically significant (38.9% vs. 9.4%, $p = 0.012$; OR 6.135, 1.346–27.78).

DISCUSSION

In this study, we determined that patients who spent longer time or had more tests below the tacrolimus therapeutic range had an increased incidence of acute pancreatic graft rejection. We also found that a cutoff of 31.5% of the time and 34% of the tests were significantly associated with an increased pancreatic rejection incidence with a very high specificity and NPV. Moreover, those with a higher number of tests below the therapeutic range were also associated with an increased incidence of dnDSA. Finally, we performed ROC analysis and found that the time and tests below the therapeutic range had a similar area under the curve compared to lipase for pancreatic graft BPAR.

Tacrolimus is a crucial part of maintenance immunosuppression in pancreas transplantation and is recommended by current guidelines despite being prescribed off-label due to lack of approval by the US Food and Drug Administration. However, sufficient evidence has proven its efficacy in improving short- and long-term pancreatic graft survival [34]. There is some data on specific dosing of immunosuppressors and dnDSA formation in pancreas transplantation. Yet, data on the impact of dosage and monitoring trough levels on the risk of rejection is lacking. That becomes of great importance as tacrolimus has a limited therapeutic threshold that needs to be constantly adjusted by transplant professionals. In this sense, there is evidence that associates the time spent within the therapeutic range of tacrolimus and various solid-organ graft survival, such as lung, heart, and kidney [35]. Nevertheless, there is currently no data on this subject in patients with SPK or PAK.

In our cohort, we found that around a third of patients were below the targeted tacrolimus therapeutic range, similar to data published by Davis et al. [33], although theirs is only from the first-year post-kidney transplantation.

Regarding BPAR, we found a rejection incidence of 12.2%, similar to the reported 10%–14% incidence published previously [36]. There is evidence evaluating the usefulness of TTR in other solid organ transplants, such as lung transplantation, where a cutoff of 30% or an increase of 10% of the baseline TTR, in turn, decreased the risk of graft rejection [20, 28]. Similarly, a study on living kidney donors determined that a TTR below 22% increased the risk of kidney graft rejection [26], while another one with deceased donors determined a cutoff of 30% [25]. In the case of heart transplants, a TTR lower than 25% was associated with more rejections [29]. In our case, we found that spending more than 31.5% of the TTR and more than 34% of the tests below the therapeutic level was significantly associated with an increased risk of acute rejection. We also found that an elevated lipase above 53 UI/L was significantly associated with an increased incidence of BPAR. To our knowledge, this is the first evidence on a specific lipase cutoff value since there is no evidence on this subject for stable pancreatic transplant recipients beyond the early postoperative scenario, where they associate a mean value of 634 ± 247 UI/L with an increased incidence of BPAR [37].

Regarding humoral response, there is evidence that lower tacrolimus levels are associated with a higher risk of dnDSA development in kidney transplant recipients [25]. The mentioned article by Davis et al. [33] found dnDSAs in 21.7% of their cohort. Their appearance was associated with a time outside the

therapeutic range of tacrolimus greater than 30%. In our cohort, 8.4% of patients developed dnDSA, and a percentage of 36% or more of tests below the therapeutic range increased the risk of developing them [37]. We did not find any significant association with lipase blood levels. This may be explained by the fact that lipase only translates to an ongoing graft injury, and while a patient with dnDSAs is at risk for rejection, it may not have occurred yet, hence there is no injury. Additionally, not all rejections are antibody-mediated, which may also explain the presence of a significant BPAR and not a dnDSA association.

A study by Torabi et al. [38] performed on pancreatic transplant recipients showed that extended-release tacrolimus was associated with fewer rejections and non-significantly with less IPV. The study by Davis et al. [25] in kidney and SPK recipients determined that an IPV greater than 44% was associated with increased dnDSA development. However, they did not perform an SPK subanalysis. In contrast, we did not find an association between IPV and BPAR and only found a non-significant difference with increased IPV and dnDSA formation.

This study has several limitations. There is a possibility that certain tacrolimus levels may not accurately represent trough levels. Concurrent medical conditions or medications may influence levels, and we did not perform a subanalysis according to patients' baseline immunological risk, given the small sample we worked with. Also, as were only evaluating immunological outcomes, we decided only to study the time below and not within the therapeutic range. Finally, this is a single-center retrospective study, which limits our capacity to determine the exact number of per cause and protocol biopsies, the interpretation of the results obtained, and their generalizability.

To conclude, this study highlights the significance of maintaining proper levels of immunosuppression in pancreas transplantation. It suggests that identifying patients at risk of rejection can potentially be done by monitoring the percentage of tests that fall below the therapeutic range. Additionally, this method could prove to be a valuable tool if combined with new rejection markers, such as donor-derived cell-free DNA [39]. This would enable identification of high-risk patients for immunological exposure, while also allowing for detection of graft damage, without incurring additional financial expenses.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

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

AUTHOR CONTRIBUTIONS

D-RE, PV-A, and FD participated in research design. D-RE, JB, and PV-A participated in the writing of the first version of the manuscript. D-RE, JB, CA, EM-M, JF, and PV-A participated in the performance of the research. MC contributed to diagnostic tools. D-RE, JB, and PV-A participated in data analysis. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

D-RE has received expenditures on travel, hospitality and conferences by Chiesi. PV-A has received expenditures on travel, hospitality and conferences by Chiesi and Astellas.

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

1. Boggi U, Vistoli F, Andres A, Arbogast HP, Badet L, Baronti W, et al. First World Consensus Conference on Pancreas Transplantation: Part II – Recommendations. *Am J Transplant* (2021) 21:17–59. doi:10.1111/ajt.16750
2. Ventura-Aguiar P, Cabello M, Beneyto I, Navarro Cabello D, Tabernero G, Alonso A, et al. Patient and Graft Survival in Pancreas Transplant Recipients: The EFISPAN Study. *Nefrología (English Edition)* (2023) 43:133–43. doi:10.1016/j.nefroe.2022.11.019
3. Kandaswamy R, Stock PG, Miller JM, White J, Booker SE, Israni AK, et al. OPTN/SRTR 2021 Annual Data Report: Pancreas. *Am J Transplant* (2023) 23:S121–S177. doi:10.1016/j.ajt.2023.02.005
4. Bechstein WO, Malaise J, Saudek F, Land W, Fernandez-Cruz L, Margreiter R, et al. Efficacy and Safety of Tacrolimus Compared With Cyclosporine Microemulsion in Primary Simultaneous Pancreas-Kidney Transplantation: 1-Year Results of a Large Multicenter Trial. *Transplantation* (2004) 77:1221–8. doi:10.1097/01.tp.0000120865.96360.df
5. Brunet M, Van Gelder T, Åsberg A, Haufroid V, Hesselink DA, Langman L, et al. Therapeutic Drug Monitoring of Tacrolimus-Personalized Therapy: Second Consensus Report. *Ther Drug Monit* (2019) 41:261–307. doi:10.1097/FTD.0000000000000640
6. Staatz CE, Tett SE. Clinical Pharmacokinetics and Pharmacodynamics of Tacrolimus in Solid Organ Transplantation. *Clin Pharmacokinet* (2004) 43:623–53. doi:10.2165/00003088-200443100-00001
7. Mendoza Rojas A, Hesselink DA, van Besouw NM, Baan CC, van Gelder T. Impact of Low Tacrolimus Exposure and High Tacrolimus Intra-Patient Variability on the Development of De Novo Anti-HLA Donor-Specific Antibodies in Kidney Transplant Recipients. *Expert Rev Clin Immunol* (2019) 15:1323–31. doi:10.1080/1744666X.2020.1693263
8. Thongprayoon C, Hansrivijit P, Kovvuru K, Kanduri SR, Bathini T, Pivovarova A, et al. Impacts of High Intra- and Inter-Individual Variability in Tacrolimus Pharmacokinetics and Fast Tacrolimus Metabolism on Outcomes of Solid Organ Transplant Recipients. *J Clin Med* (2020) 9:2193. doi:10.3390/jcm9072193
9. Hesselink DA, Bouamar R, Elens L, Van Schaik RHN, Van Gelder T. The Role of Pharmacogenetics in the Disposition of and Response to Tacrolimus in Solid

Organ Transplantation. *Clin Pharmacokinet* (2014) 53:123–39. doi:10.1007/s40262-013-0120-3
10. Ebid A-HIM, Abdel-Motaleb SMM, Mira AF, Saleh AA. Pharmacokinetics of Tacrolimus in Egyptian Liver Transplant Recipients: Role of the Classic Co-Variables. *J Adv Pharm Res* (2019) 3:182–93. doi:10.21608/aprh.2019.14237.1087
11. Andrews LM, Li Y, De Winter BCM, Shi YY, Baan CC, Van Gelder T, et al. Pharmacokinetic Considerations Related to Therapeutic Drug Monitoring of Tacrolimus in Kidney Transplant Patients. *Expert Opin Drug Metab Toxicol* (2017) 13:1225–36. doi:10.1080/17425255.2017.1395413
12. Monchaud C, De Winter BC, Knoop C, Estenne M, Reynaud-Gaubert M, Pison C, et al. Population Pharmacokinetic Modelling and Design of a Bayesian Estimator for Therapeutic Drug Monitoring of Tacrolimus in Lung Transplantation. *Clin Pharmacokinet* (2012) 51:175–86. doi:10.2165/11594760-000000000-00000
13. Kahan BD, Keown P, Levy GA, Johnston A. Therapeutic Drug Monitoring of Immunosuppressant Drugs in Clinical Practice. *Clin Ther* (2002) 24:330–50. doi:10.1016/s0149-2918(02)85038-x
14. Sikma MA, Van Maarseveen EM, Van De Graaf EA, Kirkels JH, Verhaar MC, Donker DW, et al. Pharmacokinetics and Toxicity of Tacrolimus Early After Heart and Lung Transplantation. *Am J Transplant* (2015) 15:2301–13. doi:10.1111/ajt.13309
15. Snanoudj R, Tinel C, Legendre C. Immunological Risks of Minimization Strategies. *Transpl Int: official J Eur Soc Organ Transplant* (2015) 28:901–10. doi:10.1111/tri.12570
16. Kuypers DRJ. Intrapatient Variability of Tacrolimus Exposure in Solid Organ Transplantation: A Novel Marker for Clinical Outcome. *Clin Pharmacol Ther* (2020) 107:347–58. doi:10.1002/cpt.1618
17. Shuker N, Van Gelder T, Hesselink DA. Intra-Patient Variability in Tacrolimus Exposure: Causes, Consequences for Clinical Management. *Transplant Rev (Orlando, Fla.)* (2015) 29:78–84. doi:10.1016/j.trre.2015.01.002
18. Rodrigo E, Segundo DS, Fernández-Fresnedo G, López-Hoyos M, Benito A, Ruiz JC, et al. Within-Patient Variability in Tacrolimus Blood Levels Predicts Kidney Graft Loss and Donor-Specific Antibody Development. *Transplantation* (2016) 100:2479–85. doi:10.1097/TP.0000000000001040
19. Vanhove T, Vermeulen T, Annaert P, Lerut E, Kuypers DRJ. High Intrapatient Variability of Tacrolimus Concentrations Predicts Accelerated Progression of Chronic Histologic Lesions in Renal Recipients. *Am J transplantatio* (2016) 16:2954–63. doi:10.1111/ajt.13803
20. Whalen HR, Glen JA, Harkins V, Stevens KK, Jardine AG, Geddes CC, et al. High Intrapatient Tacrolimus Variability Is Associated With Worse Outcomes in Renal Transplantation Using a Low-Dose Tacrolimus Immunosuppressive Regime. *Transplantation* (2017) 101:430–6. doi:10.1097/TP.0000000000001129
21. Gueta I, Markovits N, Yarden-Bilavsky H, Raichlin E, Freimark D, Lavee J, et al. High Tacrolimus Trough Level Variability Is Associated With Rejections After Heart Transplant. *Am J Transplant* (2018) 18:2571–8. doi:10.1111/ajt.15016
22. Gallagher HM, Sarwar G, Tse T, Sladden TM, Hii E, Yerkovich ST, et al. Erratic Tacrolimus Exposure, Assessed Using the Standard Deviation of Trough Blood Levels, Predicts Chronic Lung Allograft Dysfunction and Survival. *J Heart Lung Transplant* (2015) 34:1442–8. doi:10.1016/j.healun.2015.05.028
23. van der Veer MAA, Nangrahary N, Hesselink DA, Erler NS, Metselaar HJ, van Gelder T, et al. High Intrapatient Variability in Tacrolimus Exposure Is Not Associated With Immune-Mediated Graft Injury After Liver Transplantation. *Transplantation* (2019) 103:2329–37. doi:10.1097/TP.0000000000002680
24. Rosendaal FR, Cannegieter SC, Van Der Meer FJM, Briet E. A Method to Determine the Optimal Intensity of Oral Anticoagulant Therapy. *Stuttgart* (1993) 69:236–9. doi:10.1055/s-0038-1651587
25. Davis S, Gralla J, Klem P, Stites E, Wiseman A, Cooper JE. Tacrolimus Intrapatient Variability, Time in Therapeutic Range, and Risk of De Novo Donor-Specific Antibodies. *Transplantation* (2020) 104:881–7. doi:10.1097/TP.0000000000002913
26. Song T, Yin S, Jiang Y, Huang Z, Liu J, Wang Z, et al. Increasing Time in Therapeutic Range of Tacrolimus in the First Year Predicts Better Outcomes in Living-Donor Kidney Transplantation. *Front Immunol* (2019) 10:2912. doi:10.3389/fimmu.2019.02912
27. Yin S, Huang Z, Wang Z, Fan Y, Wang X, Song T, et al. Early Monitoring and Subsequent Gain of Tacrolimus Time-In-Therapeutic Range May Improve Clinical Outcomes After Living Kidney Transplantation. *Ther Drug Monit* (2021) 43:728–35. doi:10.1097/FTD.0000000000000881
28. Ensor CR, Iasella CJ, Harrigan KM, Morrell MR, Moore CA, Shigemura N, et al. Increasing Tacrolimus Time-In-Therapeutic Range Is Associated With Superior One-Year Outcomes in Lung Transplant Recipients. *Am J Transplant* (2018) 18:1527–33. doi:10.1111/ajt.14723
29. Adie SK, Bitar A, Konerman MC, Dorsch MP, Andrews CA, Pogue K, et al. Tacrolimus Time in Therapeutic Range and Long-Term Outcomes in Heart Transplant Recipients. *Pharmacotherapy* (2022) 42:106–11. doi:10.1002/phar.2653
30. Reiffel JA. Time in the Therapeutic Range for Patients Taking Warfarin in Clinical Trials: Useful, But Also Misleading, Misused, and Overinterpreted. *Circulation* (2017) 135:1475–7. doi:10.1161/CIRCULATIONAHA.116.026854
31. Reiffel JA. Time in the Therapeutic Range (TTR): An Overly Simplified Conundrum. *J Innov Card Rhythm Manag* (2017) 8:2643–6. doi:10.19102/icrm.2017.080302
32. Drachenberg CB, Torrealba JR, Nankivell BJ, Rangel EB, Bajema IM, Kim DU, et al. Guidelines for the Diagnosis of Antibody-Mediated Rejection in Pancreas Allografts-Updated Banff Grading Schema. *Am J Transplant* (2011) 11:1792–802. doi:10.1111/j.1600-6143.2011.03670.x
33. Davis S, Gralla J, Klem P, Tong S, Wedermyer G, Freed B, et al. Lower Tacrolimus Exposure and Time in Therapeutic Range Increase the Risk of De Novo Donor-Specific Antibodies in the First Year of Kidney Transplantation. *Am J Transplant* (2018) 18:907–15. doi:10.1111/ajt.14504
34. 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. *Pharmacother J Hum Pharmacol Drug Ther* (2022) 42:594–8. doi:10.1002/phar.2718
35. Coste G, Lemaitre F. The Role of Intra-Patient Variability of Tacrolimus Drug Concentrations in Solid Organ Transplantation: A Focus on Liver, Heart, Lung and Pancreas. *Pharmaceutics* (2022) 14:379. doi:10.3390/pharmaceutics14020379
36. Dong M, Parsaik AK, Kremers W, Sun A, Dean P, Prieto M, et al. Acute Pancreas Allograft Rejection Is Associated With Increased Risk of Graft Failure in Pancreas Transplantation. *Am J Transplant* (2013) 13:1019–25. doi:10.1111/ajt.12167
37. Sugitani A, Egidi M, Gritsch H, Corry RJ. Serum Lipase as a Marker for Pancreatic Allograft Rejection. *Transplant Proc* (1998) 30:645. doi:10.1016/s0041-1345(97)01443-7
38. Torabi J, Konicki A, Rocca JP, Ajaimy M, Campbell A, Azzi Y, et al. The Use of LCP-Tacrolimus (Envarsus XR) in Simultaneous Pancreas and Kidney (SPK) Transplant Recipients. *Am J Surg* (2020) 219:583–6. doi:10.1016/j.amjsurg.2020.02.027
39. Ventura-Aguiar P, Ramirez-Bajo MJ, Rovira J, Bañón-Maneus E, Hierro N, Lazo M, et al. Donor-Derived Cell-Free DNA Shows High Sensitivity for the Diagnosis of Pancreas Graft Rejection in Simultaneous Pancreas-Kidney Transplantation. *Transplantation* (2022) 106:1690–7. doi:10.1097/TP.0000000000004088

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ORIGINAL RESEARCH
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In Vitro Profiling of Commonly Used Post-transplant Immunosuppressants Reveals Distinct Impact on Antiviral T-cell Immunity Towards CMV

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Infectious complications, including widespread human cytomegalovirus (CMV) disease, frequently occur after hematopoietic stem cell and solid organ transplantation due to immunosuppressive treatment causing impairment of T-cell immunity. Therefore, in-depth analysis of the impact of immunosuppressants on antiviral T cells is needed. We analyzed the impact of mTOR inhibitors sirolimus (SIR/S) and everolimus (EVR/E), calcineurin inhibitor tacrolimus (TAC/T), purine synthesis inhibitor mycophenolic acid (MPA/M), glucocorticoid prednisolone (PRE/P) and common double (T+S/E/M/P) and triple (T+S/E/M+P) combinations on antiviral T-cell functionality. T-cell activation and effector molecule production upon antigenic stimulation was impaired in presence of T+P and triple combinations. SIR, EVR and MPA exclusively inhibited T-cell proliferation, TAC inhibited activation and cytokine production and PRE inhibited various aspects of T-cell functionality including cytotoxicity. This was reflected in an *in vitro* infection model, where elimination of CMV-infected human fibroblasts by CMV-specific T cells was reduced in presence of PRE and all triple combinations. CMV-specific memory T cells were inhibited by TAC and PRE, which was also reflected with double (T+P) and triple combinations. EBV- and SARS-CoV-2-specific T cells were similarly affected. These results highlight the need to optimize immune monitoring to identify patients who may benefit from individually tailored immunosuppression.

Keywords: CMV-specific T cells, immunosuppression, adoptive T-cell therapy, solid organ transplantation, hematopoietic stem cell transplantation

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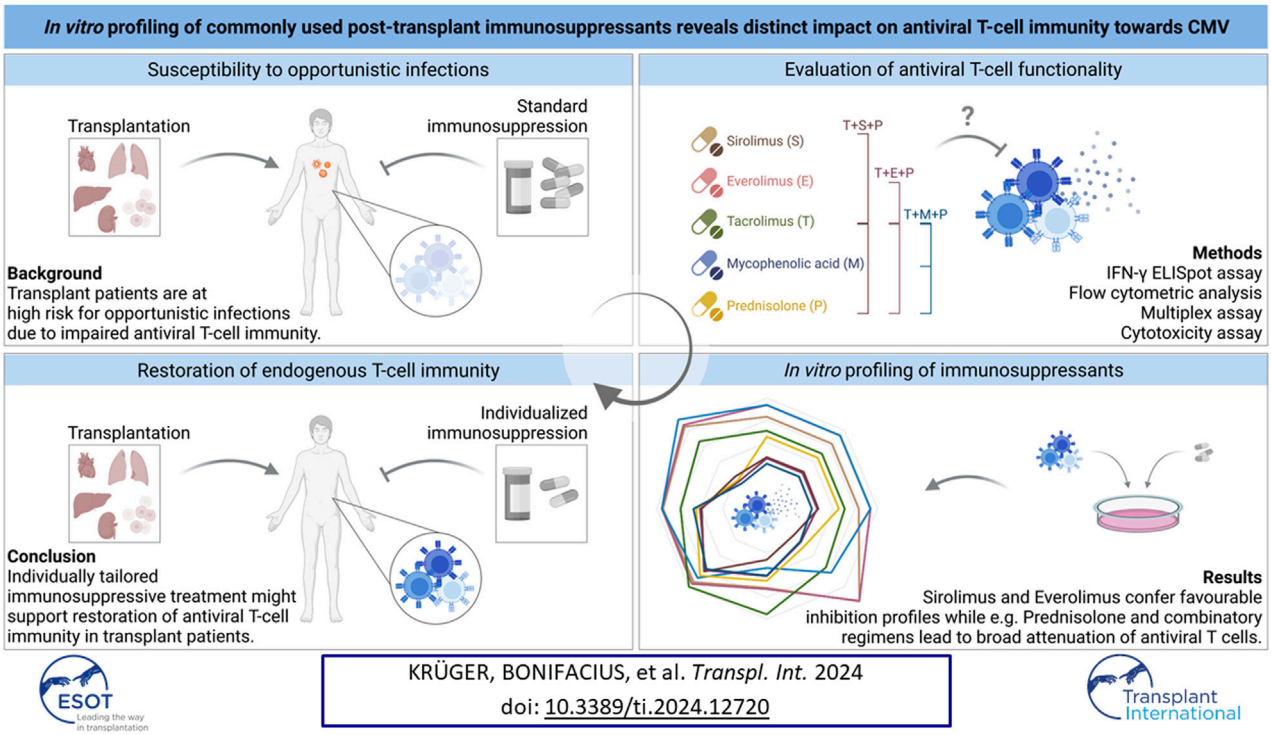
INTRODUCTION

Infectious complications following hematopoietic stem cell and solid organ transplantation (HSCT, SOT) are common due to immunosuppressive treatment for prevention of graft-versus-host disease (GvHD) and allograft rejection. Persistent herpesviruses, such as human cytomegalovirus (CMV), are particularly frequent pathogens. An association between CMV infection/reactivation, the



GRAPHICAL ABSTRACT

development and severity of GvHD and graft injury has been described in several clinical studies of HSCT and SOT [1–3]. Risk factors include *in vivo* or *in vitro* T-cell depletion, HLA-mismatched HSCT, the intensity of immunosuppression, and - in the setting of SOT - the type of transplanted organ [4, 5]. Moreover, CMV-seronegative (CMV-) SOT recipients of a graft from a CMV-seropositive (CMV+) donor (D+/R-) are at high-risk, with incidences of CMV disease up to 50% [6, 7].

The two main strategies to prevent CMV infection or disease in transplant patients are antiviral prophylaxis and preemptive therapy. Especially in high-risk SOT recipients, the most common strategy is antiviral prophylaxis, which is applied for up to 12 months after transplantation. Despite effectiveness of antiviral prophylaxis, side-effects such as nephrotoxicity or bone marrow suppression can result in discontinuation of prophylaxis and late-onset CMV disease after end of prophylaxis [8]. In addition, drug resistances can limit the efficacy of antiviral drugs [9–11]. In 2017/2018, letermovir was approved for prophylaxis after HSCT. In a recent phase III clinical trial comparing valganciclovir and letermovir prophylaxis in kidney transplant recipients (D+/R-), similar incidences of CMV disease were observed in both groups, with fewer side effects in patients receiving letermovir [12]. Preemptive treatment comprises of regular monitoring of viral load, allowing rapid therapy initiation upon detection of an increase. By this, progression to CMV disease can be prevented at an early stage of virus replication while at the same time, myelotoxicity associated with antiviral drugs is reduced [4, 13].

Mechanistically, a relationship between the magnitude of T-cell responses, especially by $CD8^+$ T cells, CMV clearance and restoration of antiviral immunity was found [14]. In line, late-onset CMV disease and mortality have been correlated with the absence of CMV-specific T cells [7, 15, 16]. In recent studies, lower incidence of late-onset CMV disease was observed in liver transplant patients receiving preemptive therapy compared to prophylaxis and this was hypothesized to be due to enhanced CMV-specific T-cell immunity [17, 18]. Assuming that preemptive treatment potentially allows early immune reconstitution and the establishment of cellular antiviral immunity due to controlled low-level CMV replication, the restoration of endogenous antiviral immunity may be sensitively disrupted or delayed by immunosuppressive therapy.

Appropriate T-cell function relies on a variety of aspects and these are targeted via different mechanisms by post-transplant immunosuppressants. Reduction of immunosuppression as tolerated is an alternative option to restore a functional antiviral immune response. CMV disease after SOT typically occurs after 30–90 days [19–22]. At this point, patients are mostly treated by maintenance therapy, e.g., triple combinations usually consisting of a calcineurin inhibitor (CNI, e.g., tacrolimus) and a corticosteroid (e.g., prednisolone), supplemented with a purine synthesis inhibitor (e.g., mycophenolate mofetil, MMF) or a mechanistic target of rapamycin inhibitor (mTORi, e.g., sirolimus, everolimus). Of note, different clinical studies including the ATHENA study showed that the use of an mTORi was associated with lower CMV infection incidences compared to MMF-based regimens [23–29].

To support the restoration of antiviral immunity in SOT recipients and thereby reduce the risk of viral infection or

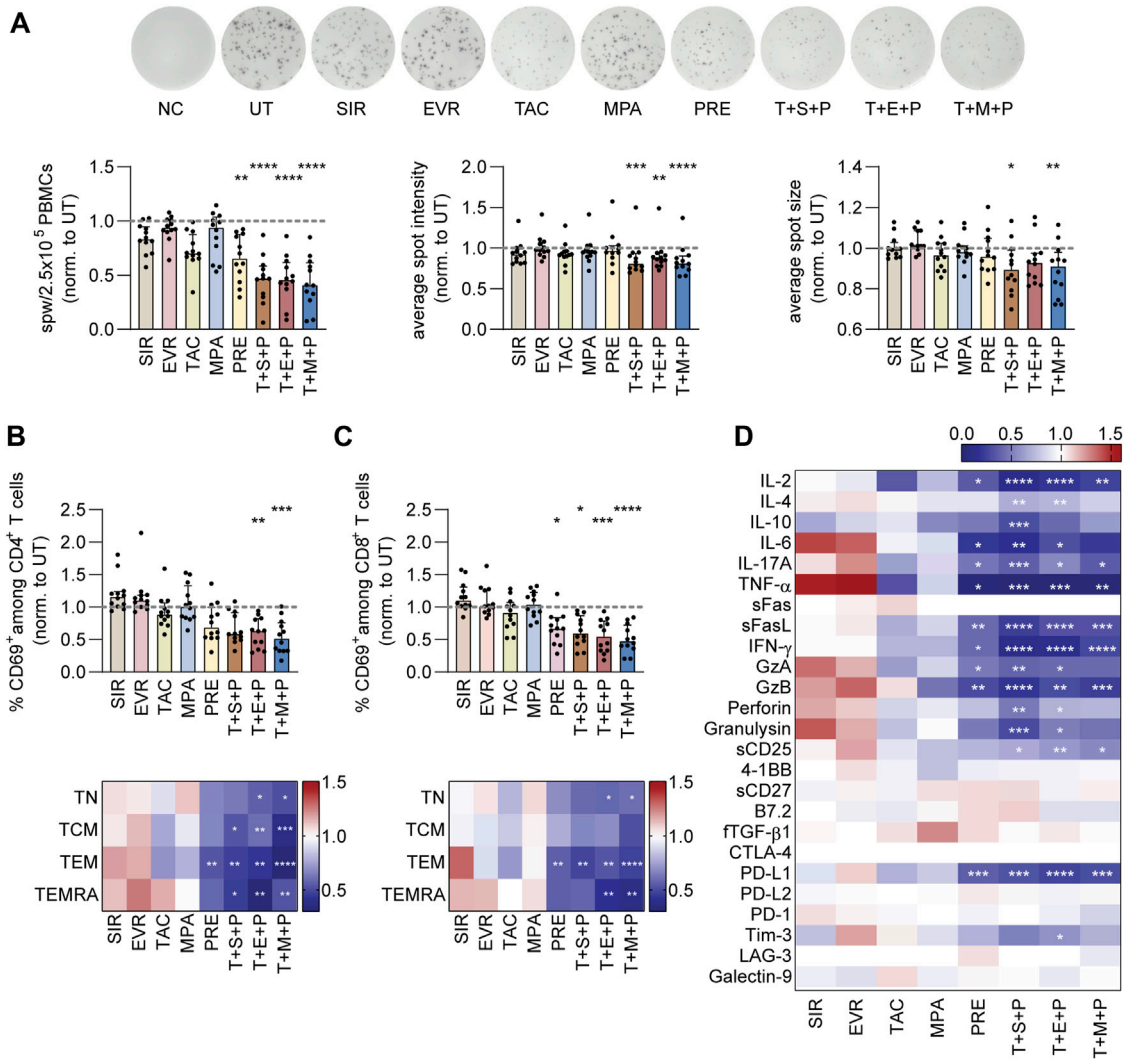


FIGURE 1 | IFN-γ ELISpot, activation and cytokine secretion of CMV_pp65-stimulated PBMCs under immunosuppression. **(A)** PBMCs were isolated from CMV+ donors, rested overnight and stimulated with CMV_pp65 on day 1 in presence and absence of indicated immunosuppressants on IFN-γ ELISpot plates. After 24h, secreted IFN-γ was detected. Representative and summarized IFN-γ ELISpot results shown as spots per well (spw)/2.5 × 10^5 PBMCs, spot intensity and spot size, normalized to untreated control (UT). **(B–D)** PBMCs were isolated from CMV+ donors, rested overnight and stimulated with CMV_pp65 on day 1 in presence and absence of indicated immunosuppressants. After 24 h cells were harvested for flow cytometric analysis and cell culture supernatants were collected for multiplex analysis. **(B)** Frequencies of $CD69^+$ cells among $CD4^+$ T cells (bar graph) and memory $CD4^+$ T-cell subsets (heat map), normalized to UT. **(C)** Summarized frequencies of $CD69^+$ cells among $CD8^+$ T cells and memory $CD8^+$ T-cell subsets (heat map), normalized to UT. **(B–C)** Bar graphs show median and interquartile range Q1-Q3, each symbol represents data from one donor (n = 12). **(B–D)** Heat maps show median values, normalized to UT (n = 12). Statistical significance (in comparison to UT) was calculated using **(A–C)** Friedman test followed by Dunn's multiple comparison and **(D)** 2way ANOVA followed by Dunnett's multiple comparison. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. NC negative control (unstimulated), UT untreated, SIR/S sirolimus, EVR/E everolimus, TAC/T tacrolimus, MPA/M mycophenolic acid, PRE/P prednisolone, TN naïve T cells ($CD45RA^+/CD62L^+$), TCM central memory T cells ($CD45RA^-/CD62L^+$), TEM effector memory T cell ($CD45RA^-/CD62L^-$), TEMRA effector memory T cell re-expressing CD45RA ($CD45RA^+/CD62L^-$).

reactivation, in-depth analysis of the effects of immunosuppressive drugs and combination regimens on antiviral T cells is required. In this study, we analyzed the impact of mTORi sirolimus (S/SIR) and everolimus (E/EVR), the CNI tacrolimus (T/TAC), the active metabolite of the purine synthesis inhibitor MMF - mycophenolic acid (M/MPA) - and the glucocorticoid prednisolone (P/PRE) [30] on CMV-specific T cells. As combination regimens are often used due to synergistic effects and lower single doses thereby minimizing toxicities, we included double (T+S/E/M/P) and triple combinations (T+S/E/M+P) in our study. Detailed assessment of CMV-specific T-cell responses *in vitro* revealed that SIR, EVR and MPA selectively inhibited T-cell proliferation, TAC slightly inhibited different aspects of CMV-specific T-cell functionality and PRE had broad inhibitory effects. Severe impairment was observed with triple combinations, and this could not be compensated by mTORi harboring partial beneficial effects on CMV-specific T cells. In line with that, T+P impaired antiviral T-cell functionality more strongly than T+S/E/M. These results, including evidence of a similar effect on T cells against

EBV and SARS-CoV-2, highlight the need to optimize monitoring of immunocompromised patients or patients with viral infection/reactivation by determining antigen-specific T-cell functionality to further individualize immunosuppressive therapy.

MATERIALS AND METHODS

For description of methods please see **Supplementary Material**.

RESULTS

PRE and Triple Combinations Reduce Antiviral T-cell Activation and Effector Molecule Production

To analyze the impact of the different immunosuppressants on the reactivity of CMV-specific memory T cells, PBMCs were isolated from CMV+ healthy donors and subjected to IFN-γ ELISpot assay using CMV_pp65 overlapping peptide pool for restimulation in absence or presence of immunosuppressants (**Figure 1A**; **Supplementary Figure S1A**). To account for inter-individual differences (**Supplementary Figure S1A**), the data were normalized to values obtained from untreated (UT; stimulated but not treated with immunosuppressants) controls (**Figure 1A**). The frequencies of reactive CMV-specific T cells were significantly decreased upon treatment with PRE and T+S/E/M+P. SIR and TAC slightly reduced detectable CMV-specific T-cell response. In addition to the number of spots, correlating to the number of reactive CMV-specific memory T cells, average spot intensities and sizes were significantly reduced in presence of triple combinations. Since all triple combinations severely impaired memory T-cell reactivity, we analyzed the impact of double combination of immunosuppressants (T+S/E/P) on the reactivity of CMV-specific T cells in a small donor cohort, revealing significantly reduced number of spots in the presence of T+P (**Supplementary Figure S1B**). Of note, EBV- and SARS-CoV-2-specific T-cell responses were similarly affected by immunosuppressive treatment (**Supplementary Figures S1C, S1D**), with SARS-CoV-2-specific T cells being more susceptible.

To gain more insights into the affected T-cell populations, PBMCs from CMV+ donors were stimulated with CMV_pp65 for 24 h in absence or presence of immunosuppressants, followed by analysis of CD69 expression as indicator of activation (**Figures 1B, C**; **Supplementary Figures S2A, S2B**). Frequencies of $CD69^+$ T cells after antigenic stimulation varied between donors and T-cell subsets (Fig. S2b) and were normalized to values obtained from UT controls (**Figures 1B, C**). Activation of $CD4^+$ T cells by CMV_pp65 was significantly reduced in presence of T+E+P and T+M+P (**Figure 1B**). Of note, within the different $CD4^+$ memory T-cell subsets, activation was significantly reduced in presence of all triple combinations. Moreover, in presence of PRE, $CD4^+$ effector memory T cells (TEM, $CD45RA^-CD62L^-$) were significantly less activated. Slightly reduced CD69 expression on $CD4^+$ central memory T cells (TCM, $CD45RA^-CD62L^+$) and TEM was detected in presence of TAC and MPA. Similarly, activation of $CD8^+$ T cells by CMV_pp65 was significantly reduced in presence of triple combinations and PRE (**Figure 1C**). The main affected $CD8^+$ memory T-cell subsets were TEM and effector memory T cells re-expressing CD45RA (TEMRA, $CD45RA^+CD62L^-$). In line with the effect of PRE on $CD4^+$ T cells, significant reduction of CD69 expression among $CD8^+$ TEM was observed in presence of PRE. Of note, slightly increased activation of $CD4^+$ and $CD8^+$ TEM and TEMRA were observed in presence of SIR and EVR. In a small donor cohort, T-cell activation was analyzed after antigenic restimulation in presence of double combinations of immunosuppressive drugs (T+S/E/M/P) and found to be slightly reduced in presence of T+P (**Supplementary Figure S2C**). Similar tendencies were observed for EBV- and SARS-CoV-2-specific T-cell responses (**Supplementary Figures S2D, S2E**). The activation of $CD4^+$ and $CD8^+$ SARS-CoV-2-specific T cells was significantly reduced in presence of T+P (**Supplementary Figure S2E**).

For a more comprehensive overview on the impact of immunosuppression on the production of cytotoxic mediators, multiplex cytokine assays were performed with supernatants of CMV_pp65-stimulated PBMCs (**Figure 1D**; **Supplementary Figure S3**). The raw values (**Supplementary Figure S3**) were normalized to the values obtained from UT controls (**Figure 1D**). While SIR and EVR induced slightly higher concentrations of, e.g., IL-6 and TNF-α, the secretion of pro-inflammatory effector molecules was slightly reduced in presence of TAC, MPA and significantly reduced in presence of PRE and T+S/E/M+P. To confirm antiviral T cells as source of the measured effector molecules, we analyzed the culture supernatants of T-cell-depleted PBMCs (**Supplementary Figure S4A**) stimulated with CMV_pp65 (**Supplementary Figure S4B**). Effector molecules such as, e.g., IL-2, TNF-α and IFN-γ were upregulated in PBMCs but not T-cell-depleted PBMCs after restimulation. Analysis of the effects of dual immunosuppression (T+S/E/M/P) on the secretion of effector molecules (**Supplementary Figure S5**) revealed significantly reduced secretion of different effector molecules by PBMCs after stimulation with CMV_pp65 in presence of T+P (**Supplementary Figure S5B**). Overall, similar patterns were observed after stimulation under the influence of immunosuppression for EBV- and SARS-CoV-2-specific T cells (**Supplementary Figure S5B**).

Taken together, PRE and triple combinations significantly reduced activation and effector molecule secretion of CMV-specific T cells. While all $CD4^+$ memory T-cell subsets were affected by triple combinations, effects on $CD8^+$ T cells were mainly attributed to TEM. Among the double combinations, T+P had the most pronounced impact on antiviral T cells. Moreover, immunosuppressive treatment resulted in impaired T-cell responses towards EBV and SARS-CoV-2.

TAC, MPA, PRE and Triple Combinations Inhibit Cytokine Production by $CD4^+$ and $CD8^+$ T-cell Subsets Upon Antigenic Stimulation

To further discriminate between $CD4^+$ and $CD8^+$ T cells, we performed intracellular cytokine staining of PBMCs stimulated

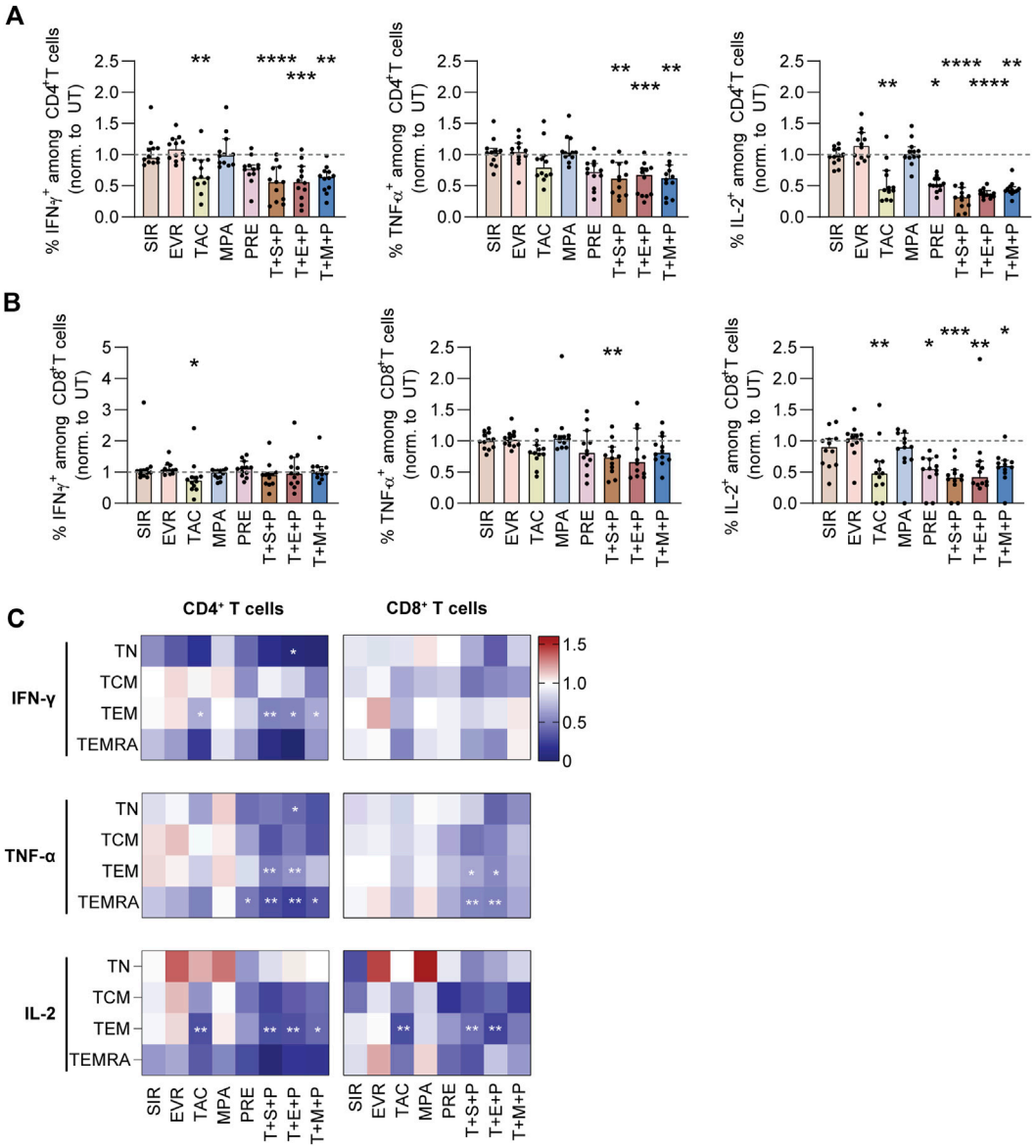


FIGURE 2 | Cytokine profiling of CMV_pp65 stimulated PBMCs under immunosuppression. PBMCs were isolated from CMV+ donors, rested overnight and stimulated with CMV_pp65 on day 1 in presence and absence of indicated immunosuppressants. After 24h, intracellular cytokine production was detected using multicolor flow cytometry and secreted cytotoxic mediators were measured using a flow cytometry-based multiplex assay (LEGENDplex). **(A,B)** Bar graphs summarize frequencies of IFN-γ$^+$, TNF-α$^+$ and IL-2$^+$ cells among **(A)** CD4$^+$ and **(B)** CD8$^+$ T cells. The data are shown as median and interquartile range Q1-Q3 (n = 12). **(C)** Heat maps summarize frequencies of IFN-γ$^+$, TNF-α$^+$ and IL-2$^+$ cells among CD4$^+$ (left) and CD8$^+$ (right) memory T-cell subsets, normalized to untreated control (UT). Data are shown as median (n = 12). Statistical significance (in comparison to UT) was calculated using Friedman test followed by Dunn's multiple comparison. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. NC negative control (unstimulated), UT untreated, SIR/S sirolimus, EVR/E everolimus, TAC/T tacrolimus, MPA/M mycophenolic acid, PRE/P prednisolone, TN naïve T cells (CD45RA$^+$/CD62L$^+$), TCM central memory T cells (CD45RA$^-$/CD62L$^+$), TEM effector memory T cell (CD45RA$^-$/CD62L$^-$), TEMRA effector memory T cell re-expressing CD45RA (CD45RA$^+$/CD62L$^-$).

with CMV_pp65 in absence or presence of immunosuppressants and triple combinations thereof (**Figure 2**; **Supplementary Figure S6**). The data were normalized to values obtained from UT controls (**Figure 2**; **Supplementary Figure S6B, S6C**). Frequencies of IFN-γ$^+$, TNF-α$^+$, and IL-2$^+$ cells within CD4$^+$ T cells were significantly reduced by triple combinations (**Figure 2A**). Moreover, IFN-γ$^+$ cells within CD4$^+$ T cells were significantly reduced by TAC and the frequencies of IL-2$^+$ cells within CD4$^+$ T cells were significantly reduced by TAC and PRE. In contrast, frequencies of IFN-γ$^+$ cells within CD8$^+$ T cells were reduced by TAC, whereas triple combinations had no impact (**Figure 2B**). TNF-α production by CD8$^+$ T cells was slightly reduced in presence of triple combinations, while IL-2 production was significantly reduced by TAC, PRE and triple combinations.

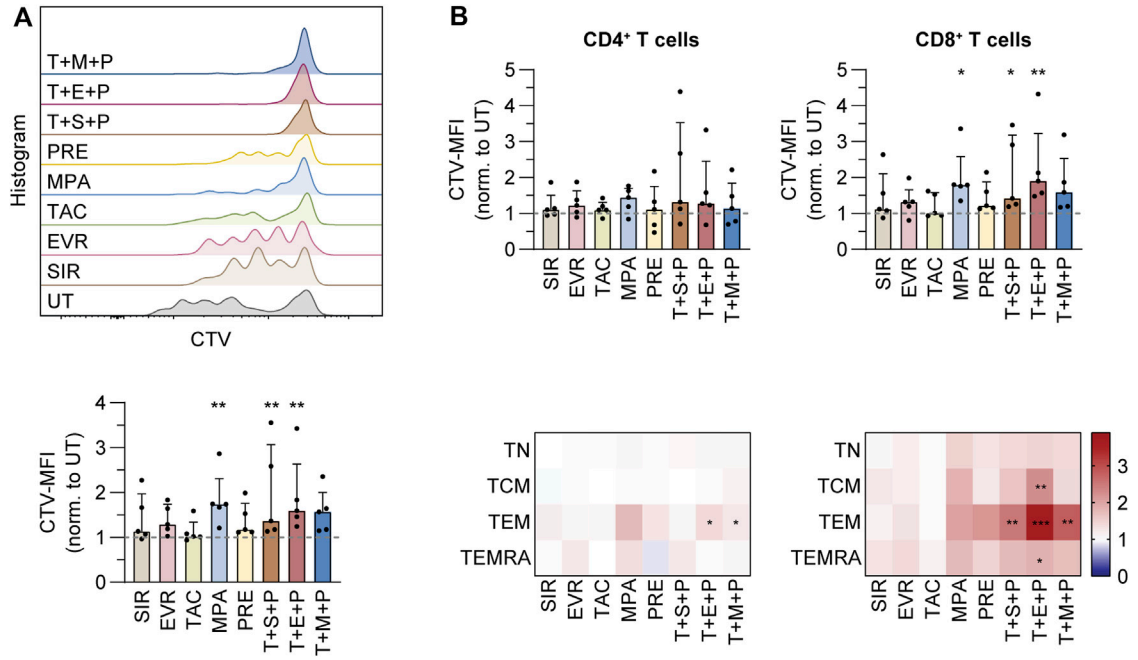


FIGURE 3 | Proliferation analysis of purified CMV-specific T cells under immunosuppression. PBMCs were isolated from CMV+ donors, labeled with CellTrace™ Violet (CTV) and rested overnight, followed by magnetic enrichment of CMV-specific T cells using Cytokine Secretion Assay and CMV_pp65 stimulation. Afterwards, the T cells were expanded on irradiated autologous PBMCs in presence or absence of indicated immunosuppressants, followed by flow cytometric analysis. **(A)** Histograms showing CTV signals of CMV-specific T cells from a representative donor after 5 days of expansion (upper graph). Bar graph shows summarized mean fluorescent intensities (MFIs) of CTV from proliferating T cells on day 5, normalized to untreated control (UT) (lower graph). **(B)** Bar graphs show summarized MFIs of CTV from proliferating $CD4^+$ (left) and $CD8^+$ (right) T cells on day 5, normalized to untreated control (UT) (upper). Heat maps show summarized MFIs of CTV from proliferating $CD4^+$ (left) and $CD8^+$ (right) memory T-cell subsets on day 5, normalized to untreated control (UT) (lower). **(A,B)** Bar graphs show median and interquartile range Q1-Q3, each symbol represents data from one donor (n = 5). Heat maps show data as median values (n = 5). Statistical significance (in comparison to UT) was calculated for each T-cell subset using Friedman test followed by Dunn's multiple comparison. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. UT untreated, SIR/S sirolimus, EVR/E everolimus, TAC/T tacrolimus, MPA/M mycophenolic acid, PRE/P prednisolone, TN naïve T cells ($CD45RA^+/CD62L^+$), TCM central memory T cells ($CD45RA^-/CD62L^+$), TEM effector memory T cell ($CD45RA^-/CD62L^-$), TEMRA effector memory T cell re-expressing CD45RA ($CD45RA^+/CD62L^-$).

Inhibitory effects on $CD4^+$ T cells were primarily focused on TEM (IFN-γ, TNF-α, IL-2) and TEMRA (TNF-α) (**Figure 2C**). Moreover, significantly reduced IFN-γ and IL-2 production by $CD4^+$ TEM was observed in presence of TAC. Among $CD8^+$ memory T-cell subsets, reduction of TNF-α and IL-2 production was comparable to $CD4^+$ T-cell subsets.

Taken together, SIR and EVR mostly preserved the release of pro-inflammatory cytokines by CMV-specific memory T cells, which is in contrast to TAC, PRE and triple combinations. Moreover, impairment of IFN-γ production by immunosuppressive treatment was mostly restricted to $CD4^+$ T cells, while IL-2 production was strongly reduced in $CD4^+$ and $CD8^+$ T cells.

MPA and Triple Combinations Inhibit CMV-specific T-cell Proliferation

To analyze the impact of immunosuppression on proliferation of CMV-specific memory T cells, we isolated CMV_pp65-specific T cells by IFN-γ cytokine secretion assay (CSA). The cells were labeled with CellTrace Violet (CTV) proliferation dye and expanded on irradiated autologous PBMCs (feeder cells) in presence or absence of immunosuppressants and combinations thereof for 4 days (**Figure 3**; **Supplementary Figure S7**). The data were normalized to values obtained from untreated controls (**Figure 3**; **Supplementary Figures S7B, S7C**). Presence of MPA, T+S+P and T+E+P resulted in significantly reduced proliferation of T cells (**Figure 3A**). Among $CD4^+$ T-cell subsets, treatment with T+E+P and T+M+P resulted in significantly reduced proliferation of TEM (**Figure 3B**). Proliferation of $CD8^+$ TEM was significantly reduced in presence of all triple combinations and $CD8^+$ TCM and TEMRA proliferation was significantly reduced in presence of T+E+P.

Taken together, treatment with MPA and triple combinations resulted in significantly impaired proliferation of CMV-specific T cells.

PRE and Triple Combinations Impair CMV-specific T-cell Activation and Cytotoxicity

For measurement of the cytotoxic capacity of CMV-specific T cells under immunosuppression, CMV_pp65-specific

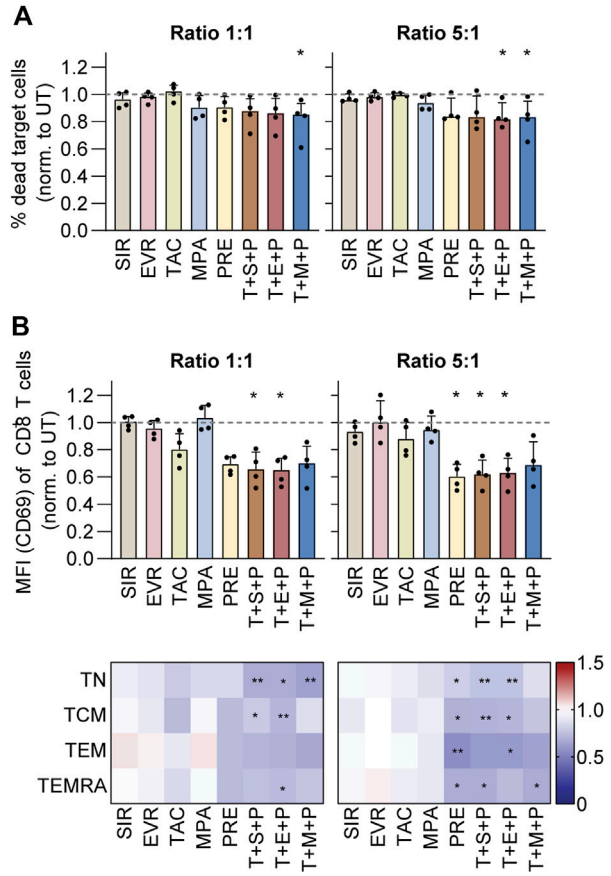


FIGURE 4 | Cytotoxic capacity and activation of CMV-specific T cells under immunosuppression. PBMCs were isolated from CMV+ donors and rested overnight, followed by magnetic enrichment of CMV-specific T cells using Cytokine Secretion Assay and CMV_pp65 stimulation. The T cells were expanded on irradiated autologous PBMCs for 11 days and subsequently co-cultured with CTV-labeled autologous CMV_pp65-loaded PBMCs in different effector-to-target ratios and in presence or absence of indicated immunosuppressants. After 4 h their cytotoxic capacity was analyzed using flow cytometry. Unloaded PBMCs served as negative control. **(A)** Bar graphs show the frequencies of dead (7-AAD$^+$) target cells, normalized to untreated control (UT). **(B)** Bar graphs show the CD69 expression (MFI) among CD8$^+$ T cells, normalized to untreated control (UT) (upper). Heat maps show the CD69 expression (MFI) among CD8$^+$ memory T-cell subsets, normalized to untreated control (UT) (lower). **(A,B)** Bar graphs show median and interquartile range Q1-Q3, each symbol represents data from one donor (n = 4). Heat maps show data as median values (n = 5). Statistical significance (in comparison to UT) was calculated using Friedman test followed by Dunn's multiple comparison. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. UT untreated, SIR/S sirolimus, EVR/E everolimus, TAC/T tacrolimus, MPA/M mycophenolic acid, PRE/P prednisolone, TN naïve T cells (CD45RA$^+$/CD62L$^+$), TCM central memory T cells (CD45RA$^-$/CD62L$^+$), TEM effector memory T cell (CD45RA$^-$/CD62L$^-$), TEMRA effector memory T cell re-expressing CD45RA (CD45RA$^+$/CD62L$^-$).

memory T cells were isolated as described before and expanded on feeder cells for 12 days, followed by co-culture with CTV-labeled autologous CMV_pp65-loaded PBMCs in presence or absence of immunosuppressants. Unloaded PBMCs served as negative control. After 4 h, the cells were harvested for flow cytometric analysis of target cell death and T-cell activation (**Figure 4**; **Supplementary Figure S8**). The data were normalized to values obtained from UT controls (**Figure 4**; **Supplementary Figure S8**). While no unspecific cytotoxicity of T cells co-cultured with unloaded PBMCs was observed (**Supplementary Figures S8A, S8B**), frequencies of dead (7-AAD$^+$) PBMCs were increased when peptide pool-loaded and co-cultured with T cells, and this effect was dose-dependent (**Figure 4A**; **Supplementary Figure S8B**). At both ratios, T+M+P resulted in reduced cytotoxicity of T cells towards loaded PBMCs. Moreover, at the 5:1 ratio, treatment with T+E+P significantly reduced cytotoxicity. Slightly reduced cytotoxicity was observed in presence of MPA, PRE and triple combinations at both ratios. In line, frequencies of CD69-expressing CD8$^+$ T cells and memory subsets were significantly reduced under treatment with PRE (5:1), T+S+P (1:1 and 5:1) and T+E+P (1:1 and 5:1) (**Figure 4B**; **Supplementary Figures S8C, S8D**).

Taken together, PRE and triple combinations resulted in comparable inhibition of cytotoxicity and activation after co-culture with autologous CMV_pp65-loaded PBMCs.

PRE and Triple Combinations Inhibit Real-Time Cytotoxicity Towards CMV-Infected Fibroblasts

To evaluate long-term effects of immunosuppressive treatment, we measured real-time cytotoxicity of CMV-specific T cells towards partially HLA-matched CMV-infected or CMV_pp65-loaded human foreskin fibroblasts (HFF) using xCelligence Real Time Cell Analyzer (RTCA) (**Figure 5**). Fluorescence microscopy confirmed the successful infection, indicated by expression of a green fluorescent protein (GFP) signal in the CMV-infected cells (**Figure 5A**). Direct comparison of growth curves for HFF cells only and HFF cells plus T cells showed reduced cell indices in presence of T cells for all three target cell conditions (**Figure 5B**). PRE and all triple combinations markedly inhibited cytotoxicity as indicated by higher cell indices. Area under the curve (AUC) values (**Supplementary Figure S9A**) were normalized to the AUC values obtained from the respective UT control (**Figure 5C**). While slightly higher normalized AUC values were measured in co-cultures treated with PRE or triple combinations, these effects were markedly stronger in co-cultures with CMV-infected HFF cells compared to the other two conditions.

Supernatants of these co-cultures were analyzed with respect to secreted cytotoxic mediators (**Supplementary Figure S9B**). Specific upregulation of IL-6, sFasL and IFN-γ was observed in co-cultures with CMV-infected HFF cells and this was slightly reduced in presence of PRE and triple combinations.

Taken together, CMV-specific T cells were unable to eliminate CMV-infected fibroblasts under immunosuppression with PRE or triple combinations, and this was accompanied by decreased effector molecule production.

Summary

Spider web graphs including all assay read-outs were created for each immunosuppressant in comparison to UT controls (**Figure 6**). While all triple combinations conferred

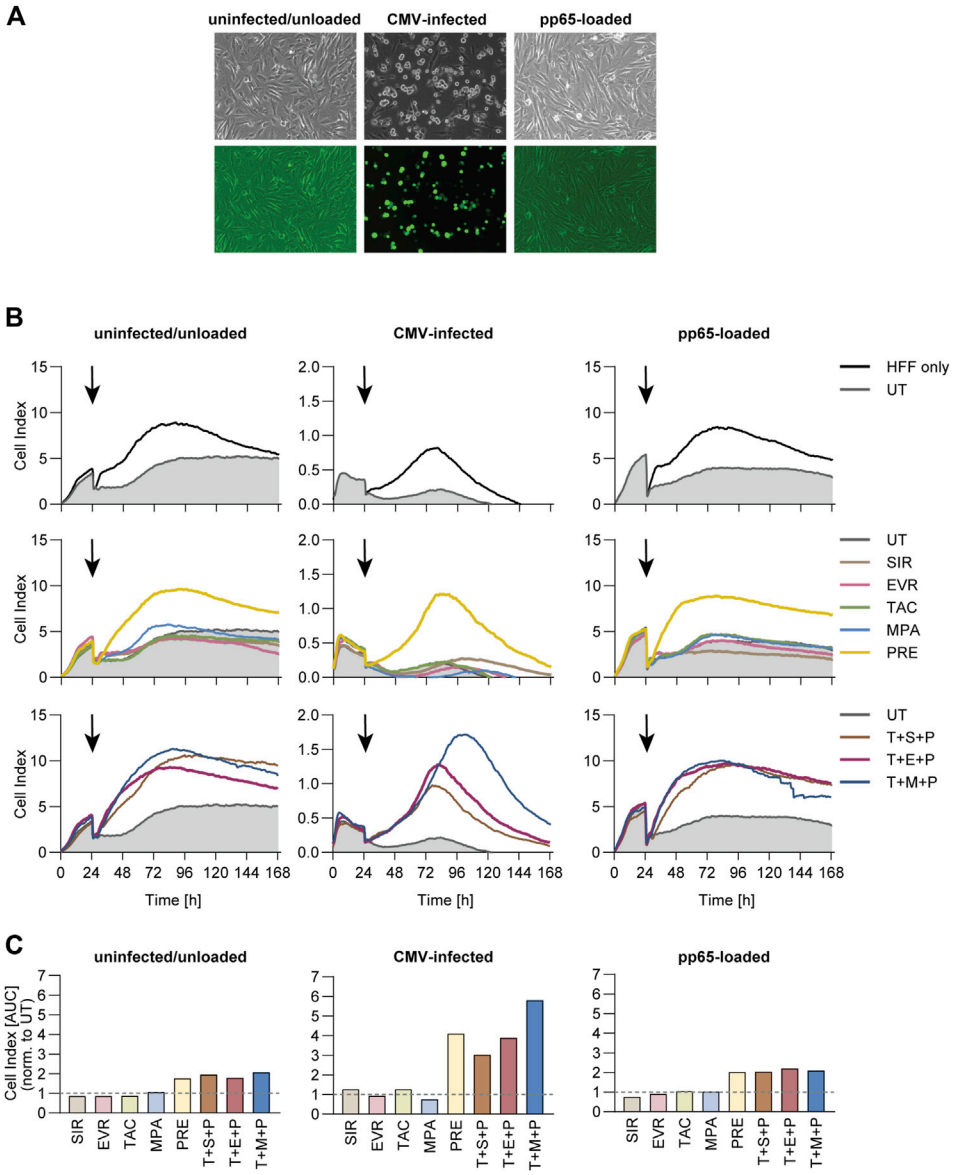


FIGURE 5 | Cytotoxic capacity of CMV-specific T cells towards CMV-infected fibroblasts under immunosuppression. PBMCs were isolated from CMV+ donors and rested overnight, followed by magnetic enrichment of CMV-specific T cells using Cytokine Secretion Assay and CMV_pp65 stimulation. The T cells were expanded on irradiated autologous PBMCs for 11 days and subsequently co-cultured with uninfected, CMV-infected or CMV_pp65-loaded Human Foreskin Fibroblasts (HFF) in an effector-to-target ratio of 1:1 and in presence or absence of indicated immunosuppressants for 7 days using an xCELLigence RTCA S16 Real Time Cell Analyzer. **(A)** Microscopic image of the different target cells prior to co-culture. **(B)** Realtime impedance-based growth curves of HFF cells cultured alone (HFF cells only) or together with CMV-specific T cells in presence or absence of indicated immunosuppressants. Black arrows indicate time of T-cell addition. **(C)** Bar graphs display the AUC of growth curves shown in **(B)**, normalized to untreated control (UT). UT untreated, SIR/S sirolimus, EVR/E everolimus, TAC/T tacrolimus, MPA/M mycophenolic acid, PRE/P prednisolone.

homogenously and broadly attenuated CMV-specific memory T cells, divergent effects of single immunosuppressants were observed. SIR and EVR slightly inhibited T-cell proliferation while mostly sparing activation and cytokine secretion. MPA selectively inhibited T-cell proliferation more profoundly. In contrast, TAC slightly inhibited different aspects of CMV-specific T-cell functionality and PRE had broad inhibitory effects on CMV-specific T cells.

DISCUSSION

The influence of post-transplant immunosuppressants on CMV susceptibility and on antiviral T cells is of high importance for choosing preventive and therapeutic measures, since T cells are required for the final control of CMV replication [31]. Appropriate T-cell function relies on different aspects such as proliferation, cytokine secretion and cytotoxicity [32] and these

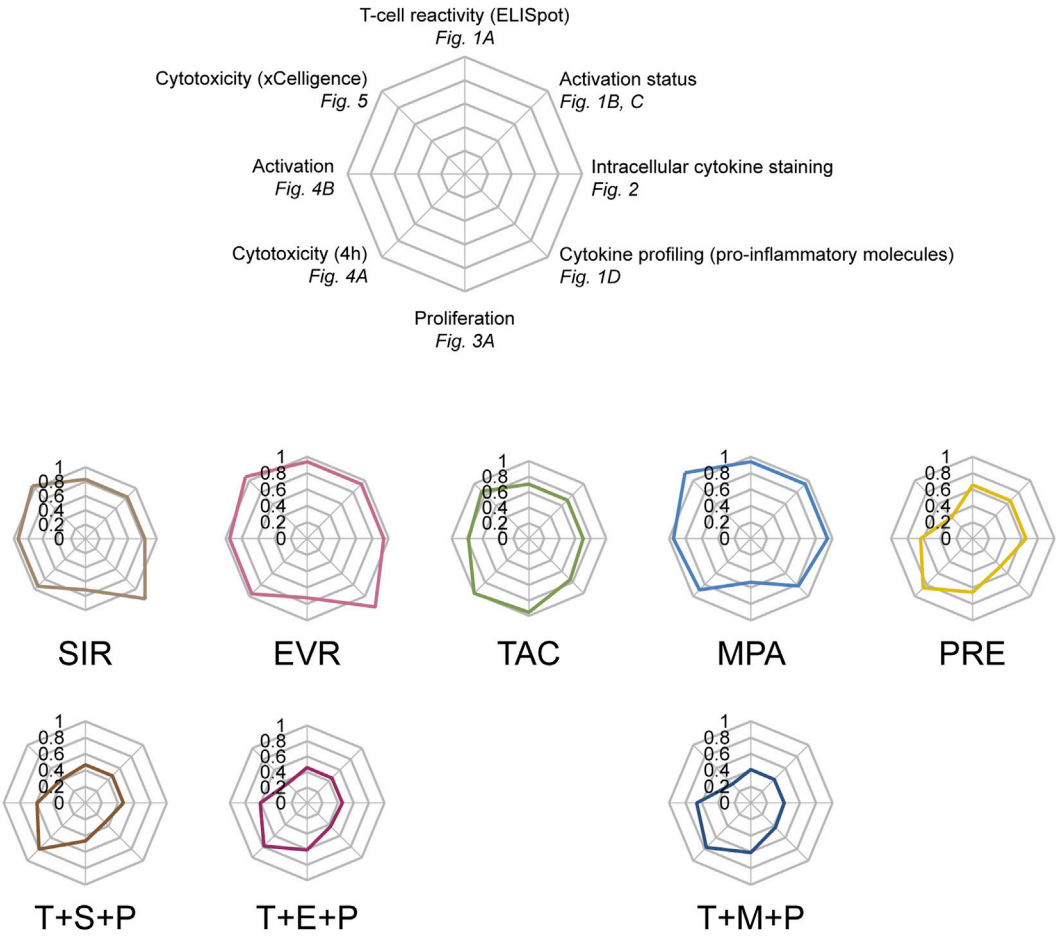


FIGURE 6 | *In vitro* profiles of commonly used post-transplant immunosuppressants in context of antiviral T-cell immunity. Spider web graphs summarizing the impact of the respective immunosuppressants on CMV-specific T cells as measured by the indicated assays and in comparison to untreated controls. Values used for the diagrams are (clockwise starting from the top): IFN-γ ELISpot (spot numbers), activation status (frequencies of $CD69^+$ $CD4^+$ and $CD8^+$ T cells), intracellular cytokine staining (cumulative frequencies of IFN-γ^+/TNF-α^+/IL-2^+ $CD4^+$ and $CD8^+$ T cells), multiplex cytokine profiling (concentrations of pro-inflammatory molecules), proliferation ($CD3^+$ T cells), cytotoxicity (4 h) (frequencies of dead target cells), activation (4 h) (CD69-MFIs of $CD8^+$ T cells), realtime cytotoxicity (area under curve). UT untreated, SIR/S sirolimus, EVR/E everolimus, TAC/T tacrolimus, MPA/M mycophenolic acid, PRE/P prednisolone.

aspects are targeted via different mechanisms by post-transplant immunosuppressants. Usually, for early prevention of allograft rejection and perioperative lowering of maintenance immunosuppressants following SOT, an induction therapy is applied. In this phase, different T cell-depleting agents are used. However, most CMV diseases following SOT typically occur after 30–90 days [19–22]. At this point, mostly a switch to maintenance therapy has been made by using triple combinations [33, 34]. Of note, immunosuppressive regimens differ regarding choice of immunosuppressants and dosages between the transplanted organs and centers. Of note, in case of resistant/refractory CMV disease, treatment options include secondary antiviral drugs and individual change of immunosuppression [35]. In case of insufficient antiviral T-cell immunity, adoptive transfer of virus-specific T cells can restore a long-lasting endogenous antiviral immune defense [36, 37]. In this study, we screened commonly used immunosuppressive drugs and combinations thereof with respect to different aspects of T-cell functionality *in vitro*.

We observed that PRE and combinations containing PRE attenuate IFN-γ secretion, which is in harmony with earlier findings [38]. PRE, the active metabolite of prednisone, is a glucocorticoid with broad immunomodulatory effects including interference with different pro-inflammatory genes and non-genomic cytosolic molecule interferences [39, 40]. IFN-γ is crucially involved in the defense against CMV and it may foreshadow the outcome prior and post transplantation [41, 42] and determines the prognosis of critically ill patients as well [43]. It was recently demonstrated that addition of methylprednisolone to regimens featuring TAC and MMF worsened the T-cell response in liver transplant recipients [44]. We did not observe significant decreases of IFN-γ secretion by the other tested immunosuppressive drugs, which is in concordance especially for SIR and EVR [45]. Of note, an

additive effect was revealed for triple combinations, exceeding the inhibitory potential of PRE. Additionally, PRE and triple combinations led to decreased expression of CD69, which is regulating T-cell differentiation and metabolism [46].

SIR and EVR are mTORi and interfere with a variety of cascades, including pathways essential for T-cell proliferation [47–50]. Despite their chemical difference, distinct pharmacokinetic characteristics and mTOR complex affinities have been summarized, creating the interest of detailed side-by-side comparisons [51]. Interestingly, clinical studies showed that mTORi-based regimens are associated with lower CMV infection incidences compared to MMF-based combinations [23–29, 52].

We extended the range of surveyed molecules using intracellular cytokine staining to measure IL-2 and TNF-α production, which are both known to play an important role in the anti-viral response [53, 54]. For the CNI TAC, which leads to a decreased activation of the nuclear factor of activated T cells (NFAT) and a lower production of pro-inflammatory stimuli [55–57], one of its main effects - the depletion of IL-2 - was reflected in our study. Furthermore, we found an inhibition pattern of TAC, PRE and triple combinations that was focused on TEM and TEMRA, which are known for secreting high amounts of cytokines [58].

Together with the production of pro-inflammatory molecules, recruitment and proliferation is required for T-cell mediated organ rejection [59] and therefore targeted by immunosuppressants. Here, MPA, the active metabolite of MMF, stood out in our study. As a purine synthesis inhibitor targeting the inosine-5′-monophosphate dehydrogenase (IMDPH), it is relatively lymphocyte specific, due to the compromised *de novo* pathway of guanosine nucleotides (lymphocytes cannot use salvage pathway of purine synthesis) and a high affinity to their IMDPH isoform. This leads to inhibited human T- and B-cell proliferation [60]. MPA has a high growth-arresting profile [61], which we conferred to be as effective as from the investigated triple combinations. Other groups described that its function extends beyond the antimetabolite pathway inhibition [62, 63], which was partly supported by our experiments, where it showed accompanying decreased cytokine release. For this, PRE and triple combinations showed severe T-cell impairment. Moreover, under triple combinations, slightly decreased cytotoxic capacity was observed, alongside reduction of T-cell activation.

Notably, the mTORi SIR and EVR showed a selective and compared to MPA less profound inhibition of CMV-specific T-cell proliferation. Our group showed earlier that SIR can augment CMV-specific effector memory T cells while inhibiting naive T cells [64], supporting the assumption that it does not only have an isolated immunosuppressive effect. Deciphering more mechanisms is a current topic, e.g., it was recently found that for kidney transplants, mTORi prevented CMV infection via αβ and γδ T-cell preservation [65]. Moreover, CMV seems to utilize mTOR for its replication, e.g., in macrophages [66]. Furthermore, for adoptive T-cell therapy, advanced strategies are being developed to overcome limitations due to immunosuppression, like the utilization of gene knockouts for creating T cell drug resistance [67, 68]. This displays an interesting approach besides providing evidence for individual changes to more favorable drugs regimens.

To evaluate functional effects of CMV-specific T cells in context of CMV infection, we established a real-time cytotoxicity model using CMV-infected human fibroblasts in which pp65 protein expression was reported as early as 1 h and up to 24 h post infection [69]. Here, we observed that PRE and triple combinations inhibited T cell-mediated elimination of CMV-infected fibroblasts, confirming our previous results. In a study by Jackson et al., $CD8^+$ T cells recognizing peptides derived from different CMV proteins (pp65, IE-1) were effective in an *in vitro* virus dissemination assay independent of their peptide specificity [70], therefore indicating that the assay developed here can be utilized to investigate T-cell responses against different viral antigens. Such assays are of broad interest, e.g., for the investigation of chimeric antigen receptor (CAR) T cells [71] and may be beneficial for future projects studying virus-specific T cells as well.

Therapeutic drug monitoring is routinely applied for CNI/mTORi and occasionally for MMF/MPA to prevent rejection and toxicities. Hence, drug concentrations investigated in this study were derived from known plasma levels to mimic a clinical situation [72–75]. Immunosuppressive protocols vary between different institutions and patients, desired ranges of combinatory sustaining therapies may lie between 5–8 ng/mL of TAC, 3–8 ng/mL EVR and 1–3.5 μg/mL MPA, for example, following liver transplantation, which was represented in our study. In a recent publication, 7.5–20 mg/d administered PRE led to a median peak plasma concentration of 0.271–0.921 μg/mL [76]. While the concentration of PRE investigated in our study was above those concentrations applied during maintenance therapy, it rather correlates to early post-transplant oral dosage. Titration studies should be conducted in the future to allow for further conclusions on dose-dependent effects. However, the results of our screening study may be useful for these further studies, including clinical trials. Further experiments comparing alloreactivity and antiviral responses side-by-side may be helpful as well. In addition, a more detailed investigation of drug interferences is of great interest, since both, TAC and SIR/EVR, bind to the FK506 binding protein at first and thus may inhibit each other [77]. Moreover, only recall responses of memory T cells but not the activation of naïve T cells was analyzed, hence future studies are needed to investigate the dose-dependent effects on memory and naïve T cells. In this study, we aimed at systematic analysis of the impact of different immunosuppressive drugs on different aspects of antiviral T-cell functionality. The impact of different immunosuppressive treatment regimens in patients with different transplantation history needs to be addressed in future studies. Especially for SOT recipients at high risk, studies on the impact of immunosuppressive drugs on the initiation of an anti-CMV immune response via activation of naïve T cells are of great interest.

To conclude, we showed that immunosuppressants administered after SOT or HSCT differentially affect CMV-specific T-cell functionality. CMV-specific T-cell responses were strongly impaired by triple combinations, while SIR, EVR and MPA selectively affected T-cell proliferation. TAC slightly inhibited activation and cytokine production. Further, PRE strongly impaired CMV-specific memory T cells, which was also reflected in the investigated triple combinations. While the focus of this study

was on the impact of immunosuppressive treatment on CMV-specific T-cell immunity, our data suggest that T-cell responses towards other clinically relevant viruses such as EBV and SARS-CoV-2 might be similarly–and in case of SARS-CoV-2 even more profoundly–affected by post-transplant immunosuppressive treatment. Based on our results on double combinations (T+S/E/M/P), it can be assumed that the discontinuation of PRE in patients receiving combinatory regimens such as T+S/E/M+P would be beneficial to restore antiviral T-cell immunity. Taken together, our data suggest potential beneficial effects of treatment with mTORi whilst, if possible, TAC, MPA, PRE and triple combinations should be used cautiously for patients at high risk or suffering from CMV disease.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by the Ethics Committee of Hannover Medical School. 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 design: MK, AB, BM-K, and BE-V. Writing of the paper: MK, AB, and BE-V. Performance of the research: MK, AB, AD, and MS. New reagents or analytic tools: UK, RB, and BE-V. Data analysis: MK, AB, AD, and MS. Funding acquisition: AB and BE-V. Supervision: AB, BN, RT, and BE-V. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.12720/full#supplementary-material

REFERENCES

1. Teira P, Battiwalla M, Ramanathan M, Barrett AJ, Ahn KW, Chen M, et al. Early Cytomegalovirus Reactivation Remains Associated with Increased Transplant-Related Mortality in the Current Era: A CIBMTR Analysis. *Blood* (2016) 127(20):2427–38. doi:10.1182/blood-2015-11-679639
2. Potena L, Valantine HA. Cytomegalovirus-Associated Allograft Rejection in Heart Transplant Patients. *Curr Opin Infect Dis* (2007) 20(4):425–31. doi:10.1097/QCO.0b013e328259c33b
3. Stern M, Hirsch H, Cusini A, van Delden C, Manuel O, Meylan P, et al. Cytomegalovirus Serology and Replication Remain Associated with Solid Organ Graft Rejection and Graft Loss in the Era of Prophylactic Treatment. *Transplantation* (2014) 98(9):1013–8. doi:10.1097/TP.0000000000000160
4. Jakharia N, Howard D, Riedel DJ. CMV Infection in Hematopoietic Stem Cell Transplantation: Prevention and Treatment Strategies. *Curr Treat Options Infect Dis* (2021) 13(3):123–40. doi:10.1007/s40506-021-00253-w
5. Ramanan P, Razonable RR. Cytomegalovirus Infections in Solid Organ Transplantation: A Review. *Infect Chemother* (2013) 45(3):260–71. doi:10.3947/ic.2013.45.3.260
6. Limaye AP, Babu TM, Boeckh M. Progress and Challenges in the Prevention, Diagnosis, and Management of Cytomegalovirus Infection in Transplantation. *Clin Microbiol Rev* (2020) 34(1):e00043. doi:10.1128/CMR.00043-19
7. Veit T, Pan M, Munker D, Arnold P, Dick A, Kunze S, et al. Association of CMV-Specific T-Cell Immunity and Risk of CMV Infection in Lung Transplant Recipients. *Clin Transpl* (2021) 35(6):e14294. doi:10.1111/ctr.14294
8. Grossi PA, Kamar N, Saliba F, Baldanti F, Aguado JM, Gottlieb J, et al. Cytomegalovirus Management in Solid Organ Transplant Recipients: A Pre-COVID-19 Survey from the Working Group of the European Society for Organ Transplantation. *Transpl Int* (2022) 35:10332. doi:10.3389/ti.2022.10332
9. Haidar G, Boeckh M, Singh N. Cytomegalovirus Infection in Solid Organ and Hematopoietic Cell Transplantation: State of the Evidence. *J Infect Dis* (2020) 221(Suppl. 1):S23–S31. doi:10.1093/infdis/jiz454
10. Tamzali Y, Pourcher V, Azoyan L, Ouali N, Barrou B, Conti F, et al. Factors Associated with Genotypic Resistance and Outcome Among Solid Organ Transplant Recipients with Refractory Cytomegalovirus Infection. *Transpl Int* (2023) 36:11295. doi:10.3389/ti.2023.11295
11. Avery RK, Arav-Boger R, Marr KA, Kraus E, Shoham S, Lees L, et al. Outcomes in Transplant Recipients Treated with Foscarnet for Ganciclovir-Resistant or Refractory Cytomegalovirus Infection. *Transplantation* (2016) 100(10):e74–80. doi:10.1097/TP.0000000000001418
12. Limaye AP, Budde K, Humar A, Vincenti F, Kuypers DRJ, Carroll RP, et al. Letermovir vs Valganciclovir for Prophylaxis of Cytomegalovirus in High-Risk Kidney Transplant Recipients: A Randomized Clinical Trial. *JAMA* (2023) 330:33–42. doi:10.1001/jama.2023.9106

13. Kotton CN, Kumar D, Caliendo AM, Huprikar S, Chou S, Danziger-Isakov L, et al. The Third International Consensus Guidelines on the Management of Cytomegalovirus in Solid-Organ Transplantation. *Transplantation* (2018) 102(6):900–31. doi:10.1097/TP.0000000000002191
14. Ozdemir E, St John LS, Gillespie G, Rowland-Jones S, Champlin RE, Molldrem JJ, et al. Cytomegalovirus Reactivation Following Allogeneic Stem Cell Transplantation Is Associated with the Presence of Dysfunctional Antigen-Specific CD8+ T Cells. *Blood* (2002) 100(10):3690–7. doi:10.1182/blood-2002-05-1387
15. Boeckh M, Leisenring W, Riddell SR, Bowden RA, Huang ML, Myerson D, et al. Late Cytomegalovirus Disease and Mortality in Recipients of Allogeneic Hematopoietic Stem Cell Transplants: Importance of Viral Load and T-Cell Immunity. *Blood* (2003) 101(2):407–14. doi:10.1182/blood-2002-03-0993
16. Donadeu L, Revilla-Lopez E, Jarque M, Crespo E, Torija A, Bravo C, et al. CMV-Specific Cell-Mediated Immunity Predicts a High Level of CMV Replication after Prophylaxis Withdrawal in Lung Transplant Recipients. *J Infect Dis* (2021) 224(3):526–31. doi:10.1093/infdis/jiaa727
17. Limaye AP, Green ML, Edmison BC, Stevens-Ayers T, Chatterton-Kirchmeier S, Geballe AP, et al. Prospective Assessment of Cytomegalovirus Immunity in High-Risk Donor-Seropositive/Recipient-Seronegative Liver Transplant Recipients Receiving Either Preemptive Therapy or Antiviral Prophylaxis. *J Infect Dis* (2019) 220(5):752–60. doi:10.1093/infdis/jiz181
18. Singh N, Winston DJ, Razonable RR, Lyon GM, Silveira FP, Wagener MM, et al. Effect of Preemptive Therapy vs Antiviral Prophylaxis on Cytomegalovirus Disease in Seronegative Liver Transplant Recipients with Seropositive Donors: A Randomized Clinical Trial. *JAMA* (2020) 323(14): 1378–87. doi:10.1001/jama.2020.3138
19. Azevedo LS, Pierrotti LC, Abdala E, Costa SF, Strabelli TM, Campos SV, et al. Cytomegalovirus Infection in Transplant Recipients. *Clinics (Sao Paulo)* (2015) 70(7):515–23. doi:10.6061/clinics/2015(07)09
20. Humar A, Limaye AP, Blumberg EA, Hauser IA, Vincenti F, Jardine AG, et al. Extended Valganciclovir Prophylaxis in D+/R-Kidney Transplant Recipients Is Associated with Long-Term Reduction in Cytomegalovirus Disease: Two-Year Results of the IMPACT Study. *Transplantation* (2010) 90(12):1427–31. doi:10.1097/tp.0b013e3181ff1493
21. Legendre C, Pascual M. Improving Outcomes for Solid-Organ Transplant Recipients at Risk from Cytomegalovirus Infection: Late-Onset Disease and Indirect Consequences. *Clin Infect Dis* (2008) 46(5):732–40. doi:10.1086/527397
22. Blyth D, Lee I, Sims KD, Gasink LB, Barton TD, Van Deerlin VM, et al. Risk Factors and Clinical Outcomes of Cytomegalovirus Disease Occurring More Than One Year post Solid Organ Transplantation. *Transpl Infect Dis* (2012) 14(2):149–55. doi:10.1111/j.1399-3062.2011.00705.x
23. Vigano M, Dengler T, Mattei MF, Poncelet A, Vanhaecke J, Vermes E, et al. Lower Incidence of Cytomegalovirus Infection with Everolimus Versus Mycophenolate Mofetil in De Novo Cardiac Transplant Recipients: A Randomized, Multicenter Study. *Transpl Infect Dis* (2010) 12(1):23–30. doi:10.1111/j.1399-3062.2009.00448.x
24. Hauser IA, Marx S, Sommerer C, Suwelack B, Dragun D, Witzke O, et al. Effect of Everolimus-Based Drug Regimens on CMV-Specific T-Cell Functionality After Renal Transplantation: 12-Month ATHENA Subcohort-Study Results. *Eur J Immunol* (2021) 51(4):943–55. doi:10.1002/eji.202048855
25. Tedesco-Silva H, Pascual J, Viklicky O, Basic-Jukic N, Cassuto E, Kim DY, et al. Safety of Everolimus with Reduced Calcineurin Inhibitor Exposure in De Novo Kidney Transplants: An Analysis from the Randomized TRANSFORM Study. *Transplantation* (2019) 103(9):1953–63. doi:10.1097/TP.0000000000002626
26. Demopoulos L, Polinsky M, Steele G, Mines D, Blum M, Caulfield M, et al. Reduced Risk of Cytomegalovirus Infection in Solid Organ Transplant Recipients Treated with Sirolimus: A Pooled Analysis of Clinical Trials. *Transpl Proc* (2008) 40(5):1407–10. doi:10.1016/j.transproceed.2008.03.084
27. Kobashigawa J, Ross H, Bara C, Delgado JF, Dengler T, Lehmkuhl HB, et al. Everolimus Is Associated with a Reduced Incidence of Cytomegalovirus Infection Following De Novo Cardiac Transplantation. *Transpl Infect Dis* (2013) 15(2):150–62. doi:10.1111/tid.12007
28. Mallat SG, Tanios BY, Itani HS, Lotfi T, McMullan C, Gabardi S, et al. CMV and BKPyV Infections in Renal Transplant Recipients Receiving an mTOR Inhibitor-Based Regimen Versus a CNI-Based Regimen: A Systematic Review and Meta-Analysis of Randomized, Controlled Trials. *Clin J Am Soc Nephrol* (2017) 12(8):1321–36. doi:10.2215/CJN.13221216
29. Sommerer C, Suwelack B, Dragun D, Schenker P, Hauser IA, Witzke O, et al. An Open-Label, Randomized Trial Indicates that Everolimus with Tacrolimus or Cyclosporine Is Comparable to Standard Immunosuppression in De Novo Kidney Transplant Patients. *Kidney Int* (2019) 96(1):231–44. doi:10.1016/j.kint.2019.01.041
30. Tonshoff B. Immunosuppressants in Organ Transplantation. *Handb Exp Pharmacol* (2020) 261:441–69. doi:10.1007/164_2019_331
31. Loewendorf A, Benedict CA. Modulation of Host Innate and Adaptive Immune Defenses by Cytomegalovirus: Timing Is Everything. *J Intern Med* (2010) 267(5):483–501. doi:10.1111/j.1365-2796.2010.02220.x
32. Klenerman P, Oxenius A. T Cell Responses to Cytomegalovirus. *Nat Rev Immunol* (2016) 16(6):367–77. doi:10.1038/nri.2016.38
33. Kidney Disease: Improving Global Outcomes Transplant Work G. KDIGO Clinical Practice Guideline for the Care of Kidney Transplant Recipients. *Am J Transpl* (2009) 9(Suppl. 3):S1–155. doi:10.1111/j.1600-6143.2009.02834.x
34. Arnol M, Naumovic R, Dimitrov EP, Racki S, Bucsa CA, Covic A, et al. Immunosuppressive Regimens Following Kidney Transplantation in Five European Countries: The Observational RECORD Study. *Transplant Rep* (2020) 5(3):100061. doi:10.1016/j.tpr.2020.100061
35. Asberg A, Jardine AG, Bignamini AA, Rollag H, Pescovitz MD, Gahlemann CC, et al. Effects of the Intensity of Immunosuppressive Therapy on Outcome of Treatment for CMV Disease in Organ Transplant Recipients. *Am J Transpl* (2010) 10(8):1881–8. doi:10.1111/j.1600-6143.2010.03114.x
36. Eiz-Vesper B, Maecker-Kolhoff B, Blasczyk R. Adoptive T-Cell Immunotherapy from Third-Party Donors: Characterization of Donors and Set up of a T-Cell Donor Registry. *Front Immunol* (2012) 3:410. doi:10.3389/fimmu.2012.00410
37. Tzannou I, Papadopoulou A, Naik S, Leung K, Martinez CA, Ramos CA, et al. Off-The-Shelf Virus-Specific T Cells to Treat BK Virus, Human Herpesvirus 6, Cytomegalovirus, Epstein-Barr Virus, and Adenovirus Infections After Allogeneic Hematopoietic Stem-Cell Transplantation. *J Clin Oncol* (2017) 35(31):3547–57. doi:10.1200/JCO.2017.73.0655
38. Gardiner BJ, Lee SJ, Cristiano Y, Levvey BJ, Sullivan LC, Snell GI, et al. Evaluation of Quantiferon®-Monitor as a Biomarker of Immunosuppression and Predictor of Infection in Lung Transplant Recipients. *Transpl Infect Dis* (2021) 23(3):e13550. doi:10.1111/tid.13550
39. Herold MJ, McPherson KG, Reichardt HM. Glucocorticoids in T Cell Apoptosis and Function. *Cell Mol Life Sci* (2006) 63(1):60–72. doi:10.1007/s00018-005-5390-y
40. Liberman AC, Budzinski ML, Sokn C, Gobbini RP, Steininger A, Arzt E. Regulatory and Mechanistic Actions of Glucocorticoids on T and Inflammatory Cells. *Front Endocrinol (Lausanne)* (2018) 9:235. doi:10.3389/fendo.2018.00235
41. Cantisan S, Lara R, Montejo M, Redel J, Rodriguez-Benot A, Gutierrez-Aroca J, et al. Pretransplant Interferon-Gamma Secretion by CMV-Specific CD8+ T Cells Informs the Risk of CMV Replication after Transplantation. *Am J Transpl* (2013) 13(3):738–45. doi:10.1111/ajt.12049
42. De Gracia-Guindo MDC, Ruiz-Fuentes MDC, Galindo-Sacristan P, Osorio-Moratalla JM, Ruiz-Fuentes N, Rodriguez Granger J, et al. Cytomegalovirus Infection Monitoring Based on Interferon Gamma Release Assay in Kidney Transplantation. *Transpl Proc* (2018) 50(2):578–80. doi:10.1016/j.transproceed.2017.09.052
43. Caston JJ, Cantisan S, Gonzalez-Gasca F, Paez-Vega A, Abdel-Hadi H, Illescas S, et al. Interferon-Gamma Production by CMV-Specific CD8+ T Lymphocytes Provides protection Against Cytomegalovirus Reactivation in Critically Ill Patients. *Intensive Care Med* (2016) 42(1):46–53. doi:10.1007/s00134-015-4077-6
44. Uemoto S, Ozawa K, Kaido T, Mori A, Fujimoto Y. Advantage of Tacrolimus/Mycophenolate Mofetil Regimen for Cytotoxic T Cell Mediated Defence and its Inhibition by Additive Steroid Administration in High-Risk Liver Transplant Recipients. *Clin Exp Immunol* (2016) 184(1):126–36. doi:10.1111/cei.12740
45. Jin N, Malcherek G, Mani J, Zurleit R, Schmitt A, Chen B, et al. Suppression of Cytomegalovirus-Specific CD8(+)T Cells by Everolimus. *Leuk Lymphoma* (2014) 55(5):1144–50. doi:10.3109/10428194.2013.822496
46. Cibrian D, Sanchez-Madrid F. CD69: From Activation Marker to Metabolic Gatekeeper. *Eur J Immunol* (2017) 47(6):946–53. doi:10.1002/eji.201646837
47. Schuurman HJ, Cottens S, Fuchs S, Joergensen J, Meerloo T, Sedrani R, et al. SDZ RAD, a New Rapamycin Derivative: Synergism with Cyclosporine. *Transplantation* (1997) 64(1):32–5. doi:10.1097/00007890-199707150-00007

48. Schuler W, Sedrani R, Cottens S, Haberlin B, Schulz M, Schuurman HJ, et al. SDZ RAD, a New Rapamycin Derivative: Pharmacological Properties *In Vitro* and *In Vivo*. *Transplantation* (1997) 64(1):36–42. doi:10.1097/00007890-199707150-00008
49. Dumont FJ, Su Q. Mechanism of Action of the Immunosuppressant Rapamycin. *Life Sci* (1996) 58(5):373–95. doi:10.1016/0024-3205(95)02233-3
50. Laplante M, Sabatini DM. mTOR Signaling in Growth Control and Disease. *Cell* (2012) 149(2):274–93. doi:10.1016/j.cell.2012.03.017
51. Klawitter J, Nashan B, Christians U. Everolimus and Sirolimus in Transplantation-Related but Different. *Expert Opin Drug Saf* (2015) 14(7): 1055–70. doi:10.1517/14740338.2015.1040388
52. Nashan B, Gaston R, Emery V, Saemann MD, Mueller NJ, Couzi L, et al. Review of Cytomegalovirus Infection Findings with Mammalian Target of Rapamycin Inhibitor-Based Immunosuppressive Therapy in De Novo Renal Transplant Recipients. *Transplantation* (2012) 93(11):1075–85. doi:10.1097/TP.0b013e31824810e6
53. Boyman O, Sprent J. The Role of Interleukin-2 During Homeostasis and Activation of the Immune System. *Nat Rev Immunol* (2012) 12(3):180–90. doi:10.1038/nri3156
54. Fiers W. Tumor Necrosis Factor. Characterization at the Molecular, Cellular and *In Vivo* Level. *FEBS Lett* (1991) 285(2):199–212. doi:10.1016/0014-5793(91)80803-b
55. Thomson AW, Bonham CA, Zeevi A. Mode of Action of Tacrolimus (FK506): Molecular and Cellular Mechanisms. *Ther Drug Monit* (1995) 17(6):584–91. doi:10.1097/00007691-199512000-00007
56. Rusnak F, Mertz P. Calcineurin: Form and Function. *Physiol Rev* (2000) 80(4): 1483–521. doi:10.1152/physrev.2000.80.4.1483
57. Ho S, Clipstone N, Timmermann L, Northrop J, Graef I, Fiorentino D, et al. The Mechanism of Action of Cyclosporin A and FK506. *Clin Immunol Immunopathol* (1996) 80(3 Pt 2):S40–5. doi:10.1006/clin.1996.0140
58. Raphael I, Joern RR, Forsthuber TG. Memory CD4(+) T Cells in Immunity and Autoimmune Diseases. *Cells* (2020) 9(3):531. doi:10.3390/cells9030531
59. Ronca V, Wootton G, Milani C, Cain O. The Immunological Basis of Liver Allograft Rejection. *Front Immunol* (2020) 11:2155. doi:10.3389/fimmu.2020.02155
60. Allison AC. Mechanisms of Action of Mycophenolate Mofetil. *Lupus.* (2005) 14(Suppl. 1):s2–8. doi:10.1191/0961203305lu2109oa
61. Staatz CE, Tett SE. Clinical Pharmacokinetics and Pharmacodynamics of Mycophenolate in Solid Organ Transplant Recipients. *Clin Pharmacokinet* (2007) 46(1):13–58. doi:10.2165/00003088-200746010-00002
62. He X, Smeets RL, Koenen HJ, Vink PM, Wagenaars J, Boots AM, et al. Mycophenolic Acid-Mediated Suppression of Human CD4+ T Cells: More Than Mere Guanine Nucleotide Deprivation. *Am J Transpl* (2011) 11(3): 439–49. doi:10.1111/j.1600-6143.2010.03413.x
63. Lemoine R, Velge-Roussel F, Herr F, Felix R, Nivet H, Lebranchu Y, et al. Interferon Gamma Licensing of Human Dendritic Cells in T-helper-Independent CD8+ Alloimmunity. *Blood* (2010) 116(16):3089–98. doi:10.1182/blood-2010-02-268623
64. Bak S, Tischer S, Dragon A, Ravens S, Pape L, Koenecke C, et al. Selective Effects of mTOR Inhibitor Sirolimus on Naive and CMV-Specific T Cells Extending its Applicable Range beyond Immunosuppression. *Front Immunol* (2018) 9:2953. doi:10.3389/fimmu.2018.02953
65. Kaminski H, Marseres G, Yared N, Nokin MJ, Pitard V, Zouine A, et al. mTOR Inhibitors Prevent CMV Infection Through the Restoration of Functional αβ and γδ T Cells in Kidney Transplantation. *J Am Soc Nephrol* (2022) 33(1): 121–37. doi:10.1681/ASN.2020121753
66. Poglitsch M, Weichhart T, Hecking M, Werzowa J, Katholnig K, Antlanger M, et al. CMV Late Phase-Induced mTOR Activation Is Essential for Efficient Virus Replication in Polarized Human Macrophages. *Am J Transpl* (2012) 12(6):1458–68. doi:10.1111/j.1600-6143.2012.04002.x
67. Amini L, Wagner DL, Rossler U, Zarrinrad G, Wagner LF, Vollmer T, et al. CRISPR-Cas9-Edited Tacrolimus-Resistant Antiviral T Cells for Advanced Adoptive Immunotherapy in Transplant Recipients. *Mol Ther* (2021) 29(1): 32–46. doi:10.1016/j.ymthe.2020.09.011
68. Kaeuferle T, Deisenberger L, Jablonowski L, Stief TA, Blaeschke F, Willier S, et al. CRISPR-Cas9-Mediated Glucocorticoid Resistance in Virus-specific T Cells for Adoptive T Cell Therapy Posttransplantation. *Mol Ther* (2020) 28(9):1965–73. doi:10.1016/j.ymthe.2020.06.002
69. Biolatti M, Dell'Oste V, Pautasso S, Gugliesi F, von Einem J, Krapp C, et al. Human Cytomegalovirus Tegument Protein Pp65 (pUL83) Dampens Type I Interferon Production by Inactivating the DNA Sensor cGAS Without Affecting STING. *J Virol* (2018) 92(6):e01774. doi:10.1128/JVI.01774-17
70. Jackson SE, Mason GM, Okecha G, Sissons JG, Wills MR. Diverse Specificities, Phenotypes, and Antiviral Activities of Cytomegalovirus-Specific CD8+ T Cells. *J Virol* (2014) 88(18):10894–908. doi:10.1128/JVI.01477-14
71. Lisby AN, Carlson RD, Baybutt TR, Weindorfer M, Snook AE. Evaluation of CAR-T Cell Cytotoxicity: Real-Time Impedance-Based Analysis. *Methods Cel Biol* (2022) 167:81–98. doi:10.1016/bs.mcb.2021.08.002
72. Bergan S, Brunet M, Hesselink DA, Johnson-Davis KL, Kunicki PK, Lemaitre F, et al. Personalized Therapy for Mycophenolate: Consensus Report by the International Association of Therapeutic Drug Monitoring and Clinical Toxicology. *Ther Drug Monit* (2021) 43(2):150–200. doi:10.1097/FTD.0000000000000871
73. Brunet M, van Gelder T, Asberg A, Haufroid V, Hesselink DA, Langman L, et al. Therapeutic Drug Monitoring of Tacrolimus-Personalized Therapy: Second Consensus Report. *Ther Drug Monit* (2019) 41(3):261–307. doi:10.1097/FTD.0000000000000640
74. Shipkova M, Hesselink DA, Holt DW, Billaud EM, van Gelder T, Kunicki PK, et al. Therapeutic Drug Monitoring of Everolimus: A Consensus Report. *Ther Drug Monit* (2016) 38(2):143–69. doi:10.1097/FTD.0000000000000260
75. MacDonald A, Scarola J, Burke JT, Zimmerman JJ. Clinical Pharmacokinetics and Therapeutic Drug Monitoring of Sirolimus. *Clin Ther* (2000) 22(Suppl. B): B101–21. doi:10.1016/s0149-2918(00)89027-x
76. Skauby RH, Gustavsen MT, Andersen AM, Bjerre A, Asberg A, Midtvedt K, et al. Prednisolone and Prednisone Pharmacokinetics in Adult Renal Transplant Recipients. *Ther Drug Monit* (2021) 43(2):247–55. doi:10.1097/FTD.0000000000000835
77. van Rossum HH, Romijn FP, Smit NP, de Fijter JW, van Pelt J. Everolimus and Sirolimus Antagonize Tacrolimus Based Calcineurin Inhibition via Competition for FK-Binding Protein 12. *Biochem Pharmacol* (2009) 77(7): 1206–12. doi:10.1016/j.bcp.2008.12.009

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Assessing Outcomes of Patients Subject to Intensive Care to Facilitate Organ Donation: A Spanish Multicenter Prospective Study

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Intensive Care to facilitate Organ Donation (ICOD) consists of the initiation or continuation of intensive care measures in patients with a devastating brain injury (DBI) in whom curative treatment is deemed futile and death by neurological criteria (DNC) is foreseen, to incorporate organ donation into their end-of-life plans. In this study we evaluate the outcomes of patients subject to ICOD and identify radiological and clinical factors associated with progression to DNC. In this first prospective multicenter study we tested by multivariate regression the association of clinical and radiological severity features with progression to DNC. Of the 194 patients, 144 (74.2%) patients fulfilled DNC after a median of 25 h (95% IQR: 17–44) from ICOD onset. Two patients (1%) shifted from ICOD to curative treatment, both were alive at discharge. Factors associated with progression to DNC included: age below 70 years, clinical score consistent with severe

Abbreviations: CI, confidence intervals; CT, computed tomography; DBI, devastating brain injury; DNC, death by neurological criteria; GCS, Glasgow Coma Scale; HR, Hazard Ratios, ICH, Intracerebral Hemorrhage Score; ICOD, Intensive Care to facilitate Organ Donation; ICU, Intensive Care Unit; IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; ONT, Organización Nacional de Trasplantes; SBD, severe brain damage; SDM, surrogate decision-makers; SEMICYUC, Spanish Society of Intensive Care and Coronary Units; WLST, withdrawal of life-sustaining treatments.

brain injury, instability, intracranial hemorrhage, midline shift ≥5 mm and certain types of brain herniation. Overall 151 (77.8%) patients progressed to organ donation. Based on these results, we conclude that ICOD is a beneficial and efficient practice that can contribute to the pool of deceased donors.

Keywords: transplantation, deceased organ donation, death by neurologic criteria, devastating brain injury, intensive care to facilitate organ donation

INTRODUCTION

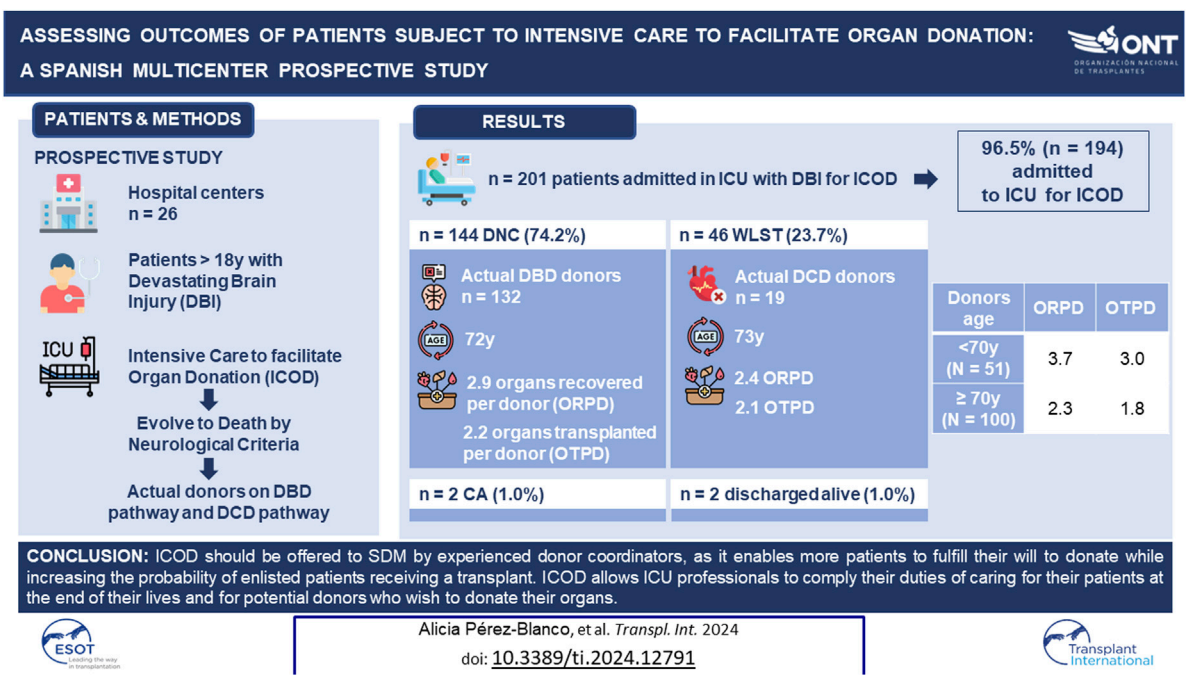
Intensive Care to facilitate Organ Donation (ICOD) is the initiation or continuation of intensive care measures in patients with a devastating brain injury (DBI) in whom curative treatment is deemed futile, and death by neurological criteria (DNC) is foreseen, with the aim of incorporating the option of organ donation into their end-of-life care plans.

ICOD is an established practice in Spain, with specific, published guidelines [1]. DBI is defined as a neurologic condition, assessed as an immediate threat to life or incompatible with good functional recovery, and where withholding or withdrawal of life-sustaining treatments (WLST) is being considered [2]. When a multidisciplinary treating team consensually decides to pursue end-of-life care, the patient is referred to the donor coordinator to evaluate donation opportunities and provide detailed information about ICOD to surrogate decision-makers (SDM). Having reflected upon the nuances of this donation process, the SDM may authorize ICOD to preserve the option of organ donation while awaiting DNC. Donor coordinators will inform the SDM that, if the patient does not meet DNC within the first 72 h or they revoke authorization, WLST will proceed.

ICOD in Spain contributes to 24%–33% of deceased donation activities, with a mean of 2.3 organs transplanted *per* donor [3–5]. Other countries—e.g. Australia [6], Canada [7], France [8], the Netherlands [9], the United Kingdom [10] and the United States [11]—have implemented similar policies, based on delaying WLST, to preserve the option of progressing to DNC. However, Spanish legislation and the ICOD national protocol permit the initiation of intensive measures, whilst several other national systems only accept their continuation [12–14].

Accurate prognosis of DBI early after the injury is difficult even for experienced clinicians, and a small percentage of patients with a DBI may be discharged alive with acceptable outcomes [10, 11, 15]. Additionally, ICOD requires the investment of expensive resources, with uncertainties about its effectiveness, and the possibility of unintended negative consequence on the patient, family and staff [3, 9, 10].

The aim of this study is to evaluate prospectively the outcomes of patients with a DBI admitted to the intensive care unit (ICU) for ICOD, and to identify clinical and radiological signs



GRAPHICAL ABSTRACT

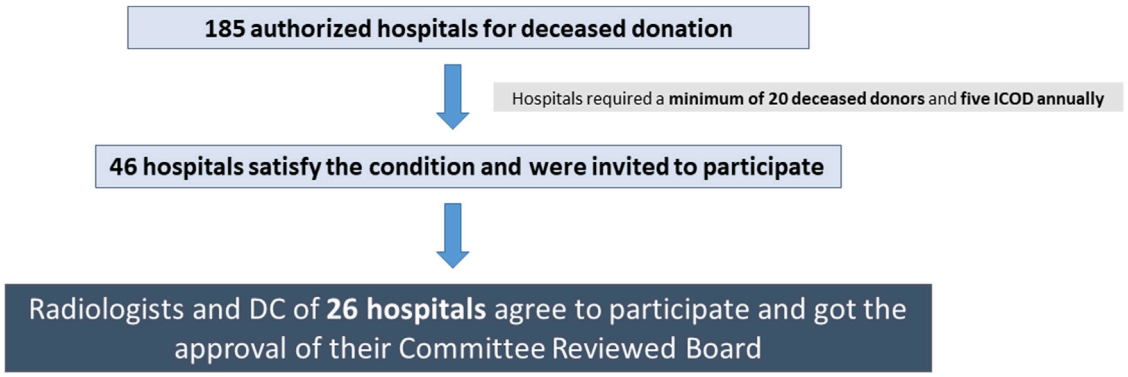


FIGURE 1 | Hospital selection process to participate in the study. Footnote: DBD, Donation after Brain Death; DCD, Donation after Circulatory Death; DC, donor coordinator.

associated with progression to DNC. With an understanding of the most reliable signs, clinicians may identify and refer in a timely manner those patients most likely to become organ donors after DNC. A secondary objective is to measure the impact of ICOD on donation and transplantation metrics. The preliminary results of this research were published as an abstract in 2021 [16].

MATERIALS AND METHODS

This is a prospective observational study conducted by the Organización Nacional de Trasplantes (ONT) and the Spanish Society of Intensive Care (SEMICYUC), performed in 26 Spanish hospitals (20 with neurosurgical units and 6 without) out of 46 hospitals invited to participate (**Figure 1**).

From July 2018 to July 2020, patients aged over 18 years, diagnosed with a DBI who had been admitted to the ICU for ICOD, were consecutively enrolled in the study.

Information was collected on patient demographics, location, and clinical and brain computed tomography (CT) scan data at the time of assessment for ICOD. Radiologists at participating centers completed a standardized form (**Supplementary Material**).

Information was also recorded on patients' outcomes and transition to actual donors, where applicable.

For this study, severe brain damage (SBD) was defined based on values of validated scores for each etiology of the DBI: ICH score ≥3 for intracerebral hemorrhage score [17], HUNT-HESS ≥ IV for aneurysmal subarachnoid hemorrhage [18], NIHSS ≥25 for ischemic stroke [19] and GCS ≤5 for traumatic brain injury [20, 21]. An unstable patient was defined by the risk of imminent respiratory arrest [1].

Qualitative data is presented as absolute numbers and percentages. Quantitative data is displayed as mean and standard deviation or median and interquartile range (IQR), depending on the dispersion of the sample. Data derived from the clinical examination and brain CT at the time of assessment for ICOD, were evaluated for their potential association with progression to DNC [21–25].

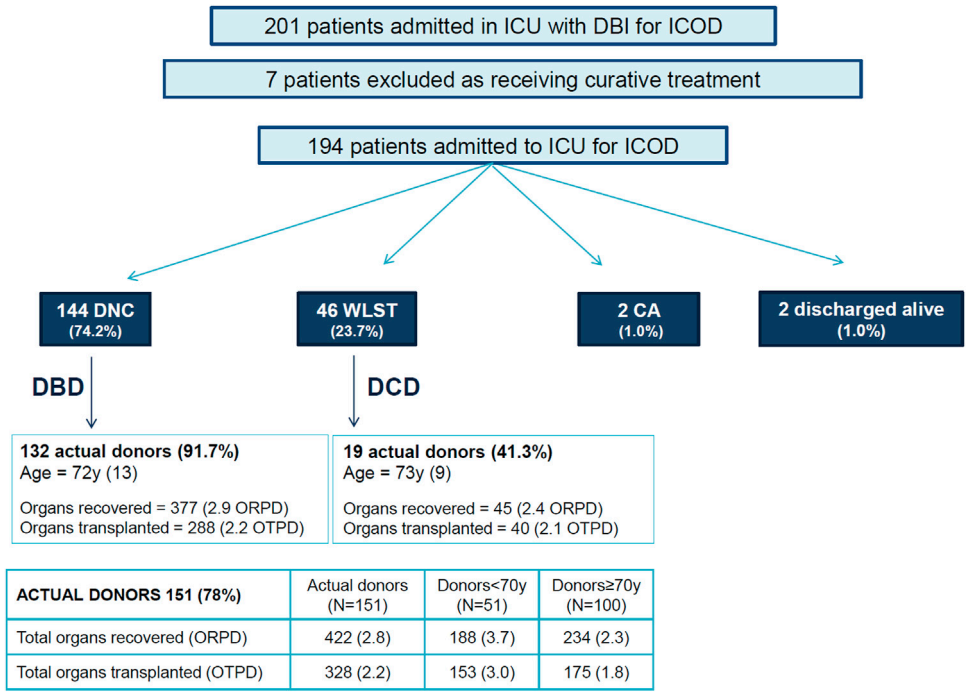
Univariate effects were analyzed using Hazard Ratios (HR) and their 95% confidence intervals (95% CI). The analysis strategy is not only based on statistical criteria. Statistically significant variables ($p < 0.05$) identified on the univariate analysis, plus likely confounding variables, were included in the multivariate Cox model. Variance inflation factor was used to study the collinearity between some explicative variables resulting after the univariate analysis. In case of collinearity, the variable with highest effect (HR) was considered the most appropriate to be included in the multivariate model. The assumption of proportionality of the models was evaluated. Discriminative ability was calculated by Harrell's C index. Two-sided tests were used and a p-value < 0.05 was considered significant. Statistical analyses were performed using Stata version 17.0.

The study was approved by the Institutional Review Boards of each participating hospital. The Ethics Committee of ONT produced a written informed consent for SDMs enrolling in the study, which was endorsed by participating centers (**Supplementary Material**). All procedures were in accordance with the Declaration of Helsinki.

RESULTS

In total, 201 patients with DBI were included in the study (**Figure 2**). Seven cases were excluded from analysis because patients had received medical treatment with curative intent within 24 h of the DBI. Data from the remaining 194 patients was analyzed.

Baseline characteristics of patients, location, results of the clinical examination and brain CT features at the time of ICOD assessment are shown in **Table 1**. The main cause of the DBI was an intracranial hemorrhage (n = 126, 88.1%). Assessment of the eligibility for ICOD was most frequently performed within the emergency department (n = 144, 74.2%). Most patients (n = 127, or 65.5%) were intubated and ventilated before the decision to apply ICOD. Brain CT showed 144 (74.2%)



ACTUAL DONORS 151 (78%)	Actual donors (N=151)	Donors<70y (N=51)	Donors≥70y (N=100)
Total organs recovered (ORPD)	422 (2.8)	188 (3.7)	234 (2.3)
Total organs transplanted (OTPD)	328 (2.2)	153 (3.0)	175 (1.8)

FIGURE 2 | Evolution of patients subject to intensive care to facilitate organ donation and effectiveness on donation and transplantation. Footnote: DNC, Death by Neurologic Criteria; DBI, Devastating Brain Injury; WLST, Withdrawal of life sustaining treatment; CA, cardiac arrest; DBD, Donation after Brain Death; DCD, Donation after Circulatory Death; Age, mean (SD); ORPD, organ recovered per donor; OTPD, organ transplanted per donor.

patients had a midline shift ≥5 mm, 150 (77.3%) had basal cistern effacement and 155 (79.9%) had some form of brain herniation.

Clinical Outcomes of Patients Subject to ICOD

Outcomes of patients subject to ICOD are displayed in **Figure 2**.

Of the 194 cases, 144 (74.2%) fulfilled the criteria for DNC after a median time of 25 h (95% IQR: 17–44) from ICOD onset, with most patients (n = 134, 69.1%) fulfilling DNC within the first 72 h from ICOD onset.

Forty-six patients (23.7%) died following the decision to WLST after a median time of 49 h (95% IQR: 24–84) from ICOD onset. The median time to death by circulatory criteria was 51 h (95% IQR: 25–84) after the initiation of ICOD. Two of the 46 patients were discharged alive from the ICU and transferred to the ward for palliative care, where they died (**Table 2**). In 21 patients, WLST took place within the first 48 h (11 due to medical contraindications and 10 because family revoked consent for ICOD). In the remaining 25 patients, WLST occurred after 48 h, in most cases because the timeframe agreed with SDM for DNC was surpassed.

Two patients (1.0%) admitted to the ICU for ICOD were later reassessed and received curative treatment. One of these patients had been diagnosed with an aneurysmal subarachnoid hemorrhage. The severity of the brain injury had been assessed close to the hemorrhage onset and SDM had refused any invasive therapeutic intervention. However, after being reassessed in the ICU, their neurological condition showed improvement, and the decision was made to apply curative treatment. After 26 days, the patient did not show any neurological improvement and was transferred to internal medicine. The second patient had been diagnosed with a traumatic brain injury and transferred from another hospital for ICOD. Clinicians reassessed the neurological status and recommended shift to curative treatment, despite the severity of the brain injury. After 15 days their neurological condition did not improve and they were discharged to a social institution.

Factors Associated With Progression to DNC

Univariate and multivariate analyses of factors associated with progression to DNC in ICOD patients is shown in **Table 3**. The variables Glasgow Coma Score and intubated patient were not included in the final multivariable model due to their collinearity with severe brain damage and unstable respectively. On the final multivariate Cox model, multiple factors were significantly associated with progression to DNC including: age under 70 years, severe brain damage, instability at the time of assessment for ICOD, intracranial hemorrhage in the temporal region, midline shift ≥5 mm and certain types of brain herniation

TABLE 1 | Characteristics of patients subject to intensive care to facilitate organ donation.

Demographic characteristics		
Sex male, n (%)	97	(50.0%)
Age (years), mean (SD)	72	(12)
Cause of DBI, n (%)		
Intracranial hemorrhage	126	(64.9%)
Traumatic brain injury	38	(19.6%)
Ischemic stroke/hypoxic brain injury	21	(10.8%)
Aneurysmal SAH	9	(4.6%)
Time and location where ICOD was assessed		
Time from DBI diagnosis to assessment for ICOD (hours), median (IQR)	1	(1–2)
Location of assessment for ICOD, n (%)		
Emergency room	144	(74.2%)
Intensive care unit	19	(9.8%)
Stroke unit	15	(7.7%)
Neurology ward	8	(4.1%)
Post-anesthesia care unit	4	(2.1%)
Other[a]	4	(2.1%)
Clinical data at the time of assessment for ICOD		
Intubated patient at the time of assessment for ICOD, n (%)	127	(65.5%)
Unstable (risk of imminent respiratory arrest), n (%)	17	(8.8%)
Glasgow Coma Score, n (%)		
3–5	155	(79.9%)
6–7	28	(14.4%)
≥8	11	(5.7%)
Severe brain damage[b], n (%)	162	(83.5%)
ICH (*intracranial hemorrhage no-SAH, n = 126*) ≥ 3	111	(88.1%)
NIHSS (*ischemic CVA, n = 21*) ≥ 25	10	(47.6%)
HUNT-HESS (*aneurysmal SAH, n = 9*) ≥IV	8	(88.9%)
Glasgow Coma Scale (GCS) (*TBI, n = 38*) ≤ 5	33	(86.8%)
Radiological data at the time of assessment for ICOD		
Intracranial hemorrhage in temporal region, n (%)	51	(42.9%)
Midline shift (mm), median (IQR)	12	(4–16)
Midline shift ≥ 5 mm, n (%)	144	(74.2%)
ONSD[c] 3 mm behind the globe (mm) (n = 104), mean (SD)	5.9	(1.3)
ONSD[c] 10 mm behind the globe (mm) (n = 104), mean (SD)	5.1	(1.7)
Hydrocephalus, n (%)	104	(53.6%)
Basal cistern effacement, n (%)	150	(77.3%)
Herniation, n (%)	155	(79.9%)
Types of brain herniation, n (%)		
No herniation	39	(20.1%)
Transtentorial alone	54	(27.8%)
Subfalcine alone	50	(25.8%)
Cerebellar tonsil alone	7	(3.6%)
Transtentorial + Subfalcine	33	(17.0%)
Cerebellar tonsil + Transtentorial and/or Subfalcine	11	(5.7%)

[a]*Internal Medicine, Neurosurgery department, transfer from another hospital.*
[b]*Severe Brain Damage is considered positive when any of the following occur: ICH≥ 3 for intracranial spontaneous hemorrhage; NIHSS ≥25 for ischemic stroke; HUNT-HESS ≥ IV, for aSAH; Glasgow ≤5 for TBI.*
[c]*ONSD: optic nerve sheath diameter.*

(cerebellar tonsillar herniation combined with transtentorial and/or subfalcine herniation).

Impact of ICOD on Organ Donation and Transplantation

Overall, 151 (77.8%) patients transitioned to actual organ donors, 132 after DNC and 19 after the circulatory determination of death (41.3% of the 46 patients who died after the WLST). In total, 2.8 organs were recovered and 2.2 organs were transplanted *per* actual donor (1.8 for donors aged ≥70 years) (**Figure 2**).

The reasons why patients with DNC did not transition to actual donation were: medical contraindications (n = 6), SDM refused consent (n = 4), no suitable recipient (n = 1) and unexpected cardiac arrest after DNC (n = 1). Corresponding reasons why patients who died after the WLST did not transition to actual donation were: medical contraindications (n = 13), age

TABLE 2 | Characteristics of the four patients discharged alive from the intensive care unit.

Age	Etiology	GCS/Hunt-Hess	WLST (h)	Outcome
69	ICH	5	13	Palliative care, died in the ward
87	ICH	8	22	Palliative care, died in the ward
83	TBI	7		Discharged Alive; GOS 3
83	aSAH	>IV		Discharged Alive; GOS 3

aSAH, aneurysmal subarachnoid hemorrhage; ICH, intracranial hemorrhage; TBI, traumatic brain injury; GOS, 3: conscious, need help for daily tasks. WLST, withdrawal of life sustaining treatment in ICU.

TABLE 3 | Analysis of the factors associated with death by neurological criteria in patients subject to intensive care to facilitate organ donation. Univariate and multivariate Cox model.

Variables	Univariate			Multivariate[a]		
	Hazard ratio	[CI 95% HR]	*p*	Hazard ratio	[CI 95% HR]	*p*
Sex male	1.06	[0.76–1.47]	0.737			
Age <70 years[b]	**1.74**	**[1.24–2.45]**	**0.002**	**1.78**	**[1.24–2.56]**	**0.002**
Cause of death			0.301			
Aneurysmal subarachnoid haemorrhage	***Ref.***					
Hemorrhagic Stroke	1.59	[0.70–3.64]	0.270			
Ischemic Stroke/Hypoxic brain injury	1.00	[0.39–2.62]	0.994			
Traumatic brain injury	1.36	[0.56–3.32]	0.497			
Glasgow Come Score			0.058			
3–5	**3.31**	**[1.22–8.98]**	**0.019**			
6–7	2.88	[0.98–8.49]	0.055			
≥8	***Ref.***					
Severe Brain Damage[c]	**1.87**	**[1.11–3.14]**	**0.019**	**2.06**	**[1.19–3.58]**	**0.010**
Time from DBI diagnosis to ICOD evaluation	0.95	[0.88–1.02]	0.131			
Intubated patient	**1.57**	**[1.10–2.25]**	**0.014**			
Unstable (risk of imminent respiratory arrest)	**1.73**	**[0.98–3.07]**	**0.059**	**3.29**	**[1.71–6.33]**	**<0.001**
Intracranial hemorrhage	1.37	[0.96–1.95]	0.079			
Intracranial hemorrhage in temporal region	**1.70**	**[1.21–2.39]**	**0.002**	**1.47**	**[1.03–2.10]**	**0.034**
Midline shift ≥5 mm	**1.68**	**[1.13–2.51]**	**0.010**	**1.77**	**[1.14–2.74]**	**0.011**
ONSD[d] 3 mm behind the globe	0.91	[0.76–1.08]	0.265			
Hydrocephalus	0.92	[0.67–1.28]	0.639			
Basal cistern effacement	1.36	[0.90–2.05]	0.146			
Type of brain herniation			**0.003**		***Ref***	
No herniation	***Ref.***					
Transtentorial	**1.98**	**[1.18–3.32]**	**0.009**			
Subfalcine	1.41	[0.83–2.40]	0.204			
Cerebellar tonsil	**2.55**	**[1.03–6.34]**	**0.043**			
Transtentorial + Subfalcine	1.63	[0.92–2.89]	0.097			
Cerebellar tonsil + Transt. And/or Subfalcine	**4.73**	**[2.15–10.40]**	**<0.001**	**1.45**	**[0.99–2.12]**	**0.054**

[a]*Discriminate analysis: Harrell Index, C 0.66.*
[b]*Cut-off stablished through ROC, curve.*
[c]*Severe Brain Damage, defined by an ICH ≥ 3 for intracranial spontaneous hemorrhage, an NIHSS ≥25 for ischemic stroke, a HUNT-HESS ≥ IV, for aneurysmal subarachnoid haemorrhage and a Glasgow Coma Score ≤5 for traumatic brain injury.*
[d]*ONSD: Optic Nerve Sheath Diameter.*
Bold means statistically significant, defined as p *equal to or less than 0.05.*

unsuitable for donation after the circulatory determination of death (DCD) (n = 12) and death not expected within a timeframe suitable for organ donation (n = 2).

DISCUSSION

Even for experts in neurocritical care, prognostication in DBI within a short timeframe from injury is challenging. With this study, we wanted to evaluate the practice of ICOD and provide detailed patient outcomes. To the best of our knowledge, this is the first multicenter study that prospectively evaluates the impact of clinical and radiological data from patients with DBI and upon the likelihood of progression to DNC in patients admitted in ICU for ICOD.

Factors Associated With Progression to DNC

Most patients subject to ICOD in our series did progress to DNC (74.2%), consistent with reports from retrospective multicenter

and single-center studies performed in Spain [3–5]. This progression to DNC was higher than that reported by both Melville et al. (65%) and Humbertjean et al. (23%) [6, 25]. Differences may be due to variation between patient cohorts and also improving ability to prognosticate over time.

Clinical factors independently associated with the progression to DNC were: age under 70 years, achieving SBD criteria (diagnosis specific), and risk of imminent respiratory arrest. Relevant radiographic factors consisted of intracranial hemorrhage in the temporal region, midline shift ≥5 mm and a combination of tonsil with transtentorial and/or subfalcine herniation.

Being older than 70 has been well described as a factor reducing the likelihood of progression to DNC [3–5, 25, 26]. This finding should not prevent clinicians considering ICOD in older patients, as our study included 100 donors aged ≥70, resulting in 1.8 organs transplanted *per* donor (**Figure 2**).

Although some have reported a cut-off value in GCS (e.g., GCS≤6) to be associated with progression to DNC [21], others do not identify a firm cut-off value [10, 15]. Aware of this limitation, we identified positive indicators for defining Severe Brain Damage (SBD) depending on the brain injury pathology (ICH≥ 3 for intracranial spontaneous hemorrhage, NIHSS ≥25 for ischemic stroke, HUNT-HESS ≥ IV for aneurysmal subarachnoid hemorrhage and a Glasgow Coma Scale ≤5 for traumatic brain injury). Our results show that meeting criteria for SBD is associated with progression to DNC in this selected cohort of ICOD patients (OR 2.06 [1.19–3.58]).

We evaluated the findings of the brain CT scan performed when ICOD was considered, to assess their association with progression to DNC. This approach is different from that of Ray A et al. who analyzed signs in the CT scan taken before DNC occurred [27]. We like others found herniation is associated with DNC [21, 25]. The combination of tonsillar plus transtentorial and/or subfalcine herniation was the combination most strongly associated with progression to DNC in patients subject to ICOD. This contrasts with Ray et al, who did not observe any association between herniation and DNC [27]. This may be due to the different timing of the brain CT to DNC and the highly selective cohort of patients in our study [21, 25, 27].

Clinical Outcomes of Patients Subject to ICOD

ICU admission for ICOD allows stabilizing hemodynamic and respiratory parameters, reassessing the neurological condition, and studying thoroughly the patient's medical history to establish eligibility for organ donation. The neurological reassessment of patients with DBI is performed daily to evaluate clinical improvement or deterioration and, when needed, a brain CT-scan is repeated.

The median time to meet DNC in our study (25 h) was relatively short compared to the average of 43 h from ICU admission (IQR 24–87) observed in all DBI patients who ultimately progressed to DNC. This latter cohort includes 2,393 patients admitted to ICU (26 centres) with DBI for either treatment with curative intent or ICOD from 2018 to 2020. The implemented ICOD practice in Spain shows that donor coordinators are highly restrictive and only consider admittance in ICU for ICOD a patient with DBI that will otherwise be admitted for terminal sedation, which explains the advanced age of our sample.

Two patients (1%) in our cohort were discharged alive (both aged 83), similar to 0.9% reported by Melville et al [6]. In both cases, the treating team decided to shift from ICOD to full treatment after observing an improvement in the neurological exam.

The main reason for not transitioning to DNC was WLST (n = 46). Reasons for the WLST in the first 48 h from ICOD onset were medical contraindications (N = 11) and SDM revoking consent for ICOD before the end of the agreed-upon timeframe (N = 10). After 48 h, WLST occurred in most cases because the timeframe agreed with SDM for DNC was surpassed (N = 23) or SDM revoked consent before surpassing it (N = 2).

The majority of the 11 medical contraindications arose as a result of serological and radiographic tests after ICU admission. We must reinforce the importance of learning from the relatives about the donor's habits, in order to perform tests before admission to ICU for ICOD.

Impact of ICOD on Organ Donation and Transplantation

ICOD requires investment of human and financial resources. Our study helps to confirm it is an efficient policy, considering 78% of patients subject to ICOD transitioned to actual donors, with a rate of 2.2 organs transplanted per donor. The percentage of ICOD patients transitioning to actual donation is lower in the series published by Melville et al. (52%) and Witjes et al (42%) [6, 9]. However, we included all cases with consent for ICOD, while theirs additionally recorded cases with declined consent for admission to the ICU to enable organ donation.

Several authors gauge the financial savings in hemodialysis, as well as the recipients' quality-adjusted life-years gained, and compare these figures to a relatively short stay in the ICU of patients admitted for ICOD. They also conclude that implementing an ICOD protocol or a 'DBI pathway' is highly efficient from the transplantation perspective [10, 26–28].

Some have questioned the additional stress placed on families agreeing to ICOD [29, 30]. Conversely, others found that long admissions of older patients with cerebrovascular injuries help their relatives grasp the reality of their loss, with a positive correlation with organ donation [31]. Many emphasized the crucial role of a positive environment around donation in the ICU, highlighting fluent communication between clinicians and donor coordinators as a means to support families' decision making [32, 33].

Twelve families expressed fatigue around the ICU admission, requesting WLST before the end of the agreed-upon timeframe.

However, there were no refusals of DCD donation in the WLST group, so the SDMs' initial decision to authorize organ donation persisted, but prolongation of waiting in ICU for DNC was not felt to be tolerable to the SDM.

ICOD Protocols Throughout the World

The efficacy of the Spanish ICOD protocol may be explained by its differences from those in other countries. First, while in Netherlands emergency care physicians approach families in the emergency department to propose ICU admission to preserve organ donation, in Spain, the donor coordinator leads the process, informing SDM about ICOD once the decision has been made not to proceed with a therapeutic purpose [9]. The special training of donor coordinators in approaching families has shown to improve the likelihood of consent to organ donation [3].

Second, though most patients in our study had been diagnosed with a DBI in the emergency room, cases were also identified in other hospital units, producing 26% of the candidates for ICOD.

A third important difference lies in the advanced age of patients subject to ICOD in our study, compared with other published articles [6, 8–11]. The mean age of ICOD patients in our study (72 years), contrasts with the Australian mean age of patients included in the "potential organ donation pathway" (55 years) [6] and with the mean age of patients admitted to the ICU for organ donation in Netherlands (59 years) [9] or in France (66 years) [25]. The advanced age of patients subject to ICOD in our series is in accordance with previous studies [3–5] and the established national policy on utilizing organs obtained from expanded-criteria donors [28].

Strengths and Limitations of This Study

This is the first prospective study in the field that may shed light on the impact of a nationwide ICOD policy on patient outcomes, and donation and transplantation metrics.

Limitations are related to sample size, resources and availability of specialized clinicians between hospitals with and without neurosurgical departments. Indeed, premature neurological assessment of the patient with the aim of transporting them to a neurosurgical center may be misleading.

The interobserver variability inherent in a multicenter study affects the interpretation of both the clinical and radiological results.

Another limitation is associated with the early performance of the brain CT. Some radiographic signs of intracranial hypertension may not be visible at the time of this exam, impeding the radiologists' ability to clearly observe the signs of impending progression to DNC. Yet, in medical practice, evaluation of a patient with DBI as an ICOD candidate is based on the results of the radiology performed during diagnosis of DBI.

Conclusion

Clinical and radiographic factors identified in our study may help clinicians identify patients potentially progressing to DNC, permitting efficient utilization of ICU resources and an effective approach to families.

ICOD should be offered to SDM by experienced donor coordinators, as it enables more patients to fulfill their will to donate while increasing the probability of enlisted patients receiving a transplant.

Our findings reinforce the importance of providing information to SDM about all the uncertainties involved in this complex process, so they can envision the potential obstacles for their loved ones to become a donor after DNC, and make a fully-informed decisions around consent. Intensivists and donor coordinators should have a plan to proceed with WLST in cases of medical contraindication, at the request of family, or if the patient does not progress to DNC by a pre-agreed timeframe.

Future large prospective studies are required to further validate and build upon these important results that may ultimately increase the number of organs available for donation.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by the ONT ETHICS COMMITTEE BOARD 2018/CIOD-02. 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

AP-B, EC, and BD-G Formulation of research goals and design of the methodology. AP-B First drafting/revision of the manuscript for content, including medical writing for content; study concept or design. AP and MA management and coordination responsibility for the research activity planning and execution. MP, ND, CF, and EC Application of statistical, mathematical, computational, or other formal techniques to analyze study data. AG, LZ, MB, AM, VC, MR, EF, JV, EF, BQ, SR, JR, AN, JM, JT, AV, EH, ÁG, MR, FG, RL, LL, FGi, and FGu, Data collection and report of each consecutive case, revision of the manuscript for content, including medical writing for content. ÁM Drafting/writing the radiologist diagnostic format enclosed as **Supplementary Material**, review the data reported by the radiologist from the participant centers. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.12791/full#supplementary-material

REFERENCES

1. Martín-Delgado MC, Martínez-Soba F, Masnou N, Martín-Delgado MC, Martínez-Soba F, Masnou N, et al. Summary of Spanish Recommendations on Intensive Care to Facilitate Organ Donation. *Am J Transpl* (2019) 19: 1782–91. doi:10.1111/ajt.15253
2. Harvey D, Butler J, Groves J, Manara A, Menon D, Thomas E, et al. Management of Perceived Devastating Brain Injury After Hospital Admission: A Consensus Statement From Stakeholder Professional Organizations. *Br J Anaesth* (2018) 120:138–45. doi:10.1016/j.bja.2017.10.002
3. Domínguez-Gil B, Coll E, Elizalde J, Herrero JE, Pont T, Quindós B, et al. Expanding the Donor Pool Through Intensive Care to Facilitate Organ Donation: Results of a Spanish Multicenter Study. *Transplantation* (2017) 101:e265–e272. doi:10.1097/TP.0000000000001701
4. Martínez-Soba F, Pérez-Villares JM, Martínez-Camarero L, Lara R, Monzón JL, Fernández-Carmona A, et al. Intensive Care to Facilitate Organ Donation: A Report on the Experience of 2 Spanish Centers With a Common Protocol. *Transplantation* (2019) 103:558–64. doi:10.1097/TP.0000000000002294
5. Mazo C, Gómez A, Sandiumenge A, Baena J, Báguena M, Nuvials FX, et al. Intensive Care to Facilitate Organ Donation: A Report on the 4-Year Experience of a Spanish Center With a Multidisciplinary Model to Promote Referrals Out of the Intensive Care Unit. *Transpl Proc* (2019) 51: 3018–26. doi:10.1016/j.transproceed.2019.08.025
6. Melville A, Kolt G, Anderson D, Mitropoulos J, Pilcher D Admission to Intensive Care for Palliative Care or Potential Organ Donation: Demographics, Circumstances, Outcomes, and Resource Use. *Crit Care Med* (2017) 45: e1050–9. doi:10.1097/CCM.0000000000002655
7. Healey A, Leeies M, Hrymak C, Chochinov A, Grunau B, Paunovic B, et al. CAEP Position Statement – Management of Devastating Brain Injuries in the Emergency Department: Enhancing Neuroprognostication and Maintaining the Opportunity for Organ and Tissue Donation. *CJEM* (2020) 22(5):658–60. doi:10.1017/cem.2020.357
8. Lesieur O, Leloup M, Gonzalez F, Mamzer M, FEPILAT Study Group. Eligibility for Organ Donation Following End-Of-Life Decisions: A Study Performed in 43 French Intensive Care Units. *Intensive Care Med* (2014) 40: 1323–31. doi:10.1007/s00134-014-3409-2
9. Witjes M, Kotsopoulos AMM, Otterspoor L, Herold IHF, Simons KS, Woittiez K, et al. The Implementation of a Multidisciplinary Approach for Potential Organ Donors in the Emergency Department. *Transplantation* (2019) 103: 2359–65. doi:10.1097/TP.0000000000002701
10. Rivers J, Manara AR, Thomas I, Derrick E. Impact of a Devastating Brain Injury Pathway on Outcomes, Resources, and Organ Donation: 3 Years' Experience in a Regional Neurosciences ICU. *Neurocrit Care* (2020) 33: 165–72. doi:10.1007/s12028-019-00879-1
11. Nelson HM, Glazier AK, Delmonico FL. Changing Patterns of Organ Donation: Brain Dead Donors Are Not Being Lost by Donation After Circulatory Death. *Transplantation* (2016) 100:446–50. doi:10.1097/TP.0000000000000954
12. Manara AR, Thomas I. Current Status of Organ Donation After Brain Death in the UK. *Anaesthesia* (2020) 75:1205–14. doi:10.1111/anae.15038
13. Opdam H. Intensive Care Solely to Facilitate Organ Donation—New Challenges. *Transplantation* (2017) 101:1746–7. doi:10.1097/TP.0000000000001748
14. de Lange DW, Soares M, Pilcher D. ICU Beds: Less Is More? No. *Intensive Care Med* (2020) 46:1597–9. doi:10.1007/s00134-020-06089-0
15. Souter MJ, Blissitt PA, Blosser S, Bonomo J, Greer D, Jichici D, et al. Recommendations for the Critical Care Management of Devastating Brain Injury: Prognostication, Psychosocial, and Ethical Management: A Position Statement for Healthcare Professionals From the Neurocritical Care Society. *Neurocrit Care* (2015) 23:4–13. doi:10.1007/s12028-015-0137-6
16. Perez A, Acevedo M, Padilla M, Perojo MD. Evolution of Patients With Devastating Brain Injury Admitted in Intensive Care Unit for Intensive Care to Facilitate Organ Donation. *Transpl Int* (2021) 34:5–404.
17. Hemphill JC, Bonovich DC, Besmertis L, Manley GT, Johnston SC. The ICH Score: A Simple, Reliable Grading Scale for Intracerebral Hemorrhage. *Stroke* (2001) 32:891–7. doi:10.1161/01.STR.32.4.891
18. Rosen DS, Macdonald RL. Subarachnoid Hemorrhage Grading Scales: A Systematic Review. *Neurocrit Care* (2005) 2:110–8. doi:10.1385/NCC:2:2:110
19. Adams HPJ, Davis PH, Leira EC, Chang KC, Bendixen BH, Clarke WR, et al. Baseline NIH Stroke Scale Score Strongly Predicts Outcome After Stroke: A Report of the Trial of Org 10172 in Acute Stroke Treatment (TOAST). *Neurology* (1999) 53:126–31. doi:10.1212/WNL.53.1.126
20. Teasdale G, Maas A, Lecky F, Manley G, Stocchetti N, Murray G. The Glasgow Coma Scale at 40 Years: Standing the Test of Time. *Lancet Neurol* (2014) 13: 844–54. doi:10.1016/S1474-4422(14)70120-6
21. Escudero D, Astola I, Balboa S, Leoz B, Meilan Á, Del Busto C, et al. Clinico-Radiological Related to Early Brain Death Factors. *Med Intensiva* (2022) 46: 1–7. doi:10.1016/j.medin.2020.06.019
22. Sekhon MS, Griesdale DE, Robba C, McGlashan N, Needham E, Walland K, et al. Optic Nerve Sheath Diameter on Computed Tomography Is Correlated With Simultaneously Measured Intracranial Pressure in Patients With Severe Traumatic Brain Injury. *Intensive Care Med* (2014) 40:1267–74. doi:10.1007/s00134-014-3392-7
23. Vaiman M, Gottlieb P, Bekerman I. Quantitative Relations Between the Eyeball, the Optic Nerve, and the Optic Canal Important for Intracranial Pressure Monitoring. *Head Face Med* (2014) 10:32. doi:10.1186/1746-160X-10-32
24. Marshall LF, Marshall SB, Klauber MR, Van Berkum Clark M, Eisenberg H, Jane JA, et al. The Diagnosis of Head Injury Requires a Classification Based on Computed Axial Tomography. *J Neurotrauma* (1992) 9(1): S287–92.
25. Humbertjean L, Mione G, Fay R, Durin L, Planel S, Lacour JC, et al. Predictive Factors of Brain Death in Severe Stroke Patients Identified by Organ Procurement and Transplant Coordination in Lorrain, France. *Transpl Int* (2016) 29:299–306. doi:10.1111/tri.12695
26. Tommasino N, Forteza D, Godino M, Mizraji R, Alvarez I. A Model to Predict Progression in Brain-Injured Patients. *Transpl Proc* (2014) 46:2950–2. doi:10.1016/j.transproceed.2014.07.002
27. Ray A, Manara AR, Mortimer AM, Thomas I. Brain Herniation on Computed Tomography Is a Poor Predictor of Whether Patients With a Devastating Brain Injury Can Be Confirmed Dead Using Neurological Criteria. *J Intensive Care Soc* (2022) 23:453–8. doi:10.1177/17511437211040019
28. Matesanz R, Domínguez-Gil B, Coll E, Mahíllo B, Marazuela R. How Spain Reached 40 Deceased Organ Donors Per Million Population. *Am J Transpl* (2017) 17:1447–54. doi:10.1111/ajt.14104
29. Cignarella A, Redley B, Bucknall T. Organ Donation Within the Intensive Care Unit: A Retrospective Audit. *Aust Crit Care* (2020) 33:167–74. doi:10.1016/j.aucc.2018.12.006
30. de Groot J, van Hoek M, Hoedemaekers C, Hoitsma A, Smeets W, Vernooij-Dassen M, et al. Decision Making on Organ Donation: The Dilemmas of Relatives of Potential Brain Dead Donors. *BMC Med Ethics* (2015) 16:64. doi:10.1186/s12910-015-0057-1
31. Soria-Oliver M, Aramayona B, López JS, Martín MJ, Martínez JM, Sáenz R, et al. Grief Reactions of Potential Organ Donors' Bereaved Relatives: An

Observational Study. *Am J Crit Care* (2020) 29:358–68. doi:10.4037/ajcc2020960

32. Kentish-Barnes N, Siminoff LA, Walker W, Urbanski M, Charpentier J, Thuong M, et al. A Narrative Review of Family Members' Experience of Organ Donation Request After Brain Death in the Critical Care Setting. *Intensive Care Med* (2019) 45:331–42. doi:10.1007/s00134-019-05575-4
33. Martin-Loeches I, Sandiumenge A, Charpentier J, Kellum JA, Gaffney AM, Procaccio F, et al. Management of Donation After Brain Death (DBD) in the ICU: The Potential Donor Is Identified, What's Next? *Intensive Care Med* (2019) 45:322–30. doi:10.1007/s00134-019-05574-5

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Impact of Asian and Black Donor and Recipient Ethnicity on the Outcomes After Deceased Donor Kidney Transplantation in the United Kingdom

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Patients of Asian and black ethnicity face disadvantage on the renal transplant waiting list in the UK, because of lack of human leucocyte antigen and blood group matched donors from an overwhelmingly white deceased donor pool. This study evaluates outcomes of renal allografts from Asian and black donors. The UK Transplant Registry was analysed for adult deceased donor kidney only transplants performed between 2001 and 2015. Asian and black ethnicity patients constituted 12.4% and 6.7% of all deceased donor recipients but only 1.6% and 1.2% of all deceased donors, respectively. Unadjusted survival analysis demonstrated significantly inferior long-term allograft outcomes associated with Asian and black donors, compared to white donors. On Cox-regression analysis, Asian donor and black recipient ethnicities were associated with poorer outcomes than white counterparts, and on ethnicity matching, compared with the white donor–white recipient baseline group and adjusting for other donor and recipient factors, 5-year graft outcomes were significantly poorer for black donor-black recipient, Asian donor-white recipient, and white donor-black recipient combinations in decreasing order of worse unadjusted 5-year graft survival. Increased deceased donation among ethnic minorities could benefit the recipient pool by increasing available organs. However, it may require a refined approach to enhance outcomes.

Keywords: kidney transplant, deceased donor, Asian, black, ethnicity

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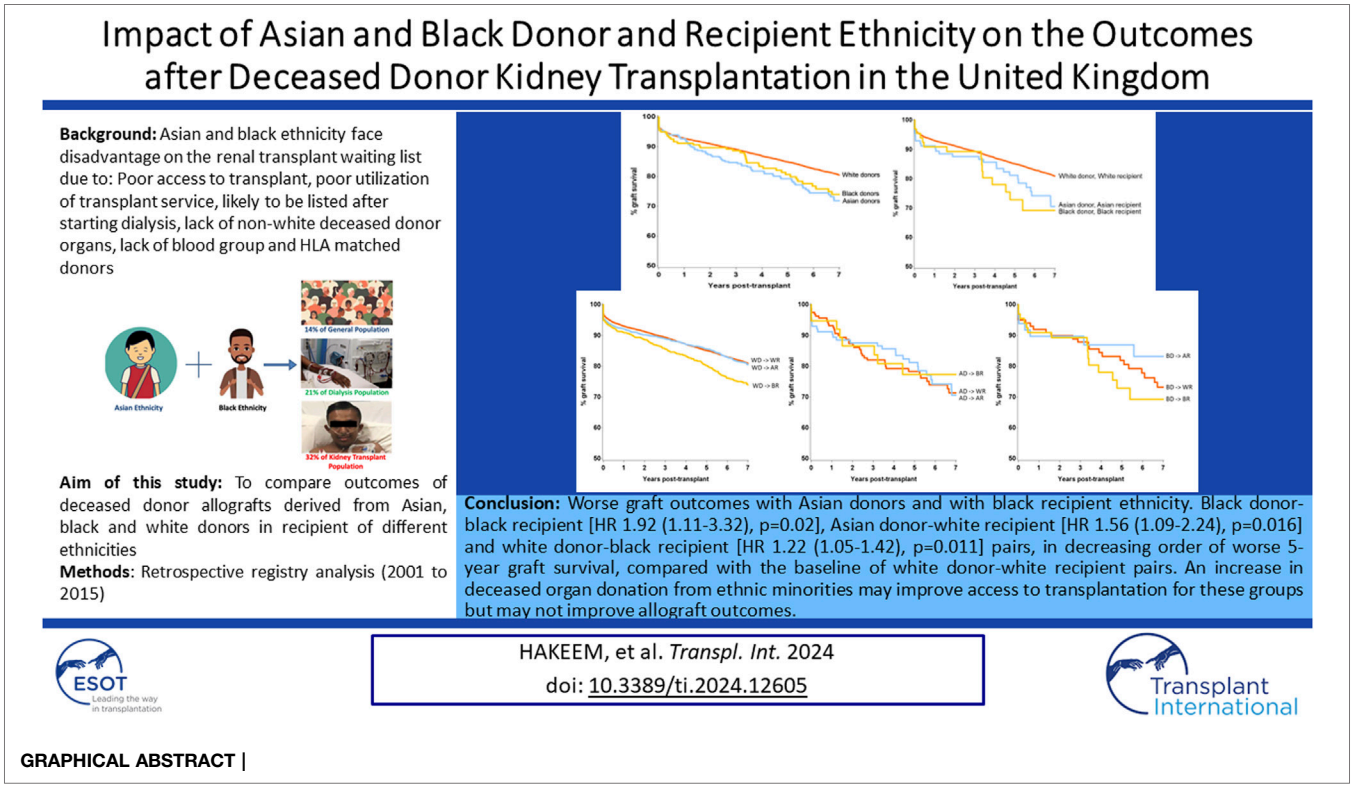
Abbreviations: ACORN, A Classification of Residential Neighbourhood; CI, Confidence Interval; CIT, Cold Ischaemia Time; CMV, Cytomegalovirus; DBD, Donation after Brain Death; DCD, Donation after Circulatory Death; ECD, Extended Criteria Donors; HLA, Human Leucocyte Antigen; HR, Hazard Ratio; MM, Mismatch; NHS, National Health Service; NHSBT, NHS Blood and Transplant; NS, Not significant; PRA, Panel-reactive antibodies; SAS, Statistical Analysis Software; SCD, Standard Criteria Donors; UK, United Kingdom; UKRR, United Kingdom Renal Registry; UKTR, United Kingdom Transplant Registry; US, United States.



GRAPHICAL ABSTRACT |

INTRODUCTION

United Kingdom (UK) residents of Asian and black ethnicity constitute 14% of the general population (based on 2011 Census estimate), but constitute 20.7% of the total dialysis population, and 32% of the patients on the renal transplant waiting list [1–3]. Poor access to and utilisation of transplant services by ethnic minority population has been well documented in the UK and elsewhere [4, 5]. There is a substantial lack of non-white deceased organ donors in the UK donor pool. Whilst organ donation from Asian and black ethnic minorities has significantly increased in the last decade, this increase is offset by the increase in the waiting list patients from these ethnicities. Currently, Asian and black ethnicity contribute 5% of all deceased donation in the United Kingdom [3]. Deceased donor kidney allocation within the UK is based on ABO-compatibility and incorporates human leukocyte antigen (HLA) matching between donor and recipient. This puts Asian and black ethnicity recipients at a disadvantage due to the relative scarcity of blood group "B" donors, as well as by challenges in optimal HLA matching with white donors [6–8], although kidney allocation changes made in 2019 sought to minimise disadvantages arising from HLA matching [9]. Significant prevalence of homozygosity of HLA alleles in these populations acts as an additional confounder [10]. In 2019/20 financial year in the United Kingdom, 10% of deceased organ donors were blood group B, compared with 19% on the renal transplant waiting list [3]. Despite efforts to improve education about transplant and organ donation among ethnic minorities, awareness remains low [11, 12].

Whilst an increase in deceased organ donation from Asian and black ethnicities is desirable to improve access by improving blood group and HLA matching for these recipients, the impact of using organs from minority donors has not been studied, in part, because of relative scarcity of such transplants. International experience with the use of non-white donors for non-white recipients, has suggested that long-term outcomes are consistently inferior to allografts obtained from white donors [13, 14]. There are multiple factors that may contribute to inferior outcome in these settings, in particular higher prevalence of hypertension, diabetes, coronary artery disease and renal disease in these populations [15, 16]. The current registry analysis was conducted to compare outcomes of deceased donor allografts derived from Asian, black and white donors in recipients of different ethnicities.

MATERIALS AND METHODS

All adult patients who had undergone first or regraft deceased donor kidney-only transplantation in the UK between 1 January 2001 and 31 December 2015 were eligible for analysis as part of this study (21,206 transplants). For the purposes of this study, "Asian" ethnicity was defined as people of Indian, Pakistani, Bangladeshi or Sri Lankan origin as recorded in the

TABLE 1 | Demographic characteristics of white, Asian and black deceased donors in the UK during 2001–2015 (*N* = 12,162).

Donor characteristic		White donors (*N* = 11,827)		Asian donors (*N* = 195)		Black donors (*N* = 140)		Overall *p*-value
		n/Mean	%/SE	n/Mean	%/SE	n/Mean	%/SE	
Donor age		47.7	0.1	45.8	1.2	40.5	1.4	**<0.001**
Donor gender	Male	6,266	53.0	112	57.4	71	50.7	0.40
	Female	5,561	47.0	83	42.6	69	49.3	
Donor height (cm)		170	0.1	165	0.9	166	1.1	**<0.001**
Blood group	O	5,558	47.0	79	40.5	72	51.4	**<0.001**
	A	4,808	40.7	42	21.5	33	23.6	
	B	1,070	9.1	60	30.8	30	21.4	
	AB	391	3.3	14	7.2	5	3.6	
Donor type	DBD	8,392	71.0	143	73.3	113	80.7	**0.032**
	DCD	3,435	29.0	52	26.7	27	19.3	
SCD/ECD donors	SCD	7,996	67.6	138	70.8	112	80.0	**0.005**
	ECD	3,831	32.4	57	29.2	28	20.0	
Cause of death	CVA	7,415	62.7	126	64.6	101	72.1	0.18
	Miscellaneous	3,056	25.8	50	25.6	32	22.9	
	Other trauma	540	4.6	9	4.6	4	2.9	
	RTA	816	6.9	10	5.1	3	2.1	
Donor creatinine >130 μmol/L	No	10,732	90.7	172	88.2	119	85.0	**0.034**
	Yes	1,095	9.3	23	11.8	21	15.0	
Donor past hypertension history[a]	No	9,060	76.6	129	66.2	100	71.4	**0.001**
	Yes	2,767	23.4	66	33.9	40	28.6	
Donor eGFR (μmol/L)		98.8	0.6	101.3	4.2	157.4	29.2	**<0.001**

CVA, cerebrovascular accident; DBD, donation after brain death; DCD, donation after circulatory death; ECD, extended criteria donors; RTA, road traffic accident; SCD, standard criteria donors.

Data presented as frequencies (percentages) or mean ± standard error.

[a]*A small number of donors with unknown past hypertension history have been assumed to have no history of hypertension.*

Bold values indicate the P *value <0.05.*

United Kingdom Transplant Registry (UKTR). "Black" ethnicity was defined as people of black, African, Caribbean and black British origin. Patients who received grafts from living donors, paediatric recipients and multiorgan recipients were excluded from the analysis. Also excluded were the transplants where either the donor or recipient ethnicity was not white, Asian or black, or where the recipient gender or HLA mismatch were unknown. There is a legal requirement for all transplant centres in the UK to report all kidney transplants undertaken to the UK Transplant Registry (UKTR) maintained by *NHS Blood and Transplant* (NHSBT), on specific donor and recipient variables in addition to graft outcomes. The study approval was provided by the NHSBT to obtain this retrospective data and no formal ethical approval was necessary.

Donor variables studied were donor ethnicity, age, gender, blood group, and cause of death. Donors were also categorised as extended or standard criteria donors (ECD or SCD). ECDs were those more than 60 years of age, or those aged 50–60 years with at least two of the following risk factors: death due to a cerebrovascular accident, history of hypertension or serum creatinine >1.5 g/dL. Both donors after brain death (DBD) and donors after circulatory death (DCD) were categorised in this way, as there was no evidence of poorer outcomes associated with DCD donors in the UK during this time period [17]. Recipient variables analysed were recipient age, gender, blood group, waiting time to transplant and ethnicity (defined as white, Asian and black ethnicities). Additional data studied included diabetic nephropathy, year of transplant, dialysis status at registration, graft number, cold ischaemia time (CIT) and HLA mismatch (MM) of the transplant (according to the four levels defined for kidney allocation in the UK): Level 1: 000 HLA-A, B, DR MM; Level 2: [0 DR+0/1 B MM]; Level 3: [0 DR+2 B MM] or [1 DR+0/1 B MM]; level 4: [2 B+1DR MM] or [2 DR MM] [18]. We did not include data on recipient panel-reactive antibodies (PRA) against HLA antigens before transplantation, as this data was not available over the study period. In addition, to study the impact of the level of deprivation and ethnicity, recipients were categorised into six different groups based on "A Classification of Residential Neighbourhood" (ACORN) geo-demographic segmentation, which gives us the demographic levels within the UK based on postcodes [19].

Statistical Analysis

Demographic and other factors were analysed for donors as well as for all recipients. White, Asian and black donor characteristics were compared using Chi-squared tests for categorical data and two-tailed t-tests for continuous variables. Data are presented as percentages, or as mean ± standard error, unless otherwise specified.

Graft survival was the primary outcome measure. Graft survival time was death-censored and defined as time from transplant to graft failure. Kaplan–Meier survival curves were used to illustrate differences in graft outcomes. Associated *p*-values were derived from the univariate log-rank test. Variables were further analysed using Cox proportional hazards regression to determine risk factors for graft failure. The interaction of donor-recipient ethnicity was tested, to assess

TABLE 2 | Demographic characteristics of recipients of kidneys from white, Asian and black donors in the UK during 2001–2015 (*N* = 20,337).

Recipient demographic		All recipients of transplants from white donors (*N* = 19,803)		All recipients of transplants from Asian donors (*N* = 317)		All recipients of transplants from black donors (*N* = 217)		Overall *p*-value
		n/ Mean	%/SE	n/ Mean	%/SE	n/ Mean	%/SE	
Recipient age		49.7	0.1	49.1	0.7	47.0	0.9	**0.010**
Recipient gender	Male	12,412	62.7	198	62.5	138	63.6	0.96
	Female	7,391	37.3	119	37.5	79	36.4	
Blood group	O	8526	43.1	112	35.3	105	48.4	**<0.001**
	A	7,941	40.1	70	22.1	47	21.7	
	B	2,362	11.9	110	34.7	53	24.4	
	AB	974	4.9	25	7.9	12	5.5	
Ethnicity	White	16,191	81.8	164	51.7	101	46.5	**<0.001**
	Asian	2,356	11.9	116	36.6	49	22.6	
	Black	1,256	6.3	37	11.7	67	30.9	
Diabetes as primary renal disease	No	18,274	92.3	292	92.1	200	92.2	0.99
	Yes	1,529	7.7	25	7.9	17	7.8	
HLA mismatch level	1	2,938	14.8	32	10.1	16	7.4	**<0.001**
	2	7,124	36.0	94	29.7	77	35.5	
	3	8,311	42.0	154	48.6	105	48.4	
	4	1,430	7.2	37	11.7	19	8.8	
Graft	First	16,822	85.0	283	89.3	191	88.0	**0.047**
	Regraft	2,981	15.0	34	10.7	26	12.0	
Median waiting time (years) and IQ range		2.2	(0.9–3.9)	2.7	(1.0–4.6)	2.8	(1.4–4.3)	**<0.001**
Median cold ischaemia time (hours) and IQ range		16.0	(13.0–19.7)	15.3	(12.6–19.0)	15.8	(12.2–21.0)	0.14
Recipient Dialysis status at registration	Haemodialysis	7,742	39.1	120	37.9	110	50.7	**0.03**
	Peritoneal dialysis	3,856	19.5	60	18.9	33	15.2	
	Not on dialysis	5,066	25.6	96	30.3	52	24.0	
	Unknown	3,139	15.9	41	12.9	22	10.1	
Recipient ACORN category	Affluent Achievers	3,658	18.5	46	14.5	33	15.2	**<0.001**
	Rising Prosperity	1,195	6.0	33	10.4	29	13.4	
	Comfortable Communities	5,202	26.3	74	23.3	48	22.1	
	Financially Stretched	5,029	25.4	63	19.9	43	19.8	
	Urban Adversity	3,793	19.2	85	26.8	56	25.8	
	Not Reported	926	4.7	16	5.1	8	3.7	

HLA, human leukocyte antigen.
Data presented as percentages or mean ± standard error unless stated otherwise.
Bold values indicate the P *value <0.05.*

the effect of different donor-recipient combinations on graft outcome. Results of the Cox regression analysis are presented as estimated hazard ratios (HRs) of groups of individuals compared with that of a baseline group. An HR of greater or less than 1.0 indicates, respectively, a higher or lower risk of failure than in the baseline group. Ninety-five percent confidence intervals (CIs) were calculated for each HR. Log cumulative hazard plots showed no evidence of non-proportionality of hazards.

A 5% level of significance was used, and all analyses were performed using the SAS software package (Version 9.1.3).

RESULTS

Of the 21,206 transplants from white, Asian or black donor or recipient ethnicity, we excluded 869 (4.1%) transplants that did not have recipient gender or HLA mismatch recorded. This gave 20,337 transplants for final analysis. A further 33 (0.2%) transplants were excluded from the Cox regression analysis due to missing data for recipient waiting time or graft survival time. The analysis cohort of 20,304 transplants from 12,162 donors thus represents 95.7% of all deceased donor kidney only transplants performed in adults in the UK over the study period. Asian (N = 195) and black (N = 140) donors constituted 1.6% and 1.2%, respectively, of the donor cohort, and the remaining 97.2% were white (N = 11,827) donors.

Comparison of white, Asian and black donors (**Table 1**) showed that black donors were significantly younger (40.5 ± 1.4 years) when compared with the white (47.7 ± 0.1 years) and Asian (45.8 ± 1.2 years) donors ($p < 0.0001$). The Asian and black donors had a significantly different blood group distribution, with higher proportions of blood group "B" (30.8% and 21.4%, respectively) and "AB" (7.2% and 3.6%, respectively) when compared with white donors (9.1% and 3.3%) ($p < 0.0001$). There were significantly more ECDs among the white donors

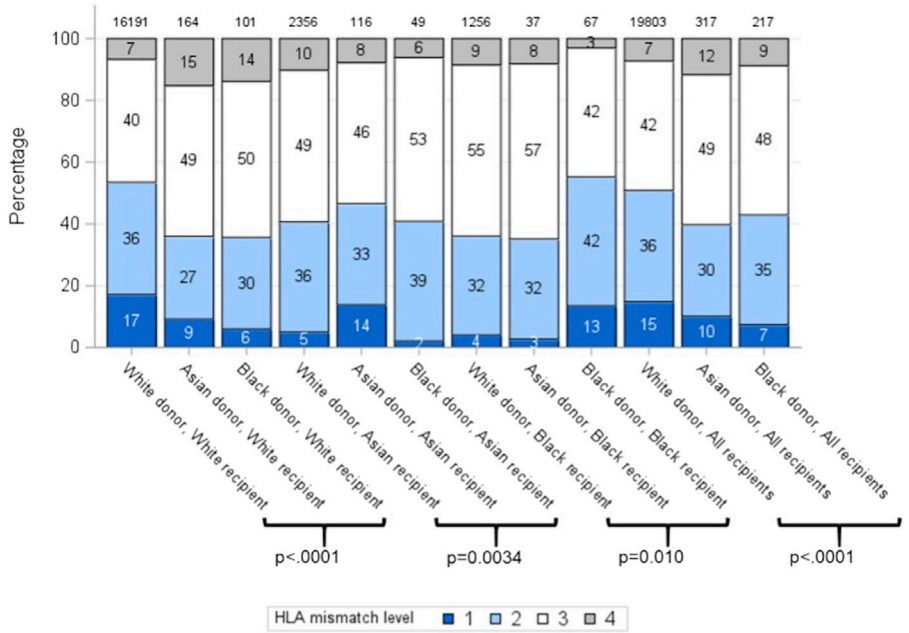


FIGURE 1 | HLA mismatch levels for white, Asian and black donor and recipient combinations (Level 1: 000 HLA-A, B, DR MM; Level 2: [0 DR+0/1 B MM]; Level 3: [0 DR+2 B MM] or [1 DR+0/1 B MM]; Level 4: [2 B+1DR MM] or [2 DR MM]).

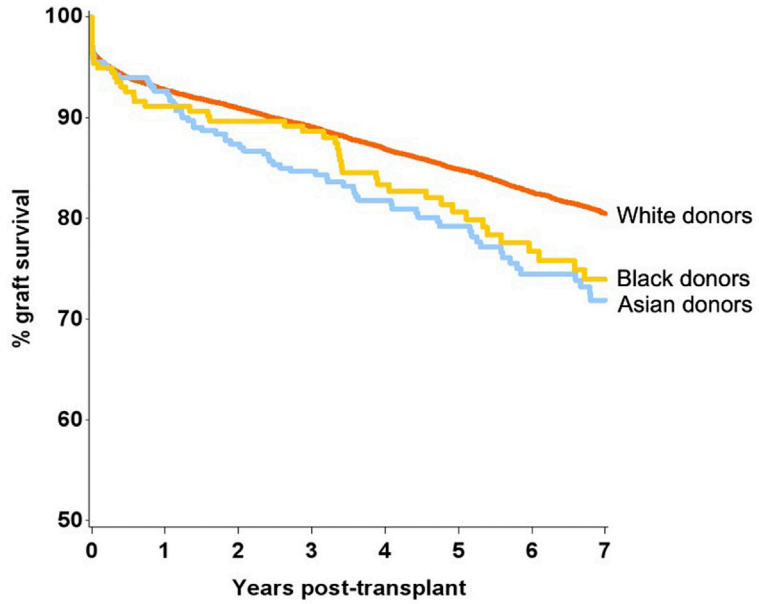
(32.4%), when compared to Asian (29.2%) and black (20.0%) donors (p = 0.0052) and also significantly more DCD donation (29.0% vs. 26.7% *vs.* 19.3%, respectively; p = 0.032). Gender distribution and the incidence of non-traumatic intracranial event as the cause of death were similar in all three groups.

Recipient demographics for the 20,337 transplants are presented according to ethnicity of the donor (**Table 2**). The recipients of kidneys from black donors (47.0 ± 0.9 years) were younger when compared to white (49.7 ± 0.1 years) or Asian (49.1 ± 0.7 years) donors (p = 0.010). There were no differences in the proportion of patients with diabetes as the primary diagnosis between the three cohorts. The median waiting time was significantly longer for the recipients who received black (2.8 years) and Asian (2.7 years) donor kidneys, when compared to white (2.2 years) donor kidneys (p < 0.0001). Unsurprisingly, kidneys from Asian and black donors were more likely to be transplanted in blood group "B" and "AB" recipients and non-white recipients. The recipients of Asian and black donor kidneys were less likely to be re-graft patients and were less well matched than recipients of white donor organs (**Table 2**).

Overall, HLA mismatch levels were superior for grafts from white donors than from Asian and black donors (p < 0.0001) (**Table 2**). 15% of all white donor kidneys were transplanted with 000 HLA-A, B, DR mismatch, compared to only 10% and 7% of Asian and black donor kidneys, respectively. Better HLA matches were achieved when the donor-recipient pair were of the same ethnicity for all three groups, with 000 HLA-A, B, DR mismatch of 17%, 14% and 13% for white, Asian and black ethnicities, respectively (**Figure 1**). The mismatch level was poorest (level 4) when white recipients received kidneys from Asian (15%) and black (14%) donors. For each recipient ethnic group, HLA match differed significantly according to donor ethnicity (p < 0.01).

Unadjusted survival analysis demonstrated significantly inferior long-term allograft outcome for Asian and black donor kidney transplants compared to white donors (7-year graft survival 71.9%, 74.0% and 80.5%; log-rank p = 0.0007, respectively) (**Figure 2**). Interestingly, further analysis revealed that survival outcomes were worse for black recipients who received grafts from black donors, as compared to kidneys from white donor or Asian donor (7-year graft survival black donor-black recipient 69.2%, compared to white donor-black recipient 74.0%, and Asian donor-black recipient 77.3%, respectively) (**Figure 3**). The graft survival rates across donor-recipient ethnicity combinations differed significantly at 3-year (p = 0.002), 5-year and 7-year follow-up (p < 0.0001), with black donor-black recipient grafts faring worse than all other donor-recipient combinations (**Figure 3**).

Multivariable analysis was performed using the Cox proportional hazards regression model (**Table 3**). Donor factors associated with 5-year graft failure were age (HR 1.02 for each additional year), male gender (HR 1.0 vs. female 0.86), donor height (HR 0.99 for every cm increase in height), donor ethnicity (HR 1.37 for Asian donors vs. white donors as baseline), type of donor (HR 1.11 for DCD donors), donor creatinine (HR 1.26 for Cr > 130 μmol/L), donor history of hypertension (HR 1.16) and CVA as cause of death (HR 1.12). Recipient factors found to significantly predict graft failure were age (HR 0.78 for each additional year over 60 years), ethnicity (HR 1.21 for black recipients *vs.* white recipient as baseline), dialysis status at transplant (HR 0.88 for peritoneal dialysis and 0.73 for not being on dialysis vs. on haemodialysis as baseline)



Donor ethnicity	% graft survival (95% confidence interval)			
	1-year	3-year	5-year	7-year
White	92·8 (92·4-93·1)	89·1 (88·6-89·5)	84·9 (84·3-85·4)	80·5 (79·9-81·1)
Asian	92·7 (89·2-95·1)	84·7 (80·1-88·3)	79·2 (74·1-83·5)	71·9 (65·7-77·2)
Black	91·1 (86·5-94·3)	88·6 (83·5-92.2)	80·7 (74·2-85·7)	74.0 (66·3-80·1)
Log-rank p-value	0·65	0·058	0·011	0·0007
Overall	92·8 (92·4-93·1)	89·0 (88·6-89·4)	84·8 (84·2-85·3)	80·3 (79·7-80·9)

Donor ethnicity	Number at risk				
	Day 0	1-year	3-year	5-year	7-year
White	19773	17653	15739	11989	8769
Asian	314	284	241	165	100
Black	217	189	155	111	69
Overall	20304	18126	16135	12265	8938

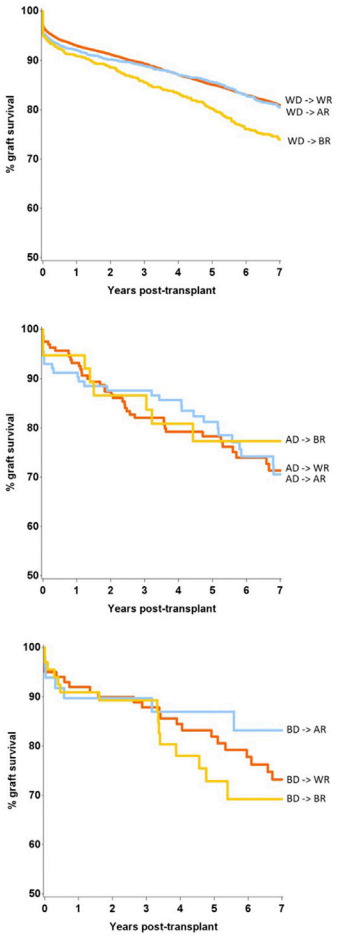
FIGURE 2 | Unadjusted graft survival by donor ethnicity (7 years).

and waiting time (HR 1.03 for each year of waiting time). Repeat graft (HR 1.37), HLA mismatch (increasing HR for higher levels of HLA mismatch), transplant year (HR 0.96) and cold ischaemia time (HR 1.01 for each minute increase) were also statistically significant (**Table 3**). The recipient ACORN categories including comfortable communities (HR 1.10), rising prosperity (1.11), financially stretched (HR 1.30) and urban adversity (1.32) showed increasing HR for graft loss, compared with affluent achievers as baseline.

Further modelling investigated the donor-recipient ethnicity interaction adjusted for all other significant factors (excluding main effects for donor and recipient ethnicity) (**Table 4**). This showed significantly poorer outcomes compared with the baseline group (white donor-white recipient) for a white donor-black recipient combination [HR 1.22 (1.05–1.42), p = 0.011], for Asian donor-white recipient combination [HR 1.56 (1.09–2.24), p = 0.016] and for black donor-black recipient combination [HR 1.92 (1.11–3.32), p = 0.02]. On comparison of graft survival for donor-recipient pairs of the same ethnicities, the white donor-white recipient pair did significantly better than the Asian-Asian and black-black donor and recipient pairs at 7-year follow-up (81.0% vs. 70.6% and 69.2%, p = 0.017). This disparity was not significant over the first 3 years post-transplant, after which time the survival curves started to diverge until the end of the study period (7 years) (**Figure 4**).

DISCUSSION

This registry analysis, conducted to examine the impact of donor-recipient ethnicity on the outcomes of deceased donor adult renal



Donor ethnicity	% graft survival (95% confidence interval)			
	1-year	3-year	5-year	7-year
White donor, White recipient	93·0 (92·6-93·4)	89·4 (88·9-89·8)	85·1 (84·5-85·7)	81·0 (80·3-81·6)
White donor, Asian recipient	92·1 (91·0-93·1)	88·9 (87·6-90·2)	85·7 (84·2-87·1)	80·5 (78·6-82·2)
White donor, Black recipient	91·0 (89·2-92·4)	85·6 (83·5-87·5)	80·2 (77·7-82·4)	74·0 (71·1-76·6)
Asian donor, White recipient	93·2 (88·0-96·2)	82·1 (75·1-87·3)	78·3 (70·8-84·1)	71·4 (62·4-78·5)
Asian donor, Asian recipient	91·2 (84·3-95·2)	87·6 (79·9-92·5)	81·2 (72·4-87·5)	70·6 (59·4-79·2)
Asian donor, Black recipient	94·7 (80·6-98·7)	86·6 (70·8-94·2)	77·3 (59·5-88·0)	77·3 (59·5-88·0)
Black donor, White recipient	92·0 (84·6-95·9)	87·8 (79·6-92·9)	81·9 (72·4-88·4)	73·2 (62·2-81·5)
Black donor, Asian recipient	89·7 (77·0-95·6)	89·7 (77·0-95·6)	87·0 (73·1-94·0)	83·2 (67·1-91·9)
Black donor, Black recipient	90·9 (80·9-95·8)	89·3 (78·8-94·8)	72·9 (57·9-83·3)	69·2 (53·1-80·8)
Log-rank p-value	0·17	0·0024	<0·0001	<0·0001
Overall	92·8 (92·4-93·1)	89·0 (88·6-89·4)	84·8 (84·2-85·3)	80·3 (79·7-80·9)

Donor ethnicity	Number at risk				
	Day 0	1-year	3-year	5-year	7-year
White donor, White recipient	16166	14473	12934	9985	7398
White donor, Asian recipient	2354	2083	1858	1333	923
White donor, Black recipient	1253	1097	947	671	448
Asian donor, White recipient	163	148	117	80	51
Asian donor, Asian recipient	114	101	94	64	33
Asian donor, Black recipient	37	35	30	21	16
Black donor, White recipient	101	90	80	62	43
Black donor, Asian recipient	49	41	34	25	15
Black donor, Black recipient	67	58	41	24	11
Overall	20304	18126	16135	12265	8938

FIGURE 3 | Effect of donor-recipient ethnicity combinations upon graft survival.

transplantation in the United Kingdom, demonstrated significantly worse graft outcomes associated with Asian donors and black recipients. When compared with white donor-white recipient combination, significantly poorer graft outcomes were observed for black donor-black recipient, Asian donor-white recipient and white donor-black recipient pairs, in decreasing order of worse 5-year graft survival. Asian and black origin patients constituted 12.4% and 6.7% of all deceased donor recipients over the study period; however only 1.6% of donors were of Asian origin and only 1.2% of donors were of black origin. Organ donation rates from Asian and black ethnicity populations have increased in recent years following sustained campaigns, yet significant disparity persists due to the increasing number of patients on the transplant waiting list from these ethnicities [3]. Black donors were significantly younger and more likely to be DBD donors and of standard criteria. The Asian and black donors had higher proportions of blood group "B" and "AB" individuals as compared to white deceased donors in the study population.

The levels of HLA mismatch for organs from Asian and black donors were significantly higher for the entire recipient pool compared to mismatch for transplants from white donors, but Asian and black recipients had more favourable HLA mismatch for organs from those ethnicities, compared to organs from white donors. Asian donor ethnicity and black recipient ethnicity were predictive of graft loss on multivariable analysis, after accounting for all identified significant factors. Further analysis suggested that black recipients of black donor organs had the poorest graft survival of all combinations (5-year and 7-year graft survival black donor-black recipient 72.9% and 69.2%, in comparison to all other pairs where the graft survival ranged from 77.3% to 87.0% and 70.6%–83.2%, respectively) (**Figure 1**).

Despite efforts to improve education about transplant and organ donation among ethnic minority groups, awareness and donation rates remain low, when compared to the white population [7, 8, 20]. Targeted community interventions have not improved deceased donation rates [12, 21, 22]. A recent study showed improved access to vulnerable population with multilevel interventions including dialysis center patient and staff education, embedding telehealth services, partnering with community providers to facilitate testing and procedures, and increased use of high-risk donors [23]. Ethnic minority patients face significant disadvantages in access to the renal transplant waitlist in the UK and may wait twice as long as white recipients for a deceased donor renal transplant [24, 25]. Barriers to

TABLE 3 | Cox regression analysis of the donor and recipient factors influencing 5-year graft survival (*N* = 20,304).

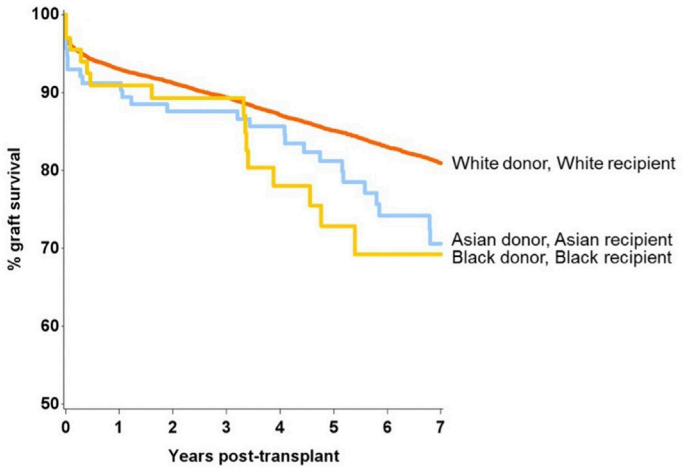
Factor	Level	*N*	HR	(95% CI)	*p*-value	Overall *p*-value for factor
Donor						
Donor age (years)		20,304	1.02	(1.02–1.02)	<0.0001	<**0.001**
Donor gender	Male	10,783	1			<**0.001**
	Female	9,521	0.86	(0.78–0.94)	0.0008	
Donor height (cm)		20,304	0.99	(0.98–0.99)	<0.0001	<**0.001**
Donor ethnicity	White	19,772	1			**0.04**
	Asian	314	1.37	(1.04–1.79)	0.023	
	Black	218	1.28	(0.91–1.80)	0.16	
Donor type	DBD	14,338	1			**0.04**
	DCD	5,966	1.11	(1.01–1.23)	0.039	
Donor creatinine >130 μmol/L	No	18,380	1			**0.001**
	Yes	1,924	1.26	(1.10–1.44)	0.0008	
Donor past hypertension history	No	15,423	1			<**0.001**
	Yes	4,881	1.16	(1.06–1.27)	0.0008	
Donor CVA as cause of death	No	7,378	1			**0.01**
	Yes	12,926	1.12	(1.02–1.22)	0.013	
Donor eGFR (10 μmol/L)		20,304	0.99	(0.98–1.00)	0.1	**0.07**
Recipient						
Recipient age (years)	18–39	4,837	1			<**0.001**
	40–59	10,141	0.72	(0.65–0.79)	<0.0001	
	60+	5,326	0.78	(0.70–0.88)	<0.0001	
Recipient ethnicity	White	16,430	1			**0.03**
	Asian	2,517	0.98	(0.87–1.11)	0.76	
	Black	1,357	1.21	(1.05–1.41)	0.0097	
Recipient ACORN category	Affluent Achievers	3,726	1			<**0.001**
	Rising Prosperity	1,264	1.11	(0.93–1.34)	0.25	
	Comfortable Communities	5,298	1.10	(0.97–1.24)	0.15	
	Financially Stretched	5,102	1.30	(1.15–1.47)	<0.0001	
	Urban Adversity	3,949	1.32	(1.16–1.50)	<0.0001	
	Other/Not Reported	965	1.03	(0.84–1.26)	0.75	
Recipient Dialysis status at registration	Haemodialysis	7,959	1			<**0.001**
	Peritoneal dialysis	3,943	0.88	(0.80–0.98)	0.019	
	Not on dialysis	5,206	0.73	(0.65–0.81)	<0.0001	
	Unknown	3,196	0.89	(0.79–1.01)	0.067	
Waiting time (years)		20,304	1.03	(1.01–1.05)	0.0008	**0.001**
Graft number	First transplant	17,269	1			<**0.001**
	Re-transplant	3,035	1.37	(1.24–1.52)	<0.0001	
HLA mismatch level	1	2,995	1			**0.01**
	2	7,320	1.11	(0.98–1.25)	0.097	
	3	8,531	1.20	(1.05–1.36)	0.0055	
	4	1,458	1.31	(1.10–1.56)	0.0029	
Transplant year		20,304	0.96	(0.95–0.98)	<0.0001	<**0.001**
Cold ischaemia time (hrs)		20,304	1.01	(1.00–1.02)	0.0012	**0.001**

ACORN, association of community organisations for reform now; CI, confidence interval; CVA, cerebrovascular accident; DBD, donation after brain death; DCD, donation after circulatory death; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; HR, hazard ratio.
Bold values indicate the P *value <0.05.*

TABLE 4 | Cox regression analysis of donor-recipient ethnicity influencing 5-year graft survival, adjusted for all factors shown in **Table 3** except donor and recipient ethnicity (*N* = 20,304).

Donor-recipient ethnicity	*N*	Number of events	HR	(95% CI)	*p*-value
White donor, White recipient	16,166	2,129	1		
White donor, Asian recipient	2,354	299	0.99	(0.874–1.12)	0.86
White donor, Black recipient	1,253	200	1.22	(1.05–1.42)	**0.011**
Asian donor, White recipient	163	30	1.56	(1.09–2.24)	**0.016**
Asian donor, Asian recipient	114	18	1.28	(0.80–2.04)	0.30
Asian donor, Black recipient	37	7	1.19	(0.56–2.50)	0.65
Black donor, White recipient	101	15	1.17	(0.70–1.95)	0.55
Black donor, Asian recipient	49	6	1.07	(0.48–2.39)	0.87
Black donor, Black recipient	67	13	1.92	(1.11–3.32)	**0.02**

Bold values indicate the P *value <0.05.*



Donor ethnicity	% graft survival (95% confidence interval)			
	1-year	3-year	5-year	7-year
White donor, White recipient	93·0 (92·6-93·4)	89·4 (88·9-89·8)	85·1 (84·5-85·7)	81·0 (80·3-81·6)
Asian donor, Asian recipient	91·2 (84·3-95·2)	87·6 (79·9-92·5)	81·2 (72·4-87·5)	70·6 (59·4-79·2)
Black donor, Black recipient	90·9 (80·9-95·8)	89·3 (78·8-94·8)	72·9 (57·9-83·3)	69·2 (53·1-80·8)
Log-rank p-value	0·5864	0·7847	0·0769	0·0174

FIGURE 4 | Graft survival from donors of same ethnicity (black-black and Asian-Asian donor and recipient combination were associated with inferior graft survival at 7 year).

transplantation include socioeconomic factors, lack of blood group "B" donors and difficulties in achieving HLA matched organs from the predominantly white donor pool [26]. Increased deceased donation among ethnic minority communities would benefit the entire recipient pool by increasing the numbers of available organs and may specifically benefit the Asian and black recipients by increasing the numbers of blood group and HLA-compatible grafts for allocation. Indeed, descriptive comparison of white and Asian donors revealed a threefold higher proportion of B blood group donors among Asian donors; organs from Asian and black donors also had a significantly better HLA mismatch among recipients of the same ethnic background.

We included only donors of white, Asian and black origin, excluding deceased donor renal grafts derived from donors of other ethnicities during the study period. These ethnicities represented 95% of all ethnic minorities on the transplant waiting list in the United Kingdom. Given the significant difference in renal risk factors between disparate populations, donor outcomes are also likely to differ significantly, particularly between Chinese and mixed populations—these transplants were excluded to remove the confounding effect of these heterogeneous groups on outcome analysis [27]. Socioeconomic status is well known to affect the outcome of patients of many different diseases, including transplant patients. Our study shows that patients in the less affluent ACORN categories do have higher graft loss compared to the affluent achievers, which is an indirect assessment of access to transplant services, compliance to immunosuppression medications and regular consultations, which all could impact on long-term graft outcomes. These differences are important public health concerns and demand further study and focused interventions in these high-risk groups as well as awareness among the transplant healthcare professionals taking care of these patients [28–30].

Kidney grafts from Asian and black donors were associated with significantly worse survival than those from white donors. Further analysis revealed that the white recipients fared better with grafts from white donors, when compared to grafts from Asian donors. Conversely, the Asian recipients had poorer outcomes from grafts of their own ethnicity, when compared to white or black donors (not statistically significant). Overall, the black recipients had the worst graft outcomes, with poorest outcomes for transplants from black donors, when compared to white or Asian donors. While the rates of early graft failure were comparable for the three ethnicity matched groups initially, the difference in outcomes becomes evident and persists from the third year onwards.

Poor outcomes for Asian and black donor-recipient combinations are likely related to a combination of donor and recipient factors. First and the foremost factor is the longer time on dialysis and longer wait for transplant. The inequity in access to transplantation in the ethnic minorities is well documented, with Access to Transplantation and Transplant Outcome Measures (ATTOM) study showing reduced access to preemptive listing for Asian and black patients and higher

likelihood of being listed after starting dialysis [31, 32] Significantly higher prevalence rates of diabetes, hypertension, coronary artery disease and death from CVA (which is one of the independent risk factors for graft loss) have been reported in these ethnic minorities [33–35]. Racial disparities in medical conditions and access to healthcare services may also exist among kidney donors [35, 36]. Ethnic minority recipients may have higher cardiac co-morbidity, or infectious complications such as cytomegalovirus (CMV) or BK virus nephropathy, but racial differences in such post-transplant events have not been well studied [36].

Sensitisation levels are usually higher in ethnic minority recipients, and failure may also be related to antibodies to HLA or unrecognised non-HLA antigens [37]. Worse outcomes have also been reported for African American DBD donor-recipient combinations as compared to white donor-African American recipient groups in US registry data [38, 39]. Minor HLA differences could play a key role in affecting long term transplant outcomes in ethnic minorities and there may be need for more comprehensive typing techniques to bring out these differences [40]. The differences in immunosuppression drug metabolism could also affect long-term outcomes, as black and mixed-race patients demonstrate very high rates of CYP3A5 expression, with a significant impact on tacrolimus pharmacokinetics and hence need for higher dosing algorithms [41].

Deceased donors from ethnic minority populations were less likely to be considered as extended criteria (29.23% of Asian and 20.0% of black donors vs. 32.39% of white donors), probably due to the younger age of death of this cohort compared to white donors. Black ethnicity increases risk of graft failure in donor-risk models and inferior graft outcomes for organs from black donors have been well documented in US-based registry data. Asian populations, like black populations, have higher rates of diabetes, hypertension and renal disease than comparable white population cohorts in the United Kingdom [42, 43]. An increased prevalence of renal diseases and co-morbidities affecting kidney function in ethnic minority populations is likely to confer added donor risk from these groups. This study supports such a hypothesis.

This study included patients who had undergone a renal transplant in the UK before 2005. Organ allocation policy for DBD donors in the UK changed in 2006, with an emphasis on equity of access, in addition to HLA matching [9]. This policy appears to have improved access to renal transplantation among ethnic minorities; however, advantages have been offset by an increase in the number of patients on the transplant waiting list [3]. Organs from DCD donors continued to be allocated according to local policy, until September 2014. In 2019 a fully integrated DBD and DCD kidney allocation scheme was introduced in the United Kingdom, simulations of which predict improvements in the equity of access to transplant across ethnic and blood groups [44].

This study has several limitations inherent to a large registry-based retrospective analysis. We lacked data on PRA, immunosuppression protocols across centres, and acute/chronic rejection outcomes, which could impact graft outcomes. Yet, center and period variation in this cohort study, along with small numbers in minority ethnicity groups, would preclude any meaningful comparison. We have adjusted for first versus regraft, so it is unlikely that inclusion of PRA data would change the outcome of this study. Ethnicity was self-reported, and this analysis offers no information on graft outcome in mixed-race recipients. Data on ethnic minority donors consisted of 2.8% of the entire study cohort, although it represents all such available data from the UK over more than a decade.

In conclusion, expanding the organ donor pool by increasing donation rates among ethnic minority groups remains a worthy goal and will improve overall access to transplantation and reduce time spent on waiting list, in particular within the ethnic minority communities. When looking at ethnicity matching between donor and recipient and compared with white-white, graft outcomes were worse for white-black, Asian-white and black-black renal transplants. Despite advantages of blood-group compatibility and improved HLA matching, black recipients of black donor grafts appear to have the poorest outcomes, and this difference cannot be explained by donor factors alone. An increase in deceased organ donation from ethnic minorities may improve access to transplantation for these groups, but may not improve allograft outcomes.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The requirement of ethical approval was waived by NHS Blood and Transplant Ethics and Data Management Committee for the studies involving humans because it used only anonymised registry data and analysis was carried out within NHSBT. Such analysis work in NHSBT falls within the General Data Protection Regulation Article 6(1)(e)—Performance of a public task. 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

NA, SA, RJ, and AH conceptualised the study design. CB and RJ obtained the registry data, performed the statistical analysis and drafted the manuscript. AH and SA prepared the final draft for submission. NA reviewed and edited the manuscript prior to submission. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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REFERENCES

1. NHSBT. *Organ Donation and Transplantation Data for Black, Asian and Minority Ethnic (BAME) Communities in the UK (April 2013 to March 2018)* (2018). Available from: https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/17883/bame-organ-donation-and-transplantation-data-2017-18.pdf (Accessed December 23, 2023).
2. Renal. *UK Renal Registry 22nd Annual Report 2018: Chapter 2: Prevalence of RRT. Adults on Renal Replacement Therapy (RRT) in the UK at the End of 2018* (2018). Available from: https://renal.org/sites/renal.org/files/22nd_UKRR_ANNUAL_REPORT_Ch2.pdf (Accessed December 23, 2023).
3. NHSBT. *NHSBT Organ Donation and Transplantation Activity Report 2019/20* (2019). Available from: https://nhsbtdbe.blob.core.windows.net/umbraco-assetscorp/19192/section-5-kidney-activity.pdf (Accessed December 23, 2023).
4. Vranic GM, Ma JZ, Keith DS. The Role of Minority Geographic Distribution in Waiting Time for Deceased Donor Kidney Transplantation. *Am J Transpl* (2014) 14:2526–34. doi:10.1111/ajt.12860
5. Rudge C, Johnson RJ, Fuggle SV, Forsythe JLR, Kidney and Pancreas Advisory Group, UK Transplant NHS BT. Renal Transplantation in the United Kingdom for Patients From Ethnic Minorities. *Transplantation* (2007) 83:1169–73. doi:10.1097/01.tp.0000259934.06233.ba
6. Johnson R, Collett D, Birch R, Fuggle S, Rudge C. Kidney Donation and Transplantation in the UK From 1998 to 2007. *Clin Transpl* (2008) 75–88.
7. Johnson RJ, Fuggle SV, O'Neill J, Start S, Bradley JA, Forsythe JLR, et al. Factors Influencing Outcome After Deceased Heart Beating Donor Kidney Transplantation in the United Kingdom: An Evidence Base for a New National Kidney Allocation Policy. *Transplantation* (2010) 89:379–86. doi:10.1097/TP.0b013e3181c90287
8. Fuggle SV, Johnson RJ, Bradley JA, Rudge CJ, Kidney Advisory Group of NHS Blood and Transplant. Impact of the 1998 UK National Allocation Scheme for Deceased Heartbeating Donor Kidneys. *Transplantation* (2010) 89:372–8. doi:10.1097/TP.0b013e3181c90270
9. NHSBT. *NHSBT Kidney Transplantation Policy: Deceased Donor Organ Allocation* (2019). Available from: https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/16915/kidney-allocation-policy-pol186.pdf (Accessed December 23, 2023).
10. Burt C, Cryer C, Fuggle S, Little AM, Dyer P. HLA-A, -B, -DR Allele Group Frequencies in 7007 Kidney Transplant List Patients in 27 UK Centres. *Int J Immunogenet* (2013) 40(3):209–15. doi:10.1111/iji.12000
11. Davis C, Randhawa G. Don't Know Enough About it!": Awareness and Attitudes Toward Organ Donation and Transplantation Among the Black Caribbean and Black African Population in Lambeth, Southwark, and Lewisham, United Kingdom. *Transplantation* (2004) 78:420–5. doi:10.1097/01.tp.0000128341.81362.0f
12. Sharif A. Prioritising Existing Donors to Receive Organs Would Boost Donation From Ethnic Minorities. *BMJ* (2013) 347:f5036. doi:10.1136/bmj.f5036
13. Callender CO, Cherikh WS, Miles PV, Hermesch A, Maddox G, Nash J, et al. Blacks as Donors for Transplantation: Suboptimal Outcomes Overcome by Transplantation Into Other Minorities. *Transpl Proc* (2008) 40:995–1000. doi:10.1016/j.transproceed.2008.03.063
14. Rao PS, Schaubel DE, Guidinger MK, Andreoni KA, Wolfe RA, Merion RM, et al. A Comprehensive Risk Quantification Score for Deceased Donor Kidneys: The Kidney Donor Risk Index. *Transplantation* (2009) 88:231–6. doi:10.1097/TP.0b013e3181ac620b
15. Pisavadia B, Arshad A, Chappelow I, Nightingale P, Anderson B, Nath J, et al. Ethnicity Matching and Outcomes After Kidney Transplantation in the United Kingdom. *PLoS one* (2018) 13(4):e0195038. doi:10.1371/journal.pone.0195038
16. Malek SK, Keys BJ, Kumar S, Milford E, Tullius SG. Racial and Ethnic Disparities in Kidney Transplantation. *Transpl Int* (2011) 24(5):419–24. doi:10.1111/j.1432-2277.2010.01205.x
17. Summers DM, Johnson RJ, Hudson A, Collett D, Watson CJ, Bradley JA. Effect of Donor Age and Cold Storage Time on Outcome in Recipients of Kidneys Donated After Circulatory Death in the UK: A Cohort Study. *Lancet* (2013) 381:727–34. doi:10.1016/S0140-6736(12)61685-7
18. Johnson RJ, Fuggle SV, Mumford L, Bradley JA, Forsythe JLR, Rudge CJ, et al. A New UK 2006 National Kidney Allocation Scheme for Deceased Heart-Beating Donor Kidneys. *Transplantation* (2010) 89:387–94. doi:10.1097/TP.0b013e3181c9029d
19. CACI. *The ACORN User Guide* (2024). Available from: https://acorn.caci.co.uk/downloads/Acorn-User-guide.pdf (Accessed December 23, 2023).
20. Jeffrey RF, Woodrow G, Mahler J, Johnson R, Newstead CG. Indo-Asian Experience of Renal Transplantation in Yorkshire: Results of a 10-Year Survey. *Transplantation* (2002) 73:1652–7. doi:10.1097/00007890-200205270-00022
21. Randhawa G. The Challenge of Kidney Transplantation Among Minority Ethnic Groups in the UK. *EDTNA ERCA J* (2004) 30:182–7. doi:10.1111/j.1755-6686.2004.tb00365.x
22. Randhawa G. Promoting Organ Donation and Transplantation Among South Asians in the United Kingdom: The Role of Social Networks in the South Asian Community. *Prog Transpl* (2005) 15:286–90. doi:10.1177/152692480501500314
23. Taber DJ, Su Z, Gebregziabher M, Mauldin PD, Morinelli TA, Mahmood AO, et al. Multilevel Intervention to Improve Racial Equity in Access to Kidney Transplant. *J Am Coll Surg* (2023) 236:721–7. doi:10.1097/XCS.0000000000000542
24. Udayaraj U, Ben-Shlomo Y, Roderick P, Casula A, Dudley C, Johnson R, et al. Social Deprivation, Ethnicity, and Access to the Deceased Donor Kidney Transplant Waiting List in England and Wales. *Transplantation* (2010) 90:279–85. doi:10.1097/TP.0b013e3181e346e3
25. Higgins RM, West N, Edmunds ME, Dukes DC, Kashi H, Jurewicz A, et al. Effect of a Strict HLA Matching Policy on Distribution of Cadaveric Kidney Transplants to Indo-Asian and White European Recipients: Regional Study. *BMJ* (1997) 315:1354–5. doi:10.1136/bmj.315.7119.1354
26. Higgins RS, Fishman JA. Disparities in Solid Organ Transplantation for Ethnic Minorities: Facts and Solutions. *Am J Transpl* (2006) 6:2556–62. doi:10.1111/j.1600-6143.2006.01514.x
27. Fan PY, Ashby VB, Fuller DS, Boulware LE, Kao A, Norman SP, et al. Access and Outcomes Among Minority Transplant Patients, 1999-2008, With a Focus on Determinants of Kidney Graft Survival. *Am J Transpl* (2010) 10:1090–107. doi:10.1111/j.1600-6143.2009.03009.x
28. Taylor DM, Bradley JA, Bradley C, Draper H, Dudley C, Fogarty D, et al. Limited Health Literacy Is Associated With Reduced Access to Kidney Transplantation. *Kidney Int* (2019) 95(5):1244–52. doi:10.1016/j.kint.2018.12.021
29. Bailey PK, Caskey FJ, MacNeill S, Tomson CRV, Dor FJMF, Ben-Shlomo Y. Mediators of Socioeconomic Inequity in Living-Donor Kidney Transplantation: Results From a UK Multicenter Case-Control Study. *Transpl Direct* (2020) 6(4):e540. doi:10.1097/TXD.0000000000000986
30. Wong K, Owen-Smith A, Caskey F, MacNeill S, Tomson CRV, Dor FJMF, et al. Investigating Ethnic Disparity in Living-Donor Kidney Transplantation in the UK: Patient-Identified Reasons for Non-Donation Among Family Members. *J Clin Med* (2020) 9(11):3751. doi:10.3390/jcm9113751
31. Pruthi R, Robb ML, Oniscu GC, Tomson C, Bradley A, Forsythe JL, et al. Inequity in Access to Transplantation in the United Kingdom. *Clin J Am Soc Nephrol* (2020) 15(6):830–42. doi:10.2215/CJN.11460919

32. Wu DA, Robb ML, Watson CJE, Forsythe JLR, Tomson CRV, Cairns J, et al. Barriers to Living Donor Kidney Transplantation in the United Kingdom: A National Observational Study. *Nephrol Dial Transplan* (2017) 32(5):890–900. doi:10.1093/ndt/gfx036
33. Wilkinson E, Brettle A, Wawar M, Randhawa G. Inequalities and Outcomes: End Stage Kidney Disease in Ethnic Minorities. *BMC Nephrol* (2019) 20(1): 234. doi:10.1186/s12882-019-1410-2
34. Chisholm-Burns MA, Spivey CA, Tsang CCS, Wang J. Racial and Ethnic Disparities Due to Medicare Part D Star Ratings Criteria Among Kidney Transplant Patients With Diabetes, Hypertension, And/or Dyslipidemia. *J Manag Care Spec Pharm* (2022) 28(6):688–99. doi:10.18553/jmcp.2022.28.6.688
35. Burton H, Perisanidou LI, Steenkamp R, Evans R, Mumford L, Evans KM, et al. Causes of Renal Allograft Failure in the UK: Trends in UK Renal Registry and National Health Service Blood and Transplant Data From 2000 to 2013. *Nephrol Dial Transpl* (2019) 34(2):355–64. doi:10.1093/ndt/gfy168
36. Williams A, Richardson C, McCready J, Anderson B, Khalil K, Tahir S, et al. Black Ethnicity Is Not a Risk Factor for Mortality or Graft Loss After Kidney Transplant in the United Kingdom. *Exp Clin Transpl* (2018) 16(6):682–9. doi:10.6002/ect.2018.0241
37. Lowe M, Payton A, Verma A, Gemmell I, Worthington J, Hamilton P, et al. Human Leukocyte Antigen Associations With Renal Function Among Ethnic Minorities in the United Kingdom. *HLA* (2020) 96(6):697–708. doi:10.1111/tan.14078
38. Tahir S, Gillott H, Jackson-Spence F, Nath J, Mytton J, Evison F, et al. Do Outcomes After Kidney Transplantation Differ for Black Patients in England Versus New York State? A Comparative, Population-Cohort Analysis. *BMJ Open* (2017) 7(5):e014069. doi:10.1136/bmjopen-2016-014069
39. Sureshkumar KK, Nashar K, Chopra B. Donor Ethnicity and Kidney Transplant Outcomes in African Americans. *Transpl Proc* (2021) 53(3): 885–8. doi:10.1016/j.transproceed.2020.06.042
40. Alelign T, Ahmed MM, Bobosha K, Tadesse Y, Howe R, Petros B. Kidney Transplantation: The Challenge of Human Leukocyte Antigen and Its Therapeutic Strategies. *J Immunol Res* (2018) 2018:5986740. doi:10.1155/2018/5986740
41. Muller WK, Dandara C, Manning K, Mhandire D, Ensor J, Barday Z, et al. CYP3A5 Polymorphisms and Their Effects on Tacrolimus Exposure in an Ethnically Diverse South African Renal Transplant Population. *S Afr Med J* (2020) 110(2):159–66. doi:10.7196/SAMJ.2020.v110i2.13969
42. Ravanan R, Udayaraj U, Ansell D, Collett D, Johnson R, O'Neill J, et al. Variation Between Centres in Access to Renal Transplantation in UK: Longitudinal Cohort Study. *BMJ* (2010) 341:c3451. doi:10.1136/bmj.c3451
43. Major RW, Shepherd D, Medcalf JF, Xu G, Gray LJ, Brunskill NJ. Comorbidities and Outcomes in South Asian Individuals With Chronic Kidney Disease: An Observational Primary Care Cohort. *Nephrol Dial Transpl* (2021) 37(1):108–14. doi:10.1093/ndt/gfaa291
44. Watson CJE, Johnson RJ, Mumford L. Overview of the Evolution of the UK Kidney Allocation Schemes. *Curr Transplant Rep* (2020) 7:140–4. doi:10.1007/s40472-020-00270-6

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LETTER TO THE EDITOR
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Belatacept in Pancreas Transplantation: Promising Insights From a Cohort Series

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Keywords: pancreas transplantation, belatacept conversion, calcineurin inhibitor toxicity, pancreas allograft function, rejection

Dear Editors,

Belatacept has proven its efficacy as maintenance therapy in kidney transplant recipients (KTR), allowing a reduction in calcineurin inhibitor (CNI) allograft injuries. Despite being of interest for pancreas transplant recipients due to the β-cell toxicity of the CNI, data on the subject are scarce and suggest a high risk of pancreas rejection when used *de novo* [1].

We report our experience with 8 pancreas transplant recipients converted to belatacept (5 mg/kg day 1, 15, 28, and then monthly) during their follow-up, because of pancreas dysfunction (i.e., hyperglycemia not requiring insulin, $n = 2$) or kidney dysfunction ($n = 6$). The median time to conversion was 31 months **Table 1**. Of note, no systematic pancreatic biopsies were performed before conversion to rule out rejection episode. Nevertheless, among the 6 patients treated because of kidney dysfunction, 4 underwent a kidney allograft biopsy before belatacept in order to assess the etiology of dysfunction and rule out rejection.

Two patients were converted to belatacept in order to preserve β-cell function (Patient 1 and Patient 2). For Patient 1, Belatacept was interrupted 3 months later due to the patient's convenience (refusal of injections). Patient 2 had a marginal β-cell function 2 years after transplantation related to the donor's characteristics, persisting despite a switch from tacrolimus to CsA and addition of oral antidiabetics (metformin + GLP agonists). At belatacept conversion, CsA was withdrawn and replaced with low dose mTOR inhibitors in addition to low dose Mycophenolate Acid (MPA, 360 mg twice daily). At 2 years' follow-up, we observed a significant improvement in fasting glycemia in addition to improvement in the kidney allograft function. HbA1c level decreased from 7.7% to 6% 2 years after conversion to belatacept, without any other medication modifications (and notably no change in his oral antidiabetic drugs).

Among the six patients converted for nephroprotection, there were 2 Pancreas Transplant Alone (PTA) and 4 Simultaneous Pancreas Kidney (SPK). Two SPK patients were on dialysis when initiating belatacept, (Patient 5 and Patient 8). Belatacept was interrupted after a few months in Patient 5 due to a poor renal prognosis and massive glomerulosclerosis and fibrosis on kidney biopsy. Causes of kidney impairment in other patients were CNI toxicity added to previous diabetic nephropathy in the PTA patients (Patient 3 and Patient 6), thrombotic microangiopathy related to CNI (Patient 4), sequelae of kidney allograft rejection (Patient 7) and kidney infarction in the

Abbreviations: SPK, simultaneous pancreas-kidney; PAK, pancreas after kidney; PTA, pancreas transplantation alone; CNI, calcineurin inhibitors; CsA, cyclosporin A; MPA, mycophenolate acid; eGFR, estimated glomerular filtration rate.

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TABLE 1 | Summary of pancreas transplant recipients who underwent belatacept conversion.

Patient	Sex	Age at transplant	Category	Indication for conversion	Immunosuppressive regiment before belatacept	Time from belatacept introduction (months)	Total duration of belatacept at last follow-up (months)	Associated immunosuppression	eGFR at conversion (mL/min)	βcell function at conversion (IgIs criterion)	Oral antidiabetics	Insulin	eGFR at 1 year (mL/min)	βcell function at 1 year (IgIs criterion)	Occurrence of rejection and/or DSA	Infectious complication
1	F	28	SPK	Pancreas	CsA + low MPA	80	3	Low CsA + low MPA	87	Good	DPP4 inh	None	NA	NA	None	None
2	M	36	SPK	Pancreas	CsA + low MPA + steroids	10	36	Low mTOR inh. + low MPA	51	Marginal/ Good	Metformin + GLP1-a	None	77	Optimal	None	None
3	F	35	PTA	Kidney	Tac + low MPA	9	20	Low Tac + low MPA	44	Optimal	None	None	55	Optimal	None	None
4	F	34	SPK	Kidney	Tac + low MPA	20	22	Low Tac + low MPA	49	Optimal	None	None	105	Optimal	None	None
5	M	47	SPK	Kidney	Tac + low MPA	150	4	Low MPA + low steroids	5	Optimal	None	None	NA	NA	None	None
6	M	53	PTA	Kidney	Tac + Steroids	48	40	Low mTOR inh. + low MPA	26	Optimal	None	None	39	Optimal	None	None
7	F	27	SPK	Kidney	Tac + Aza + Steroids	43	28	Low Tac + low MPA	24	Optimal	None	None	25	Optimal	None	None
8	F	49	SPK	Kidney	Tac + low MPA	1	30	Low Tac + low MPA	5	Good	None	None	18	Optimal	None	None

SPK, simultaneous pancreas kidney; PTA, pancreas transplant alone; CsA, cyclosporine; MPA, mycophenolic acid; Tac, tacrolimus; Aza, azathioprine; DPP4 Inh, DPP4 inhibitors; GLP1-a, GLP1-agonists.

immediate post-transplantation period (Patient 8). Associated immunosuppression was low tacrolimus (trough level between 3 and 5 ng/mL) plus low MPA (360 mg twice daily) in 4/6 patients and low everolimus (trough level between 3 and 5 ng/mL) plus low MPA in one patient. No steroids were used except for one patient who received neither CNI nor mTOR inhibitors.

Apart from Patient 5 who presented severe chronic injuries, belatacept conversion improved kidney allograft function in all patients. Notably, interruption of dialysis was allowed for Patient 8 who presented a primary non function following SPK transplantation due to ischemic complication. One year after conversion, the average improvement of estimated glomerular function (eGFR) was 20 mL/min (median = +13 mL/min), **Supplementary Figure S1**. All HbA1c levels remained excellent with optimal β cell function after conversion.

Importantly, during the complete follow-up (at least 18-month), we did not observe any suspicion of pancreas and/or kidney rejection nor appearance of donor specific antibodies (DSA). In our institution, patients are usually followed-up monthly following conversion, and pancreas rejection is suspected when unexplained significant elevation in lipasemia associated with glycemic imbalance. DSA were monitored yearly. Additionally, no serious infections were observed (notably no CMV/BKV), despite the use of low tacrolimus/mTor inhibitor in addition to belatacept.

Impairment of kidney function is not unusual in pancreas transplantation and might require CNI reduction. Even though mTOR inhibitors have been validated in a clinical trial conducted by our group, their use is associated with a wide range of side effects often leading to treatment interruption [2]. Moreover, the association of belatacept with a low dose of mTOR inhibitors, allows a significant improvement in pancreatic function and HbA1c in one patient with pancreatic dysfunction. Similar observations were made in recipients of islets transplant [3] or in diabetic KTR [4].

Importantly, no rejection episodes were observed among our patients. Even if we assume that the low number prevents any definitive conclusion, late conversion to belatacept may carry a lower risk of rejection compared to the *de novo* strategy. Moreover, the associated immunosuppression (mostly low-dose tacrolimus), probably participated in the prevention of rejection. A recent series of at-risk kidney transplant recipients converted to belatacept reported an eGFR improvement despite continuation of low-dose CNI [5]. Finally, no serious infectious complications were observed in our patients, suggesting that our strategy was quite efficient and safe.

In conclusion, our series highlights the feasibility of belatacept in pancreas transplant recipients. Whilst a larger dataset is obviously required, belatacept does allow CNI reduction (and even withdrawal), thus leading to improvement in kidney and pancreatic allograft functions. Importantly, we did not observe any pancreas/kidney rejection nor infectious complications, providing promising insights regarding its use in pancreatic and potentially islets 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.

ETHICS STATEMENT

The studies involving humans were approved by Commission nationale de l'informatique et des libertés numéro 914184. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements. 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

DC elaborated the design and research project, supervised analysis, helped in writing the manuscript and critically revised it. CM collected and analyzed the data and wrote the manuscript. All authors participated in writing and revising the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.12778/full#supplementary-material

REFERENCES

1. Stock PG, Mannon RB, Armstrong B, Watson N, Ikle D, Robien MA, et al. Challenges of Calcineurin Inhibitor Withdrawal Following Combined Pancreas and Kidney Transplantation: Results of a Prospective, Randomized Clinical Trial. *Am J Transpl* (2020) 20:1668–78. doi:10.1111/ajt.15817
2. Cantarovich D, Kervella D, Karam G, Dantal J, Blancho G, Giral M, et al. Tacrolimus-Versus Sirolimus-Based Immunosuppression After Simultaneous Pancreas and Kidney Transplantation: 5-Year Results of a Randomized Trial. *Am J Transpl* (2020) 20:1679–90. doi:10.1111/ajt.15809
3. Wisel SA, Posselt AM, Szot GL, Nunez M, Santos-Parker K, Gardner JM, et al. A Multi-Modal Approach to Islet and Pancreas Transplantation With Calcineurin-Sparing Immunosuppression Maintains Long-Term Insulin Independence in Patients With Type I Diabetes. *Transpl Int* (2023) 36:11367. doi:10.3389/ti.2023.11367
4. Terrec F, Jouve T, Naciri-Bennani H, Benhamou PY, Malvezzi P, Janbon B, et al. Late Conversion From Calcineurin Inhibitors to Belatacept in Kidney-Transplant Recipients Has a Significant Beneficial Impact on Glycemic Parameters. *Transpl Direct* (2020) 6:e517. doi:10.1097/TXD.0000000000000964
5. 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:e0240335. doi:10.1371/journal.pone.0240335

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LETTER TO THE EDITOR
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Belatacept Rescue Therapy in the Early Period After Simultaneous Kidney-Pancreas Transplantation

Laure Esposito[1], Emmanuel Cuellar[2], Olivier Marion[1,3], Arnaud Del Bello[1], Anne Laure Hebral[1], Federico Sallusto[4], Fabrice Muscari[2,5], Thomas Prudhomme[4,5] and Nassim Kamar[1,3,5]†*

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Keywords: belatacept, kidney transplantation, pancreas transplantation, gastroparesis, delayed graft function

Dear Editors,

Belatacept has been used as a rescue therapy in kidney-transplant patients and in a heart-liver-kidney transplant patient with prolonged delayed graft function (DGF) [1, 2]. Although, encouraging results were observed in kidney-transplant patients with preexisting diabetes [3], very few simultaneous kidney-pancreas-transplant (SKPT) patients were given belatacept [4, 5]. Gastroparesis, a common complication in diabetic patients, is a syndrome defined by symptoms and delayed gastric emptying in the absence of mechanical obstruction [6]. Typical symptoms include nausea, vomiting, abdominal pain, and early satiety [6]. Gastroparesis can be responsible at impaired drug absorption, including immunosuppressants [6].

Herein, we report the use of belatacept as rescue therapy in four SKPT because of severe gastroparesis responsible for large tacrolimus trough levels variability (n = 3) and/or prolonged delayed graft function (n = 2) (**Table 1**).

At transplantation, all patients had been given polyclonal antibodies (Thymoglobulins®, Sanofi; 3.75 mg/kg total dose), tacrolimus (Prograf®, Astellas Pharma) and mycophenolic acid. Steroids were scheduled to be stopped within the first 10 days after transplantation. Only one patient was maintained on prednisone (5 mg/d) for 3 months.

Since in the BENEFIT phase III trials, an increased risk of acute rejection was observed in belatacept-treated patients compared to those given cyclosporine A-based therapy [7, 8], when belatacept was initiated in our patients, it was given with low-dose tacrolimus, and MPA (500 mg b.i.d that remained unchanged). Belatacept was administrated at the dose of 6 mg/kg at days 0 and 15 and then every 4 weeks. All patients were Epstein Barr Virus IgG positive.

Patient 1 started belatacept at day 15 post-transplantation because of severe gastroparesis, vomiting, tacrolimus malabsorption and large variations of tacrolimus trough levels (Tac C0) that ranged between 3.8 and 52 ng/mL (median = 12) using tacrolimus at 7–10 mg/d. At belatacept initiation, it was at 12.8 ng/mL. When associated to belatacept, Tac C0 was maintained between 4 and 5 ng/mL. Belatacept was stopped 1.5 months later (after 3 doses) when gastrointestinal symptoms had disappeared. After belatacept stop, Tac C0 ranged between 7 and 8 ng/mL using tacrolimus 6 mg/d. Serum creatinine level decreased from 174 µmol/L at belatacept initiation to 116 µmol/L when it was stopped.

Patient 2 had gastroparesis symptoms and prolonged DGF. Belatacept was started at day 23 while she had still had gastrointestinal symptoms and was still requiring dialysis. A kidney allograft biopsy revealed the presence of isolated acute tubular necrosis (ATN). Tac C0 ranged between 4.4 and 12 ng/mL while Tac dose was unchanged (12 mg/d), and was at 11 ng/mL at belatacept initiation. When associated to belatacept, Tac C0 was maintained between 4 and 5 ng/mL. After the initiation of

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TABLE 1 | Patients' characteristics and outcome.

	Age at transplantation (years)	Gender	Anti-HLA antibodies/ Preformed donor specific antibodies	Time between transplantation and belatacept initiations (days)	Serum creatinine level at the initiation of belatacept (μmol/L)	Duration of belatacept (months)	Serum creatinine level at belatacept stop (μmol/L)	Time between transplantation and last follow-up (months)	Serum creatinine level at last-follow-up (μmol/L)
Patient 1	38	Male	No/No	15	174	1.5	116	110	96
Patient 2	55	Female	Yes/No	23	Dialysis	Ongoing	-	3	150
Patient 3	40	Female	No/No	81	Dialysis	1.5	123	8	100
Patient 4	32	Male	Yes/No	170	269	Ongoing	122	7	122

belatacept, gastrointestinal symptoms improved and kidney function recovered. At last follow-up, i.e. 3 months after transplantation, she is still given belatacept-based therapy and her serum creatinine level is at 150 μmol/L.

Patient 3 experienced several complications after transplantation, namely, infections of peripancreatic fluid collections requiring antibiotics and antifungal therapies. She had a prolonged DGF. At day 81, she was still requiring hemodialysis. A kidney allograft biopsy showed isolated severe ATN. Hence, belatacept was started. Tac C0 was at 8 ng/mL. When associated to belatacept, Tac C0 was at 4 ng/mL. Kidney function recovered rapidly and belatacept was stopped after 3 administrations, i.e. 1.5 months after its initiation. After belatacept stop, Tac C0 ranged between 7 and 8 ng/mL. Serum creatinine level had decreased to 123 μmol/L.

Finally, patient 4 presented several episodes of gastrointestinal symptoms attributed to gastroparesis after transplantation. This was associated each time with an impairment of kidney function. At 5 months post-transplantation he was admitted for a severe gastroparesis episodes associated with large Tac C0 variations and acute kidney injury. A kidney allograft biopsy revealed the presence of isolated ATN belatacept was initiated and is still pursued until last follow-up, i.e.,7 months after transplantation. Tac C0 ranged between 5.6 and 18 ng/mL while tacrolimus dose was unchanged (8 mg/d), and was at 11 ng/mL at belatacept initiation. When associated to belatacept, Tac C0 was maintained at 5 ng/mL. Serum creatinine level decreased from 269 at the initiation of belatacept to 122 at last follow-up.

At the initiation of belatacept, only one patient (patient 2) who was receiving parenteral nutrition was still given insulin while all other three patients were insulin-free. At last follow-up, none of the patient was given insulin and c-peptide level was at 3.75 (3.3–4.7) ng/mL. No acute rejection, *de novo* DSA or infection occurred after the initiation of belatacept. BK virus DNAemia was negative in all patients In the 2 patients in whom belatacept was stopped and tacrolimus doses re-increased, no episode of gastrointestinal symptoms occurred after belatacept stop. In the two other patients, it was decided to pursue belatacept and to stop tacrolimus at one-year posttransplant.

In a phase II prospective study, *de novo* SKPT patients were randomized to receive a tacrolimus based immunosuppressive regimen or belatacept and low-dose tacrolimus [5]. At Week 40, in the absence of an history of acute rejection and in patients having stable grafts' functions, tacrolimus was withdrawn. The biopsy proven acute rejection rates of the pancreas and the kidney were low and similar in both arms before tacrolimus withdrawal in the belatacept arm [5]. However, an increased risk of pancreas rejection was observed during and after tacrolimus withdrawal [5]. The authors concluded that belatacept did not provide sufficient immunosuppression to reliably prevent pancreas rejection in SKPT patients undergoing calcineurin inhibitors withdrawal. Conversely, late conversion to belatacept in SKPT patients was found to be safe [4]. In our report, since the initiation of belatacept was done within the first months after transplantation, we have chosen to maintain a low-dose of tacrolimus in addition to belatacept. This strategy was safe.

Our short case series suggests that in selected SKPT patients with severe gastroparesis responsible for immunosuppressants malabsorption and/or in those presenting a prolonged DGF, a transient or prolonged course of belatacept associated with low-dose tacrolimus can be considered. Further studies including a larger number of patients are required to confirm theses preliminary data.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical approval was not required for the studies involving humans because this a retrospective study. According to the Loi Jarde in France, ethical approval is not required. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements because this a retrospective study.

AUTHOR CONTRIBUTIONS

LE designed the study, did the patients' follow-up and reviewed the paper. EC and FM did the pancreas transplantations; FS and TP did the kidney transplantations; OM, AD, and AH

participated to the patients' follow-up and reviewed the paper; NK designed the study and wrote the paper. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

LE has received speakers' fees and participated to advisory boards for Astellas, Chiesi, Sanofi, Sandoz, Takeda. NK has received speakers' fees and participated to advisory boards for Astellas, AstraZeneca, Biotest, BMS, CSL Behring, Chiesi, ExeViR, Gilead, Hansa, MSD, Glasgow Smith Kline, Neovii, Novartis Pharma, Roche, Sanofi, Sandoz, Takeda.

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

1. Wojciechowski D, Chandran S, Vincenti F. Early Post-Transplant Conversion From Tacrolimus to Belatacept for Prolonged Delayed Graft Function Improves Renal Function in Kidney Transplant Recipients. *Clin Transpl* (2017) 31: e12930. doi:10.1111/ctr.12930
2. Kumar D, Yakubu I, Cooke RH, Halloran PF, Gupta G. Belatacept Rescue for Delayed Kidney Allograft Function in a Patient With Previous Combined Heart-Liver Transplant. *Am J Transpl* (2018) 18:2613–4. doi:10.1111/ajt.15003
3. Rostaing L, Massari P, Garcia VD, Mancilla-Urrea E, Nainan G, del Carmen Rial M, et al. Switching From Calcineurin Inhibitor-Based Regimens to a Belatacept-Based Regimen in Renal Transplant Recipients: A Randomized Phase II Study. *Clin J Am Soc Nephrol* (2011) 6:430–9. doi:10.2215/CJN.05840710
4. Mujtaba MA, Sharfuddin AA, Taber T, Chen J, Phillips CL, Goble M, et al. Conversion From Tacrolimus to Belatacept to Prevent the Progression of Chronic Kidney Disease in Pancreas Transplantation: Case Report of Two Patients. *Am J Transpl* (2014) 14:2657–61. doi:10.1111/ajt.12863
5. Stock PG, Mannon RB, Armstrong B, Watson N, Ikle D, Robien MA, et al. Challenges of Calcineurin Inhibitor Withdrawal Following Combined Pancreas and Kidney Transplantation: Results of a Prospective, Randomized Clinical Trial. *Am J Transpl* (2020) 20:1668–78. doi:10.1111/ajt.15817
6. Ma J, Rayner CK, Jones KL, Horowitz M. Diabetic Gastroparesis: Diagnosis and Management. *Drugs* (2009) 69:971–86. doi:10.2165/00003495-200969080-00003
7. Vincenti F, Charpentier B, Vanrenterghem Y, Rostaing L, Bresnahan B, Darji P, et al. A Phase III Study of Belatacept-Based Immunosuppression Regimens Versus Cyclosporine in Renal Transplant Recipients (BENEFIT Study). *Am J Transpl* (2010) 10:535–46. doi:10.1111/j.1600-6143.2009.03005.x
8. Durrbach A, Pestana JM, Pearson T, Vincenti F, Garcia VD, Campistol J, et al. A Phase III Study of Belatacept Versus Cyclosporine in Kidney Transplants From Extended Criteria Donors (BENEFIT-EXT Study). *Am J Transpl* (2010) 10:547–57. doi:10.1111/j.1600-6143.2010.03016.x

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