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TRANSPLANT TRIAL WATCH
published: 28 November 2024
doi: 10.3389/ti.2024.14062

Transplant Trial Watch

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

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Keywords: randomised controlled trial, systematic review/meta-analysis, kidney transplantation (KT), heart transplantation, hypothermic oxygenated machine perfusion

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.

SYSTEMATIC REVIEW

Antihypertensive Treatment for Kidney Transplant Recipients.

by Natale, P., et al. Cochrane Database of Systematic Reviews 2024; 7: CD003598.

Aims

This study aimed to compare the outcomes associated with different classes and combinations of antihypertensive drugs in renal transplant patients.

Interventions

A literature search was conducted using the Cochrane Kidney and Transplant Register of Studies. Study selection and data extraction were performed by two independent reviewers. The risk of bias was assessed using the Cochrane risk of bias tool.

Participants

97 studies were included in the review.

Outcomes

The primary outcomes were death (all-causes), death-censored graft loss and kidney function. The secondary outcomes were cardiovascular death and other cardiovascular events, blood pressure, acute rejection, proteinuria, haemoglobin (Hb) and/or hematocrit (HCT), serum potassium and/or hyperkalaemia, infection, cancer, life participation, dementia, falls, fatigue, hypoglycemia and other adverse effects.

Follow-Up

N/A.

CET Conclusion

by Reshma Rana Magar

This systematic review looks at the benefits and harms associated with antihypertensive drugs in kidney transplant recipients. This is an updated version of the 2009 Cochrane review. A total of

Transplant Trial Watch

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97 studies were included, all of which were randomised controlled trials, apart from one which had a quasi-randomised design. Study selection, data extraction and quality assessment were performed independently by two reviewers. The GRADE approach was used to rate the certainty of evidence. The study found that, compared to standard care alone or placebo, calcium channel blockers (CCB) treatment significantly reduced all-cause death and graft loss, while angiotensin receptor blockers (ARB) was found to reduce graft loss. However, the certainty of evidence was moderate for CCB and low for ARB. Overall, the methodological quality of this paper is good and provides a granular analysis of the available data. Where heterogeneity was observed, attempts were made to explore it using subgroup analysis and meta-regression. However, for some of the outcomes, the number of studies included in the analyses were to low (1 or 2). Data were not analysed separately for living versus deceased donor transplants.

Trial Registration
N/A.

Funding Source
No funding received.

RANDOMISED CONTROLLED TRIAL 2

A Randomized Phase 2 Trial of Felzartamab in Antibody-Mediated Rejection.

by Mayer, K. A., et al. New England Journal of Medicine 2024 [record in progress].

Aims
This study aimed to compare short-term outcomes of continuous, hypothermic oxygenated machine perfusion (HOPE) versus static cold storage (SCS).

Interventions
Donor hearts were randomised to either preservation with HOPE or SCS.

Participants
229 adults (aged ≥18 years) in the waitlist for heart transplantation. Donors criteria were adults (age ≥18 to ≤70 years) accepted as a heart donor by the transplantation team.

Outcomes
The primary endpoint was the time to first event of a composite measure (including graft failure, cardiac-related death, cellular rejection of at least grade 2R, moderate or severe primary graft dysfunction (PGD) of the left ventricle or PGD of the right ventricle). Secondary endpoints were the composite primary endpoint, duration of stay at the intensive care unit, cardiac injury markers, echocardiography data, incidence and duration of any postoperative mechanical circulatory support, incidence of major adverse cardiac transplant events (MACTE), and overall success or failure.

Follow-Up
30 days post-transplantation.

CET Conclusion
by Simon Knight

This multicentre RCT investigates the role of hypothermic oxygenated machine preservation (HOPE) of the DBD heart prior to transplantation. 229 patients were randomised to HOPE or static cold storage. 100 donor hearts underwent HOPE, and all were transplanted. The primary endpoint of cardiac-related death, graft dysfunction, rejection or graft failure was numerically lower in the study group (HR 0.56), but did not quite reach statistical significance (p = 0.059). Most of the difference in the primary endpoint appears to be driven by a significant reduction in risk of primary graft dysfunction with use of HOPE. The study is well-designed and well conducted, with an inclusive donor and recipient population reflective of clinical practice. Allocation concealment is good with centralised randomisation and stratification, and ITT analysis is used. Use of a complex primary endpoint with components of different severity is questionable, and outcomes were only measured for 30-day post-transplant. Whilst the primary endpoint is not quite met, the study provides compelling evidence that use of HOPE is safe in the short-term and can reduce the risk of primary graft dysfunction following DBD cardiac transplantation.

Jadad Score
3.

Data Analysis
Strict intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
ClinicalTrials.gov - NCT03991923.

Funding Source
Industry funded.

CLINICAL IMPACT SUMMARY

by John O'Callaghan

This is a large and well-conducted RCT in heart transplantation comparing Hypothermic Oxygenated machine Perfusion (HOPE) to static cold storage. It was conducted across

multiple transplant centres in 8 European countries. The methods of randomisation, data analysis and full follow up make the results reliable. The study builds on work in pre-clinical and clinical feasibility studies in heart transplantation using HOPE, as well as a now-considerable evidence base in the preservation of other organs.

The device used to deliver HOPE in this study was the XVIVO Heart Assist Transport (XVIVO Group, Gothenburg, Sweden) and primed with the cardioplegic solution from the same company, XVIVO Heart Solution, with additional recipient matched blood or erythrocytes, antibiotics, and insulin. This is a portable and automated device, taken to the donor centre so that the heart could be placed inside as soon as possible after retrieval. Of the 100 donor hearts preserved using the HOPE device, 3 were not transplanted, and this was for reasons unrelated to the device or preservation.

The primary outcome was a composite of cardiac-related death, specific grades of Primary Graft Dysfunction (PGD) or cellular rejection and early graft failure. There was a substantial reduction in this primary outcome associated with HOPE preservation (19% versus 30%, HR = 0.56) but with the statistical analysis plan this did not reach statistical significance in this study size (p = 0.59). The sample size was predicated on a 60% reduction in the primary endpoint, which may have been selected to achieve a reasonable study size and considering prior work. However, a reduction of 44% in the primary outcome, as seen here, would certainly be clinically significant. Also, there was a significant reduction in PGD (11% versus 28%) and severe PGD (5% versus 20%) when looked at alone. This is despite an overall longer median preservation time of hearts in the HOPE group (240 min versus 215 min).

This study clearly supports the use of HOPE for DBD cardiac allograft preservation compared to static cold storage. Further work should now be done to see if there is benefit in using HOPE to expand the potential donor pool, and if there is a role in DCD heart preservation.

Clinical Impact Rating

5/5.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

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

GENERATIVE AI STATEMENT

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

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NEWS AND VIEWS
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Shedding Light on Microvascular Inflammation: Understanding Outcomes, But What Sparks the Flame?

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Keywords: kidney transplantation, antibody-mediated rejection, biomarkers, microvascular inflammation, targeted therapies

A Commentary on

Microvascular Inflammation of Kidney Allografts and Clinical Outcomes
by Sablik M, Sannier A, Raynaud M, Goutaudier V, Divard G, Astor BC, Weng P, Smith J, Garro R, Warady BA, Zahr RS, Twombley K, Dharnidharka VR, Dandamudi RS, Fila M, Huang E, Sellier-Leclerc A-L, Tönshoff B, Rabant M, Verine J, del Bello A, Berney T, Boyer O, Catar RA, Danger R, Giral M, Yoo D, Girardin FR, Alsadi A, Gourraud P-A, Morelon E, Le Quintrec M, Try M, Villard J, Zhong W, Bestard O, Budde K, Chauveau B, Couzi L, Brouard S, Hogan J, Legendre C, Anglicheau D, Aubert O, Kamar N, Lefaucheur C and Loupy A (2024). N Engl J Med. doi: 10.1056/NEJMoa2408835

In their recent article, Drs. Sablik and Sannier, along with more than 40 international collaborators, examined the impact of different microvascular inflammation (MVI) phenotypes on allograft outcomes by analyzing a total of 16,293 allograft biopsies from 6,798 patients across over 30 transplant centers in Europe and North America [1]. Clinical and pathological data was used to reclassify biopsy specimens according to the 2022 BANFF Classification of Renal Allograft Pathology now including the two new diagnostic categories of probable antibody-mediated rejection (ABMR) and MVI without evidence of an antibody-mediated response [2]. The newly identified phenotypes were present in 788 specimens, of which 641 were previously categorized as no rejection by the BANFF 2019 classification [3].

In terms of graft loss, patients with *ABMR* and those with the newly considered histopathological phenotype, *MVI without antibody-mediated response* (DSA-/C4d-) showed an increased risk of 2.7 (95% 2.2–3.3) and 2.1 (95% CI 1.5–3.1), respectively, when compared to non-rejection cases, whereas patients with the diagnosis of *probable ABMR* did not show an increased risk through the following 5 years after biopsy (Hazard Ratio [HR] of 1.3; 95% CI 0.8–2.1). In terms of progression to ABMR, patients with DSA-/C4d- MVI and those with probable ABMR showed a comparable risk of progression, with an intermediate cumulative incidence of ABMR during follow-up, positioned between patients without MVI and those with active ABMR (subdistribution HRs of 0.4 [95% CI, 0.3–0.5] and 0.7 [95% CI, 0.4–1.2], respectively). Finally, when analyzing the risk of progression to transplant glomerulopathy, the DSA-/C4d- MVI group showed, once more, a similar risk to that in the probable ABMR group, again falling in between the risks seen in those without MVI and those with active ABMR.

In short, this extensive population-based study, utilizing a remarkable dataset of allograft biopsies, compellingly demonstrates the importance of recognizing MVI as distinct histopathological

News & Views

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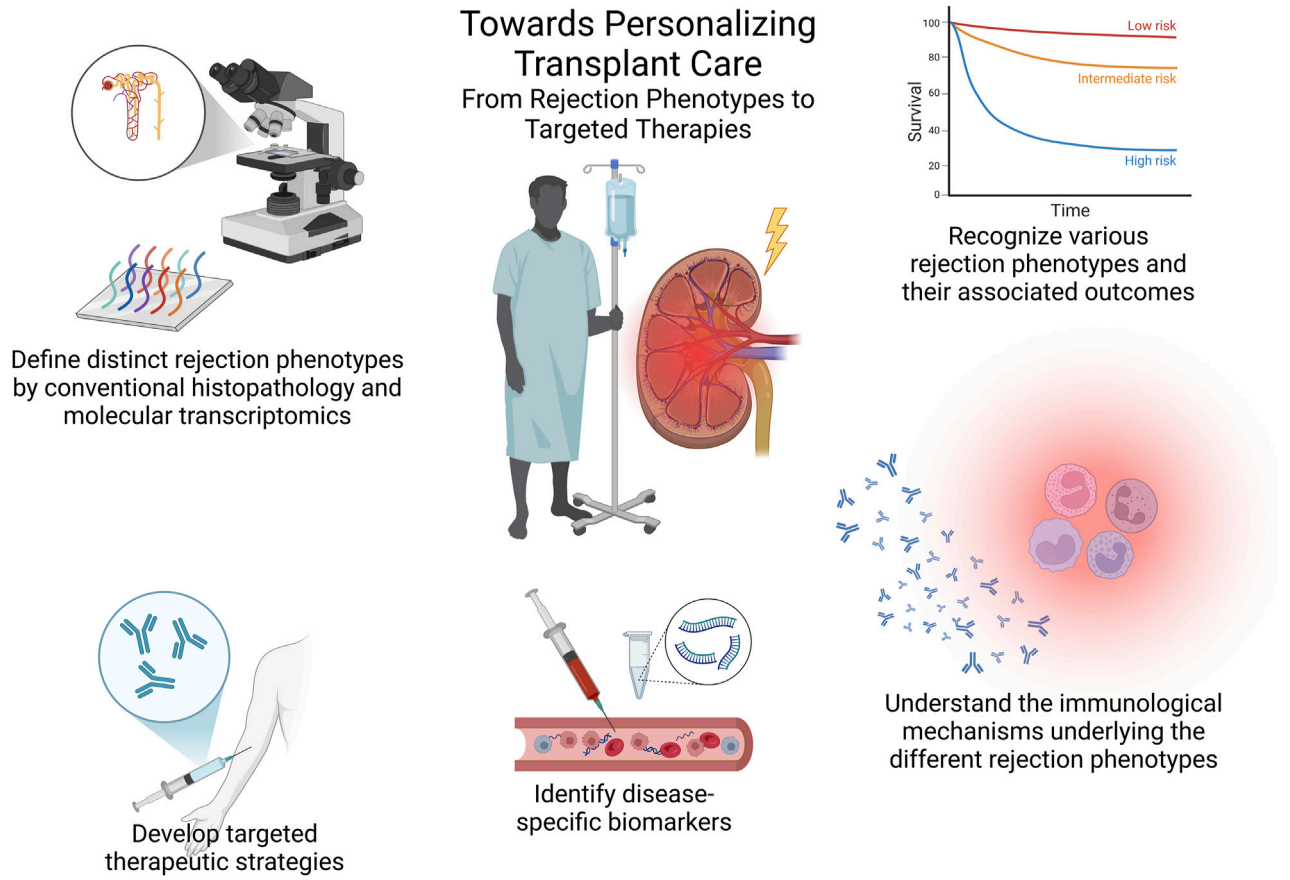

FIGURE 1 | Towards personalizing transplant care – from rejection phenotypes to targeted therapies. This summary figure illustrates the essential steps toward personalized transplant care, including the classification of rejection phenotypes through conventional histopathology and molecular tools, understanding associated outcomes, exploring underlying immunological mechanisms, identifying disease-specific biomarkers, and ultimately developing targeted therapies based on these insights.

phenotypes that associate with different disease progression and allograft failure. Notably, patients with MVI fulfilling the complete ABMR diagnosis display worse graft outcome, aligning with prior studies suggesting that patients with MVI and incomplete humoral phenotypes display better outcomes than those with full ABMR but worse than patients without rejection [4–6]. Crucially, the study emphasizes the necessity for broader acknowledgment of the MVI phenotypes in clinical practice, which have frequently been overlooked until recently. The discussed findings highlight the advantages of the 2022 BANFF classification in capturing the clinical, histological, and prognostic diversity of MVI over the previous version, thus establishing a foundation for standardizing future trials aimed at elucidating the immunological mechanisms behind these distinct phenotypes and potentially guiding tailored therapeutic strategies. Interestingly, the authors further propose that their findings may extend to other solid-organ transplants, where MVI is also a key diagnostic feature of ABMR, indicating possible similarities in pathophysiological processes that merit further study.

Authors are to be commended for their collaborative effort in assembling this substantial dataset to investigate the newly defined BANFF phenotypes in relation to the advent of distinct allograft outcomes. Nonetheless, the precise pathophysiological mechanisms underlying the development of these newly considered histopathological phenotypes, and especially MVI without evidence of an antibody-response (DSA-/C4d-) still remain elusive, leaving the question of what truly sets the spark for MVI. This is of paramount importance as the identification of main effector mechanisms orchestrating such specific graft injuries would allow to consistently design guided therapeutic strategies within interventional clinical trials. Indeed, a clear example underscoring such endeavor was delineated already in 2001, when the diagnostic feature of ABMR was first incorporated into the Banff classification by including the basic histopathological lesions of MVI and key immunological parameters such as serum DSA or C4d deposition [7], with an expansion of the histopathological ABMR criteria later in 2013 to include endarteritis, when concomitantly found in presence of serum DSA [8]. While the causality link between the two features

may not strictly be confirmed, the strong associations described between such specific allograft lesions and the presence of DSA, the downstream effector mechanism of an anti-donor B-cell alloimmune response, has provided the solidest basis for this histological diagnosis. Notably, advances in molecular transcriptomics have helped to further refine distinct histopathological features, especially T-cell mediated rejection (TCMR) and ABMR, thus ultimately allowing reclassification of allograft lesions not fully captured with the conventional light microscope [9–13]. Nevertheless, while some recent works have shown overlapping transcriptional signatures between ABMR and DSA-/C4d- MVI, suggesting a common ethiopathological origin [14, 15], it may be argued that such common gene perturbation merely illustrates the similar cellular infiltrate composition, rather than the mechanisms driving its development. Notably, growing evidence suggests that DSA-/C4d- MVI may be more closely linked to an innate immune response, with natural killer (NK) cell–driven allorecognition potentially playing a key role in allograft injury [16–22]. Yet, the precise role of NK cells in MVI remains unclear [16], as they constitute only a small portion of the inflammatory infiltrate in MVI, which seems otherwise largely dominated by macrophages and T-cells [22–24]. Indeed, recent multi-omic profiling has shown a notable T-cell presence and activity, suggesting a T-cell effector dominant phenotype [25]. It is also plausible that other innate immune effector mechanisms, including myeloid-and monocyte-driven allorecognition could lead to similar histological/molecular pictures [26]. Importantly, it is highly likely that these diverse alloimmune effector mechanisms are not mutually exclusive but may, in fact, rather interconnect in complex ways [16].

Additionally, current clinical trials are exploring various blood- and urine-biomarkers indicative of graft injury, frequently caused by rejection, with donor-derived cell-free DNA (dd-cfDNA) emerging as particularly promising for differentiating microvascular injury in ABMR [27–38]. Consequently, dd-cfDNA has already been cleverly implemented into recent trials targeting ABMR. For instance, treatment with the anti-IL6 monoclonal antibody clazakizumab did not result in significant changes in dd-cfDNA levels, indicating ongoing allograft injury [39], whereas, only recently, treatment with anti-CD38 monoclonal antibody felzartamab demonstrated notable changes in dd-cfDNA, suggesting a beneficial therapeutic effect with the apparent resolution of injury [40]. Notably, while biomarkers like dd-cfDNA signal graft injury, they provide only limited insights into the underlying mechanisms driving graft damage [41]. Ideally, biomarkers would also reflect lesion pathophysiology or track alloimmune responses, allowing a more comprehensive understanding of the graft injury process. Thus, further research is needed to explore how biomarkers can aid in differentiating the various rejection phenotypes, understand rejection pathophysiology, assist in monitoring treatment responses, and be used to predict patient outcomes.

In consequence, as we now acknowledge novel kidney allograft rejection phenotypes and their different associated outcomes, it is essential to deepen our understanding of the main mechanisms driving these histopathological lesions by means of exploring immunological biomarkers and functional diagnostic tools tracking alloimmune responses, beyond conventional histology and DSA measurements. These advancements, along with others, will then represent a significant step forward in personalized care to optimize patient and allograft outcomes (**Figure 1**).

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

LB and OB both drafted the article and revised it critically. Both 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.

GENERATIVE AI STATEMENT

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

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Burnout Among Physicians of Specialties Dedicated to Liver Transplantation

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Burnout is increasingly relevant among healthcare professionals. The aim of this study is to describe the prevalence of burnout and other parameters of professional satisfaction among different specialists dedicated to Liver Transplantation (LT) in transplant teams. A working group from the Spanish Society of LT designed a survey with 39 questions evaluating the prevalence of parameters related to professional satisfaction, including burnout. It was distributed among 496 specialists dedicated to liver transplantation in Spanish transplant teams. Responders included surgeons (49%), hepatologists (27%), anesthesiologists (16%), intensivists (4%), and other specialties (4%). Among responders, 78% reported some degree of burnout. Moreover, 46% of responders did not see themselves working in transplantation in 5 years. The rates of burnout and dissatisfaction among anesthesiologists and surgeons were higher than other specialists. The highest levels of dissatisfaction were in economic remuneration and work–life balance. Being younger than 60 years old and non-head of department showed to be risk factors of burnout. In conclusion, the prevalence of burnout among LT physicians in Spain was notably high. Among the various specialties, anesthesiologists and surgeons exhibited the highest dissatisfaction rates. The results of this work may be of interest to healthcare management and planning.

Keywords: liver transplantation, burnout, physician, healthcare professionals, transplant teams

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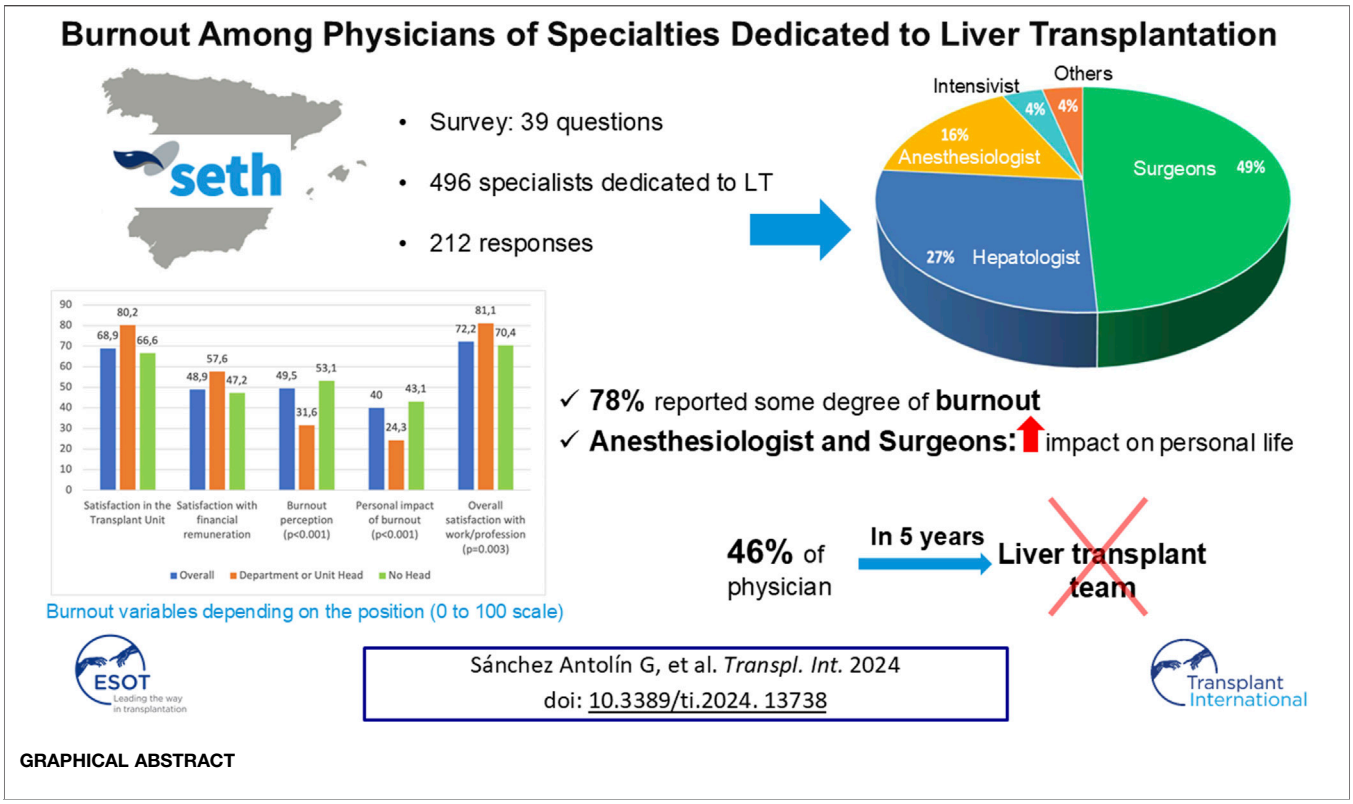
INTRODUCTION

Burnout, first described in 1974 by Herbert Freudenberger [1], is a syndrome typically encountered in high-demand jobs [2]. The syndrome is increasingly relevant among healthcare professionals and is well-documented among surgeons, gastroenterologists, and hepatologists [3–6]. Burnout results in emotional exhaustion, loss of interest in work, depersonalization or lack of empathy toward patients

Abbreviations: LT, Liver transplantation; SETH, Spanish Society of Liver Transplantation.



GRAPHICAL ABSTRACT

and colleagues, and job dissatisfaction [7]. It is also associated with a higher risk of medical errors, sadness and, depression [8].

Liver transplantation (LT) is a complex treatment requiring high levels of professional competence from surgeons, hepatologists, anesthetists, intensivists, and other specialists. Some studies show that abdominal transplant surgeons are on call more nights per week than other surgical specialists and experience an alarmingly high prevalence of burnout and depression [9–11]. Hepatologists are also known to have high burnout rates due to work-time distribution, peer support, and affect [6]. Although burnout has been studied among anesthesiologists and intensivists, there is no research specifically related to LT [12, 13]. Overall, the existing literature on burnout has typically focused on its effects in specific specialties [14–17].

The aim of this study is to describe the prevalence of burnout and other parameters of professional satisfaction among different specialists dedicated to LT in Spanish transplant teams.

MATERIAL AND METHODS

Study Population

Participants were invited from medical specialists dedicated to LT, including surgery, hepatology, anesthesiology, intensive care, pediatrics, and pediatric surgery. The survey was sent to all members of Spanish Society of Liver Transplantation (SETH), and to achieve wider dissemination, the directors of transplant teams were contacted and asked to send the survey to specialists in their transplant units who were not members of SETH.

The survey was also sent to medical residents from the same specialty as the other staff doctors. They were residents in the specialties of general and digestive surgery, gastroenterology and hepatology, anaesthesiology and intensive care medicine.

Survey Design

The Scientific committee of SETH designed a survey based on the questions of the Maslach model [18], adapted to the socio-labor structure of our environment, with 39 questions evaluating emotional exhaustion with a loss of interest in work, depersonalization or lack of empathy for patients and colleagues, and professional dissatisfaction. Questions were included about the impact of stress on professional life, personal life, and team support, as well as the approach to burnout and the attitude toward its therapeutic options. Personality refers to the subjective perception of personal character. The item "felt recognized" was referred to subjective feel of recognition of your own work by colleagues related and no related to LT.

Survey Dissemination

Once designed and agreed upon by the working group, the survey was created using SurveyMonkey[1]. Personal or specific workplace

[1]www.surveymonkey.com

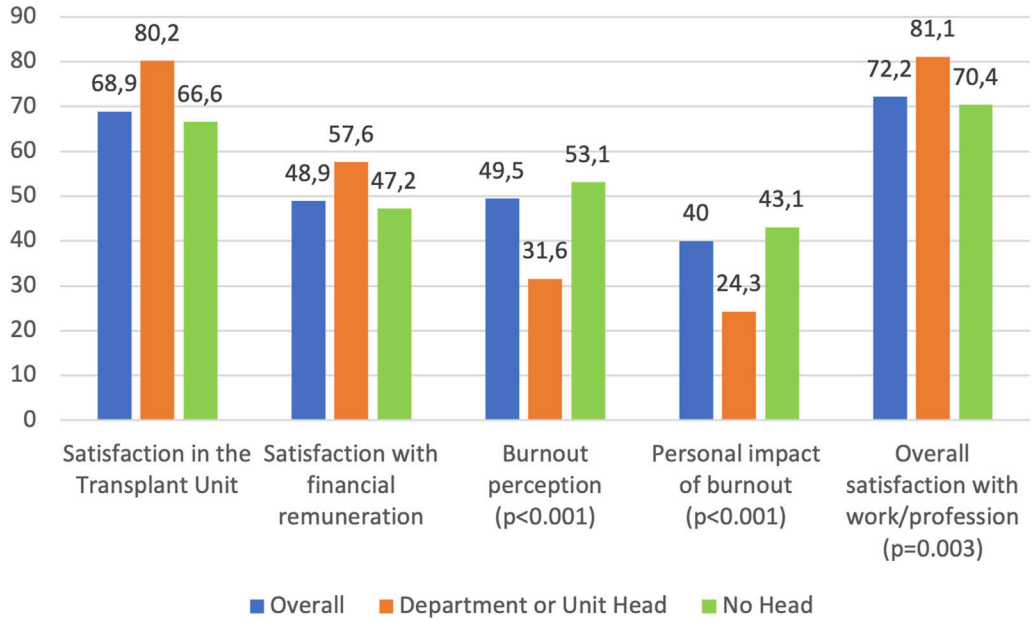


FIGURE 1 | Burnout variables depending on the position (0–100 scale).

TABLE 1 | Satisfaction and consequences of burnout according to specialty.

		Anesthesiology	Surgery	Hepatology	Intensive care	Others	Overall	p
Overall satisfaction (Media/Standar Desviation)	Residents and fellows included	60.4 (27.7)	65.5 (26.3)	75.4 (17)	85.4 (9.2)	85.4 (10.5)	68.9 (24.3)	0.001
	Residents and fellows excluded	60.2 (28.2)	66 (26.7)	76.3 (16.8)	85.4 (9.2)	85.4 (10.5)	69.6 (24.4)	0.001
Satisfaction with remuneration (Media/ Standar Desviation)	Residents and fellows included	25.9 (23.1)	46.9 (29)	63.2 (20.1)	57.1 (16.4)	60.1 (11.4)	48.9 (27.7)	<0.001
	Residents and fellows excluded	26.1 (23.4)	49.8 (28.3)	63.6 (20.7)	57.1 (16.4)	60.1 (11.4)	50.4 (27.4)	<0.001
Perception of burnout (Media/Standar Desviation)	Residents and fellows included	59.4 (34.4)	52.4 (32.5)	45 (27.2)	31.5 (27.1)	22.7 (23.2)	49.5 (31.7)	0.0001
	Residents and fellows excluded	60.8 (34.1)	49.7 (32.5)	43.7 (27.5)	31.5 (27.1)	22.7 (23.2)	48.1 (31.7)	0.009
Consequences of burnout on life (Media/ Standar Desviation)	Residents and fellows included	44.2 (30.3)	44.9 (29.7)	33.6 (23.2)	15.4 (17.7)	28.3 (26.5)	40 (28.4)	0.001
	Residents and fellows excluded	44.8 (30.6)	41.6 (29.1)	32.1 (23)	15.4 (17.7)	28.3 (26.5)	37.8 (27.9)	0.02
Overall satisfaction with your work (Media/ Standar Desviation)	Residents and fellows included	66.7 (22.3)	69.6 (22)	76.8 (12.2)	81.1 (10.8)	89 (8.6)	72.2 (19.7)	0.0002
	Residents and fellows excluded	66.3 (22.5)	71 (21.8)	77.4 (12.1)	81.1 (10.8)	89 (8.6)	73.1	0.006

data were not requested to ensure anonymity. An email was sent to eligible participants that included a cover letter explaining the purpose of the study, encouragement to participate, and a web link to the survey.

Statistical Analysis

Descriptive statistics were estimated using frequencies (n) and percentages (%) for categorical data and means and standard deviations (SD) for continuous data. Differences between groups were analyzed using the non-parametric Mann–Whitney *U* test for quantitative variables, and differences between percentages or frequencies were assessed using Pearson's chi-square test or Fisher's exact probability test. A p-value <0.05 was considered significant. The IBM SPSS statistical software (version 22.0; IBM Corp., Armonk, NY) was used for calculations.

TABLE 2 | Variables related to burnout according to sex.

Recognition with respect to others colleagues			
	Male n = 105	Female n = 107	p
Undervalued n (%)	4 (3.8%)	5 (4.7%)	0.07
Well recognized or highly recognized n (%)	77 (77.3%)	63 (58.9%)	
Neither recognized nor undervalued n (%)	24 (22.9%)	39 (36.4%)	
Satisfaction with work flexibility and family life balance			
	Male n = 105	Female n = 107	p
Dissatisfied or very dissatisfied n (%)	40 (38.1%)	52 (48.6%)	0.014
Satisfied or very satisfied . . . n (%)	42 (40%)	23 (21.5%)	
Neither satisfied nor dissatisfied n (%)	23 (21.9%)	32 (29.9%)	
Access to continued education and research and innovation tasks			
	Male n = 105	Female n = 107	p
Access to continued education never or almost never n (%)	21 (20%)	21 (19.6%)	0.5
Possibility of carrying out research or innovation tasks never or almost never n (%)	17 (6.2%)	34 (31.8%)	0.008
Work in the transplantation unit within 5 years (excluding residents and >60 years old)			
	Male n = 54	Female n = 78	
I will not be or I would like not to be n (%)	19 (26%)	8 (9.3%)	0.005

RESULTS

Demographics

The survey was distributed to 496 physicians and had a 43% response rate (n = 212 responses). Of these, 78% (n = 165) were SETH members, with an even distribution between males and females (50.9% females), and a mean age of 45.17 ± 11.6 years (range 26–70 years). The mean age was significantly higher for males (48.6 ± 12.4 vs. 42.3 ± 10 years old (p = 0.00008). Of note, 30% of participants had been working in the same unit for >20 years and 44% had been in the same unit for <10 years. Nearly half of the respondents were surgeons (49%), followed by hepatologists (27.8%), anesthesiologists (16%), intensivists (3.8%), and others from various specialties (3.3%). Anesthesiologists (67.6%), intensivists (62.5%), and hepatologists (54.2%) were more often women, while only 42.3% were female surgeons (p = 0.101). Regarding the positions of respondents, 69.3% (n = 147) were attending physicians, 9.9% (n = 21) were residents, and 20.75% (n = 44) were department or unit heads. Only 20.5% of the heads were women. Overall 59%, 31%, and 6% of respondents worked in centers performing 21–50, 51–100, and >100 LTs annually.

Satisfaction and Perception of Burnout

On a scale of 1–100, mean overall satisfaction with activities within the transplant unit was 68.86 (SD 24.3), while satisfaction with economic remuneration was the worst-rated aspect at 48.9 (SD 27.7) points. Department or unit heads were generally more satisfied and had a lower perceived burnout (**Figure 1**). Significant differences were observed in overall satisfaction, satisfaction with remuneration, perception of burnout, the consequences of burnout on life, and overall job satisfaction when analyzing data by specialty; notably, anesthesiologists and surgeons were most dissatisfied and had the highest reported burnout. These differences remained after excluding medical residents from the analysis (**Table 1**). Women also reported higher perceived burnout (55 ± 29.6 vs. 44 ± 32.9; p = 0.01) and impact on personal life (44.3 ± 26.9 vs. 35.6 ± 29.3; p = 0.02) (**Table 2**). Among respondents, 78% (n = 165) believed they suffered from some degree of burnout, all agreeing that it affected their work in some way. Regarding the impact on personal life, 27.4% (n = 58) reported that this was affected to a moderate-to-severe degree, with this especially common among women (33.6% vs 21%; p = 0.02) and surgeons (**Table 3**).

Factors related to burnout that affected the personal life of respondents are shown in **Table 4**. Among the reported symptoms, tiredness was the most frequent (n = 74), followed by irritability (n = 47) and lack of motivation (n = 37). Depression was present in 24 (11.3%) participants, with 9 (4.2%) acknowledging a need for treatment. There were no differences between males and females in the rate of depression (12.4% vs. 10.3%; p = 0.6) or in acknowledging the need for treatment (2.9% vs 5.6%; p = 0.3). However, although there were no differences among specialties in the rate of depression, there were differences in acknowledging the need for treatment (**Table 3**).

Regarding burnout management, 26.9% of participants (n = 57) sought support from family or friends, 34.9% (n = 74) turned to physical exercise, and only 4.2% (n = 9) reported needing the support of mental health services or pharmacological treatment. Although 78.8% (n = 167) reported that they would find it interesting if their institution offered support for stress or burnout, only 44% (n = 74) acknowledged they would use such a service, with no differences in response by gender or specialty (**Table 3**). Regarding the influence of personality on the onset of burnout, 10.9% (n = 23) were unsure if it affected them, 71.2% (n = 151) attributed their burnout to external factors, and 17.9% (n = 38) believed their personality played a role, with differences noted across specialties (**Table 3**). There were no differences between men and women (17.1% vs. 18.7%; p = 0.6). Factors recognized as affecting burnout were excessive working hours, excessive bureaucratic tasks, and lack of respect from the institution, bosses, and colleagues (**Figure 2**).

Univariate and multivariate analyses were conducted to assess variables associated with burnout. The results indicated that being younger than 60 years old (OR 2.89; 95% CI 1.09–7.61; p = 0.032), not being head of service or transplant unit (OR 4.14 95% CI 1.83–9.38; p = 0.001) and being an intensivist (OR 0.98

TABLE 3 | Variables related to burnout according to specialty.

	Anesthesiology n = 34	Surgery n = 104	Hepatology n = 59	Intensive care n = 8	Others n = 7	Overall n = 212	p
Depression	4 (11.8%)	14 (13.5%)	5 (8.5%)	0 (0%)	1 (14.3%)	24 (11.3%)	0.7
Need for depression treatment	3 (8.8%)	6 (5.8%)	0 (0%)	0 (0%)	0 (0%)	9 (4.2%)	0.03
Burnout moderately or severely affects personal life	8 (23.5%)	36 (34.6%)	14 (23.7%)	0 (0%)	0 (0%)	58 (27.4%)	0.002
Influence of personality on the onset of burnout	1 (2.9%)	21 (20.2%)	15 (25.4%)	0 (0%)	1 (14.3%)	38 (17.9%)	0.02
You would like the institution to offer support for stress or burnout?	30 (88.2%)	79 (76%)	45 (76.3%)	7 (87.5%)	6 (85.6%)	167 (78.8%)	0.6
You would use support from the institution	13 (38.2%)	43 (41.3%)	29 (49.2%)	4 (50%)	3 (42.9%)	92 (43.4%)	0.8

TABLE 4 | Factors associated with burnout that affect personal life.

	Burnout that affect personal life	p
Sex	72.4%	0.058
Male	83.2%	
Female		
Department or unit head	51.4%	0.000
Yes	83.1%	
Not		
Age <60 years	81.7%	0.000
Yes	46.2%	
Not		
Access to continued education	73.5%	0.002
Yes	95.2%	
Never or almost never		
Access to research	68.1%	0.007
Frequently	81.4%	
Sometimes	90.2%	
Never or almost never		
Specialty	94.1%	0.001
Anesthesiology	78.8%	
Surgery	76.3%	
Hepatology	37.5%	
Intensive care	42.9%	
Others		

95% CI 0.021–0.448; p = 0.003) were significantly correlated with the risk of experiencing burnout (**Table 5**).

Regarding to satisfaction with the work performed in the transplant unit (scale 0–100 points) we performed a multiple linear regression analysis and found that the independent factors related to this were: being a surgeon (correlation coefficient −0.257; p < 0.001), being an anesthesiologist (correlation coefficient −0.238; p = 0.001) and not being the head of the transplant unit (correlation coefficient −0.203; p = 0.002).

Perception of Problems and Professional Recognition

When respondents were asked to rank issues within their transplant unit from least to most important on a scale of 1–5, three critical issues were identified: lack of personnel, lack of economic compensation, and lack of organization and leadership (**Figure 3**). Lack of the appropriate technological tools was the only factor perceived to be a more significant problem by men compared to women, with average scores of 2.77 ± 1.5 and 3.36 ± 1.5, respectively (p = 0.005). The other problems were rated similarly by both sexes. Differences were observed by specialty in the importance attributed to lack of economic reward, technological tools, and personnel (**Table 6**) (**Figure 3**). Additionally, compared with other physicians, department or unit heads considered the lack of technological tools more important (4.09 ± 1.36 vs. 2.86 ± 1.5; p < 0.001) and the lack of organization and leadership less important (2.68 ± 1.57 vs. 3.66 ± 1.86; p < 0.002). There was no significant differences whether they were women or men. Another noteworthy aspect is that heads respond that they can make their own decisions almost always or always more often than physicians who are not heads (80% vs. 45.8%; p < 0.001).

Assessment of time constraints revealed high levels of clinical pressure among 25.5% of participants (n = 54), who felt they almost never had enough time to perform their tasks well; another 43.9% (n = 93) felt this way sometimes. Of the 212 respondents, 33% (n = 70) felt their opinions were not considered within the team, though they were allowed to express them, and 30.7% (n = 65) believed their achievements were never or almost never recognized. Despite 4.25% (n = 9) feeling undervalued compared to other physicians not involved in LT, most (66%) felt recognized or highly recognized, although this sentiment varied by specialty (**Table 6**). None of the department or unit heads felt undervalued, as compared with 5.1% of other professionals. Likewise, 94.3% of department or unit heads felt recognized or very recognized compared with 60.5% of other professionals (p = 0.02). No statistically significant differences were found by gender. Colleague support was present always or almost always for 65% of respondents.

Continued Education, Performance, and Professional Future

Regarding decision-making in professional performance, 51.4% (n = 109) had the ability to make decisions and only 45.8% (n = 98) believed their work was well organized almost always or always. Concerning opportunities for professional development, 29.7% (n = 63) were dissatisfied or very dissatisfied. Up to 43.4% (n = 92) were dissatisfied or very dissatisfied with work flexibility and family life balance. This parameter showed the highest dissatisfaction levels among anesthesiologists (70.6%) and the lowest levels among hepatologists (15.3%; p < 0.001) (**Table 6**). By gender, women reported higher rates of being dissatisfied or very dissatisfied with work flexibility and family life balance

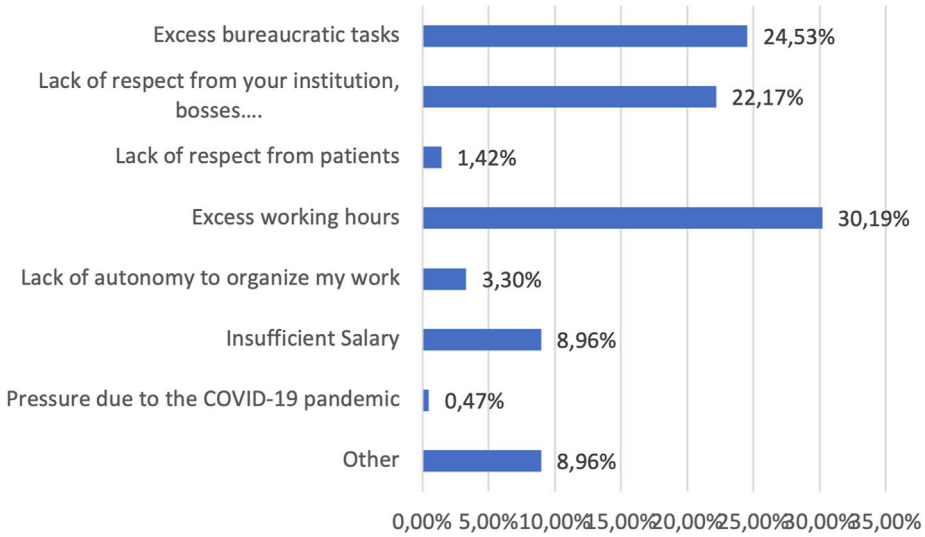


FIGURE 2 | Frequency of factors associated with stress.

TABLE 5 | Univariate and multivariate analysis of possible factors associated with burnout.

Variable	Univariate			Multivariate	
	Burnout	No Burnout	$p < 0.05$	OR (CI 95%)	OR (CI 95%)
Sex (female)	89 (83.2%)	18 (16.8%)	0.042		
SETH member	124 (75.6%)	40 (24.4%)	0.403		
Age <60 years	147 (81.7%)	33 (18.3%)	<0.001	2.89 (1.09–7.61)	0.032
Resident	21 (100%)	0	0.005		
No head	147 (83.1%)	30 (16.9%)	<0.001	4.14 (1.83–9.38)	0.001
Anesthesiologist	32 (94.1%)	2 (4.3%)	0.012		
Surgeon	82 (78.8%)	22 (21.2%)	0.744		
Intensivist	3 (37.5%)	5 (62.5%)	0.014	0.98 (0.021–0.448)	0.003
Hepatologist	44 (75.9%)	14 (24.1%)	0.712		
LT per year ≥ 50	63 (78.8%)	17 (21.3%)	0.867		

SETH, spanish society of liver transplantation; OR, odds ratio; LT, liver transplant.

(48.6% vs. 38.1%; p = 0.014). We carried out a multivariate analysis to study which variables influence dissatisfaction with work-life balance, we found that gender was not a statistically significant variable, however, the following variables had a greater influence on dissatisfaction: Not being head of service or head of a department (OR 4.119 CI 95% 1.624–10.45; p = 0.003), being anesthesiologist (OR 10.663 CI 95% 4.073–27.917; p < 0.001) or surgeon (OR 5.948 CI 95% 2.862–12.362; p < 0.001).

Continued education was seen as always or almost always accessible for 42% of respondents (n = 89), while 19.8% (n = 42) admitted they never or almost never had access. No gender differences were detected (**Table 2**), but variations were observed by specialty, with anesthesiologists reporting the least access (**Table 6**). A similar pattern emerged for participation in research and innovation, with 24.1% (n = 51) stating they never or almost never had the opportunity to participate. Differences were observed among specialties, with anesthesiologists again reporting the least access (**Table 6**). Additionally, twice as many women as men reported that access to research and innovation was never or almost never possible (16.2% vs. 31.8%; p = 0.008) (**Table 2**).

Overall, 147 respondents (69.3%) reported that going to work was satisfying or very satisfying, and 104 (49%) considered their working conditions good or very good. Despite the challenges faces, 91% of transplant physicians felt committed or very committed to their work.

Regarding their futures within transplant teams, 54.3% of physicians (n = 115) saw themselves as part of the team in 5 years, while 21.2% (n = 45) would either not be or prefer not to be in the team. Importantly, 24.5% (n = 52) expressed a desire to stay, but they were unsure if they could withstand the pressure. To refine the analysis of how many physicians did not wish to remain in the transplant unit within 5 years, we excluded residents and those over 65 years of age. Among the remaining 132 respondents, 27 (17%) indicated they would not want to be part of the team. This percentage was higher among men than women (26% vs. 9.3%; p = 0.005); among the different specialties, it was highest among anesthesiologists (34.5%; p = 0.001) (**Table 6**). Among the surgery residents, it was notable that 27.8% thought they would not work or would not like to work in a LT unit.

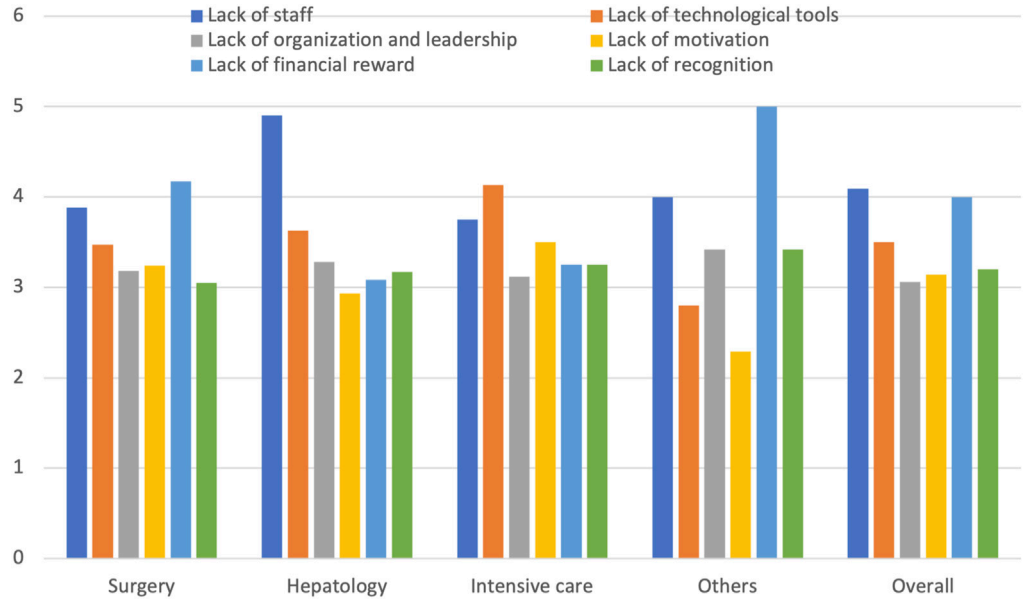


FIGURE 3 | Importance of the most relevant problems depending on type of specialist.

DISCUSSION

In this survey of principal medical specialties involved in LT, we found that burnout was present in 78%, a rate that is higher than previously reported [3–6]. The high rate of burnout, especially among anesthesiologists and surgeons, was associated with lack of support and recognition from the team, superiors, and the institution. The greatest dissatisfaction centered on economic incentives, especially for anesthesiologists and surgeons.

We designed an adapted burnout survey for transplant doctors, rather than using a validated one like the MBI, to better capture the specific challenges of transplant work, such as the emotional and organizational pressures unique to this field. Additionally, the survey is tailored to the Spanish healthcare context and offers greater flexibility to address specific factors like workload in transplant units and lack of resources. Importantly, the survey maintains the structure of a validated tool by assessing key dimensions such as emotional exhaustion, depersonalization (or cynicism), and reduced personal accomplishment, ensuring that it covers the core aspects of burnout. The goal is to obtain practical and immediate results that help implement targeted interventions to improve the wellbeing of the team.

A 43% response rate in a burnout survey among transplant doctors is acceptable for this type of population. While it isn't high, it's common for surveys in busy professional groups like doctors, where response rates typically range from 30% to 60%. Although a higher rate would be ideal, this level of participation can still yield valuable insights.

A 2015 national survey among transplant surgeons in the United States showed high levels of emotional exhaustion (40.1%), depersonalization, and low personal satisfaction. Lack of autonomy in decision-making, lack of support from superiors, and high patient demands were associated with higher levels of burnout [19]. A study of burnout among abdominal transplant surgeons in Europe also found that nearly a third exhibited emotional exhaustion, but that levels of depersonalization were low, suggesting that commitment to their work remained despite feeling exhausted [20]. Our data support the importance of physician commitment to their work, with 91% of respondents feeling committed or very committed.

Among intensivists, severe burnout has been described at rates of up to 50% [21]. Although dissatisfaction in our series was lower than that of anesthesiologists, surgeons, and hepatologists, the low number of participants mean that our results should be interpreted with caution. Studies among anesthesiologists show one of the highest prevalences of burnout, with higher rates of suicide and addiction than in the general population. Autonomy, control of the work environment, professional relationships, leadership, and organizational justice are considered the most important factors in job satisfaction [22].

A factor associated with dissatisfaction in our study was the difficulty maintaining a work–life balance, especially for anesthesiologists, consistent with the results of other studies [22]. It is also noteworthy that dissatisfaction with work–life balance was higher among women involved in LT, although this variable was not statistically significant in the multivariate analysis. A systematic review exploring the influence of gender on physician burnout found that both men and women experience high rates of burnout, but that it is more likely to develop in females, especially emotional exhaustion [23].

The rate of perceived burnout did not change when excluding medical residents from the analysis, suggesting they are affected similarly to other physicians. A study conducted among surgical transplant residents in the United States found that up to 17% exhibited symptoms of burnout, and that those working >100 h

TABLE 6 | Perception of the most important problems, professional recognition with respect to colleagues and access to continued education and research according to specialty.

	Anesthesiology n = 34	Surgery n = 104	Hepatology n = 59	Intensive care n = 8	Others n = 7	Overall n = 212	p
Perception of the most important problems[a]							
Lack of staff (media +/−SD)	3.44 ± 1.48	3.88 ± 1.78	4.9 ± 1.35	3.75 ± 1.67	4.0 ± 1.63	4.09 ± 1.68	<0.001
Lack of organization and leadership (media +/−SD)	3.35 ± 1.74	3.47 ± 1.97	3.63 ± 1.76	4.13 ± 1.81	2.86 ± 1.21	3.5 ± 1.84	0.6
Lack of technological tools (media +/−SD)	2.21 ± 1.22	3.18 ± 1.56	3.28 ± 1.51	3.12 ± 1.73	3.42 ± 1.72	3.06 ± 1.54	0.01
Lack of motivation (media +/−SD)	3.26 ± 1.44	3.24 ± 1.5	2.93 ± 1.67	3.5 ± 1.51	2.29 ± 1.6	3.14 ± 1.55	0.37
Lack of financial rewards (media +/−SD)	5.05 ± 1.58	4.17 ± 1.56	3.08 ± 1.65	3.25 ± 1.83	5.0 ± 1.15	4.0 ± 1.72	<0.001
Lack of recognition (media +/−SD)	3.67 ± 1.55	3.05 ± 1.59	3.17 ± 1.55	3.25 ± 2.12	3.42 ± 2.07	3.2 ± 1.61	0.4
Recognition with respect to others colleagues							
	Anesthesiology n = 34	Surgery n = 104	Hepatology n = 59	Intensive care n = 8	Others n = 7	Overall n = 212	p
Undervalued n (%)	1 (2.9%)	6 (5.8%)	2 (3.4%)	0 (0%)	0 (0%)	9 (4.2%)	0.001
Well recognized or highly recognized n (%)	12 (35.3%)	67 (64.4%)	49 (83.1%)	6 (75%)	6 (85.7%)	140 (66%)	
Neither recognized nor undervalued n (%)	21 (61.8%)	31 (29.8%)	8 (13.6%)	2 (25%)	1 (14.3%)	63 (29.7%	
Satisfaction with work flexibility and family life balance							
	Anesthesiology n = 34	Surgery n = 104	Hepatology n = 59	Intensive care n = 8	Others n = 7	Overall n = 212	p
Dissatisfied or very dissatisfied. n (%)	24 (70.6%)	55 (52.9%)	9 (15.3%)	2 (25%)	2 (28.6%)	92 (43.4%	<0.001
Satisfied or very satisfied n (%)	4 (11.8%)	29 (27.9%)	26 (44.1%)	3 (37.5%)	3 (42.9%)	65 (30.7%)	
Neither satisfied nor dissatisfied n (%)	6 (17.6%)	20 (19.2%)	24 (40.7%)	3 (37.5%)	2 (28.6%)	55 (25.9%)	
Access to continued education and research and innovation tasks							
	Anesthesiology n = 34	Surgery n = 104	Hepatology n = 59	Intensive care n = 8	Others n = 7	Overall n = 212	p
Access to continued education never or almost never n (%)	19 (55.9%)	18 (17.3%)	5 (8.5%)	0 (0%)	0 (0%)	42	p < 0.001
Possibility of carrying out research or innovation tasks never or almost never n (%)	17 (50%)	17 (16.3%)	15 (25.4%)	2 (25%)	0 (0%)	51 (24.1%)	0.02
Work in the transplantation unit within 5 years (excluding residents and >60 years old)							
	Anesthesiology n = 29	Surgery n = 70	Hepatology n = 48	Intensive care n = 7	Others n = 5	Overall n = 132	p
I will not be or I would like not to be	10 (34.5%)	13 (18.6%)	4 (8.3%)	0 (0%)	0 (0%)	27 (17.0%)	0.001

[a]*1 (least important) to 6 (most important).*

per week were more likely to experience severe stress, contemplate leaving their residency, or commit a medical error [2]. High levels of burnout and suicide have also been described among medical trainees in intensive care and anesthesiology [24]. West et al. has reported that physician burnout leads to dysfunction in the healthcare system by losing organizational talent, reducing patient care quality, and ultimately causing severe mental health damage to professionals [25].

It was notable that almost half of the respondents felt that they could not make decisions within the team, and that only 46% felt that their work was well organized always or almost always. The value given to organizational factors and decision-making might explain the lower perceived rate of burnout among service or unit heads, who can make these decisions. In public hospitals in Spain, department heads typically work around 37.5 h per week, not including on-call shifts. Although there is no fixed national regulation, it is common for 20%–30% of this time to be reserved for management duties, such as resource planning and team coordination. This protected time can vary depending on the autonomous community or the hospital, and may also depend on the clinical workload of the department. The regulation of this time is often governed by specific labor agreements in each region.

Regarding burnout management, it was striking that only 4.2% of respondents had sought professional help, and that, despite recognizing institutional support as interesting, only 35% would use it if implemented by their institution. We have no information on why professionals would not use support to treat or prevent burnout even if their institution provided it. This is likely due to fear of being labelled or fear of losing anonymity. This could be a relevant aspect for future research. The literature also highlights low adherence by physicians to support programs, which is considered to reflect their tendency to care for others but not themselves [26]. Moreover, there is little evidence of their benefit, and given the complexity of implementing preventive measures due to the heterogeneity of workers and the causes of workplace stress, results cannot be extrapolated [27, 28].

Another finding was that up to a quarter of participants acknowledged not having access to research, with concern that this issue presents twice as much in women compared to men. A

recent study analyzing authorship of published papers between 2012 and 2021 in the United States observed that, despite an increase in women as first or last authors, there is still a significant gender gap. However, a female last author is associated with the presence of a female first author, highlighting the importance of mentoring young women entering transplantation [29]. Regulated continuous education and mentorship are considered essential to ensure the generational replacement of physicians [30].

Our results indicate that approximately 21% of respondents recognize that they will not be, or would not like to be, working in LT in 5 years. This is especially worrying in the case of residents, where the percentage rises to 27.8%. A study among surgery residents in Spain showed that most surgery residents did not want to dedicate themselves to transplantation because they considered the specialism too demanding [31]. This lack of motivation to dedicate themselves to transplantation has been highlighted by other authors [32–34].

As limitations of our study, we highlight that it relied on self-perception and lacked standardization, with bias toward a higher response rate among professionals more sensitized to burnout. Furthermore, the characteristics of the Spanish healthcare system are probably associated with greater dissatisfaction, due to the low salaries of professionals, which may complicate comparisons with series from other countries.

The survey did not include descriptive variables. The main reasons of dissatisfaction were remuneration, and work-life balance specially in women; thus, we may conclude that these are the reasons why physicians would not want to be in LT in the future.

Salary comparisons across specialties cannot be performed because we have no specific no data about remuneration for LT among the different specialist in Spain. This fact is not regulated in Spain, and each hospital, and each department has his own rules. Physician's salaries are among the lowest in Europe, but not only in the transplant setting [35]. Regulation of salary and comparison across specialties in Spain with international teams may help to mitigate dissatisfaction. Parallelly to regulation of salaries, several regulatory, phycological and institutional solutions should be implemented at individual and organization-level [25]. Effective solutions should align with the drivers described at our study. Due to excessive workload, and low ratio of physicians, in most Spanish LT departments, research is not clearly scheduled, and there is no protected time to do it. This is clearly a field to be improved. Another limitation of our study is that physicians of the same specialty who do not work in transplantation teams have not been surveyed in order to make objective comparisons. Comparisons were based on the self-perception of the respondents. Nevertheless, this study has several strengths: the response rate was high, it covered the whole of Spain (a country with consolidated experience in LT), and included all major specialties involved in LT together, and not in isolation. The results of this work may be of interest to healthcare management and planning.

The high degree of burnout among LT physicians is the main conclusion of our study, and we consider it to be a warning to all healthcare stakeholders, especially the responsible of healthcare organizations. We should implement all needed interventions to improve the degree of burnout and mitigate dissatisfaction. It is imperative to avoid the decreasing number of professionals dedicated to LT, and evermore to avoid an increase of adverse events and effects on patients care that are related to burnout [25].

Previous studies have reported possible solutions to improve the degree of burnout and their outcomes. Efforts may be focused on salary, job security and flexibility, protected workload and professional development [25, 36]. The results of our survey suggest that healthcare system leaders and hospital administrators should implement strategies, not only economic ones, to minimize burnout among transplant professionals. These strategies should focus on increasing professional recognition, improving work-life balance, facilitating career progression, reducing excessive workloads, and providing emotional and psychological support.

In conclusion physicians dedicated to LT in Spain show high levels of commitment to their work. However, burnout rates were high (78%), being among anesthesiologists and surgeons higher than those of other specialists involved in LT. The highest levels of dissatisfaction were experienced for the perceived economic remuneration and the impact on balance with family life, with the latter especially common among women.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board also waived the requirement of written informed consent for the participation of participants or participants' legal guardians/ next of kin because this study was based on data provided by the participants themselves, who are liver transplant professionals, and does not include patient data.

AUTHOR CONTRIBUTIONS

GS-A conceptualised the study. GS-A, GB-F, LL, IC-V, PR, JA, AO, and SP designed the study. GB-F performed the statistical analysis. GS-A, GB-F, and LL developed the content of the article. GS-A, GB-F and LL wrote the initial draft of the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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.2024.13738/full#supplementary-material

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ORIGINAL RESEARCH
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Acceptance of Organs from Deceased Donors With Resolved or Active SARS-CoV-2 Infection: A Survey From the Council of Europe

Maddalena Peghin[1†], Elena Graziano[1†], Maria De Martino[2†], Maria Luisa Balsamo[1†], Miriam Isola[2†], Marta López-Fraga[3†], Massimo Cardillo[4†], Giuseppe Feltrin[4†], Beatriz Domínguez-Gil González[5†], Paolo Antonio Grossi[1†] and The COVIDonors COE Study Group*

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SARS-CoV-2 infection represents a new challenge for solid organ transplantation (SOT) with evolving recommendations. A cross-sectional survey was performed (February–June 2024) to describe practices among Member States of the Council of Europe (COE) on the use of organs from deceased donors with resolved or active SARS-CoV-2 infection. Overall, 32 out of 47 Member States with a transplant program participated in the study. Four (12.5%) countries did not use organs from deceased donors either with resolved or with active SARS-CoV-2 infection and 8 (25%) countries accepted organs only from deceased donors with resolved SARS-CoV-2 infection. Donor evaluation for SARS-CoV-2 included universal screening with standard PCR testing on respiratory specimens generally (61.4%) performed within 24 h prior to organ recovery. Further microbiological, immunological and radiological investigations varied. Most waitlisted patients receiving organs from a deceased donor with active (94.5%) or resolved (61.5%) SARS-CoV-2 infection were preferred to have natural, vaccine-induced or hybrid SARS-CoV-2 immunity. Most countries did not require recipients to undergo specific anti-SARS-CoV-2 treatment as pre-exposure (0%), post-exposure prophylaxis (15.4%) or modification of immunosuppression regimen (24%). This study highlights similarities and heterogeneities in the management of SARS-CoV-2 positive donors between COE countries, and a potential to safely expand donors' pool.

Keywords: DDI, donor, recipient, Sars-CoV-2, COVID-19, donor derived infections

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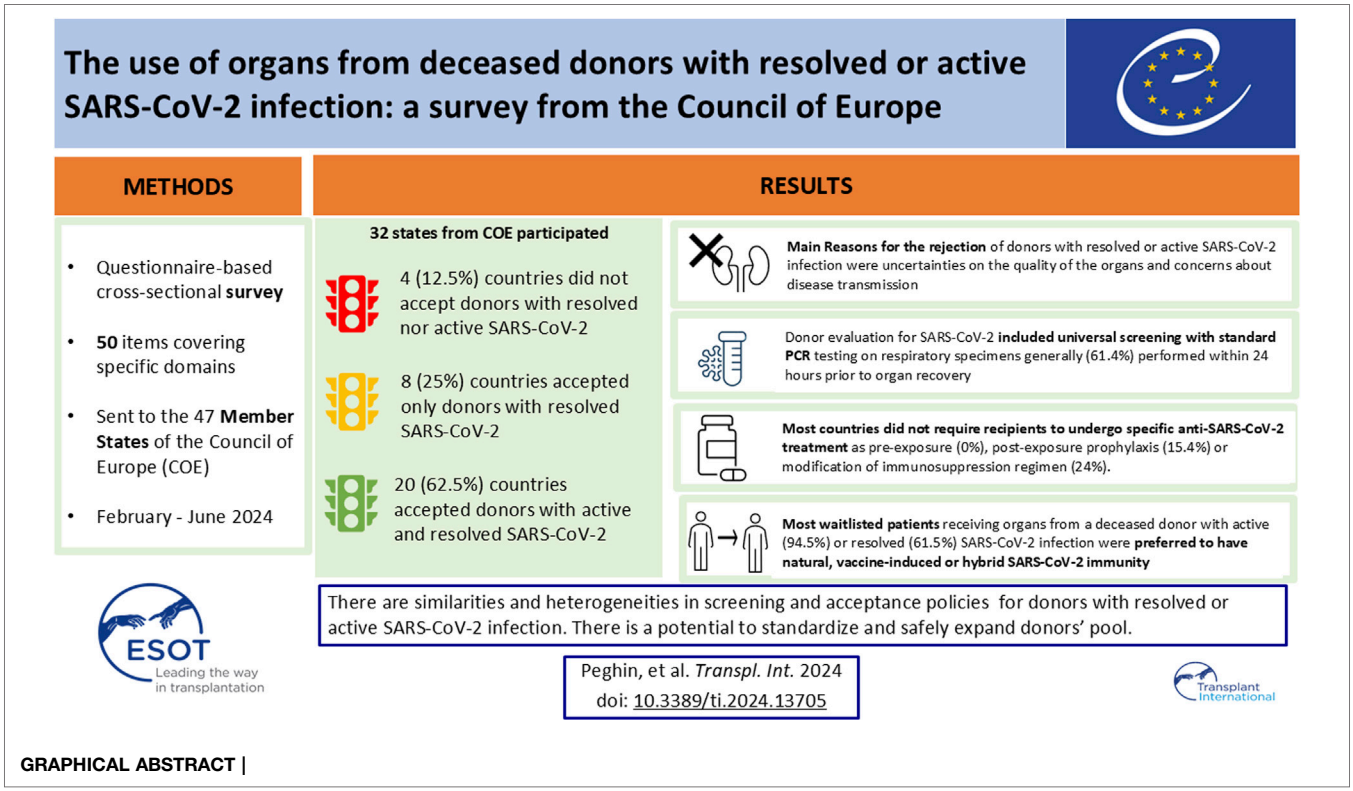
Abbreviations: COVID-19, coronavirus disease 2019; COE, Council of Europe; Ct, cycle threshold; CT, computed tomography; LRT, lower respiratory tract; NPS, nasopharyngeal swab; PCR, polymerase chain reaction; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; SOT, solid organ transplant.



GRAPHICAL ABSTRACT |

INTRODUCTION

The increasing gap between patients on the waiting list and organ availability has led to the use of organs from donors with well-known and emerging infections, supported by the improvement of risk mitigation strategies to avoid donor derived infections [1].

At the beginning of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, uncertainties existed regarding the route of transmission of SARS-CoV-2; this created pressure on transplant systems to recommend universal donor screening and advise against solid organ transplantation (SOT) from donors testing positive for SARS-CoV-2 [2]. Such restrictive policies resulted in the loss of a significant number of lifesaving and life-enhancing organs [3]. Based on growing evidence of the biology of SARS-CoV-2, along with the availability of effective vaccines and new treatment options, these recommendations have been challenged and transplant systems worldwide have adopted various policies and regularly updated guidance for organ acceptance and recipient management [2, 4, 5].

This manuscript describes current transplantation practices in Member States of the Council of Europe (COE) with regards to the use of organs from deceased donors with resolved or active SARS-CoV-2 infection.

MATERIALS AND METHODS

A questionnaire-based cross-sectional survey was developed based on a scoping review of the literature and was independently reviewed by four study investigators (PAG, MP, EG, MLB) who were part of an expert panel. A definitive questionnaire with 50 items covering specific domains was based on the full consensus of the investigators. The survey was sent by the European Directorate for the Quality of Medicines and HealthCare (EDQM) of the Council of Europe (COE) to Member States of the COE. The survey was hosted on a cloud-based software (SurveyMonkey®, San Mateo, CA, United States) between February and June 2024. Member States who were willing to participate were included.

A deceased donor with resolved SARS-CoV-2 infection was defined as a donor who died after the resolution of symptoms and had viral clearance documented by a negative SARS-CoV-2 RT-PCR or antigenic test in respiratory samples. A deceased donor with active SARS-CoV-2 infection was defined as a donor who died and had a positive SARS-CoV-2 RT-PCR or antigenic test in respiratory samples.

The reference European committee on organ transplantation of the COE (CD-P-TO) approved the study and all procedures were in accordance with established ethical standards (TO129).

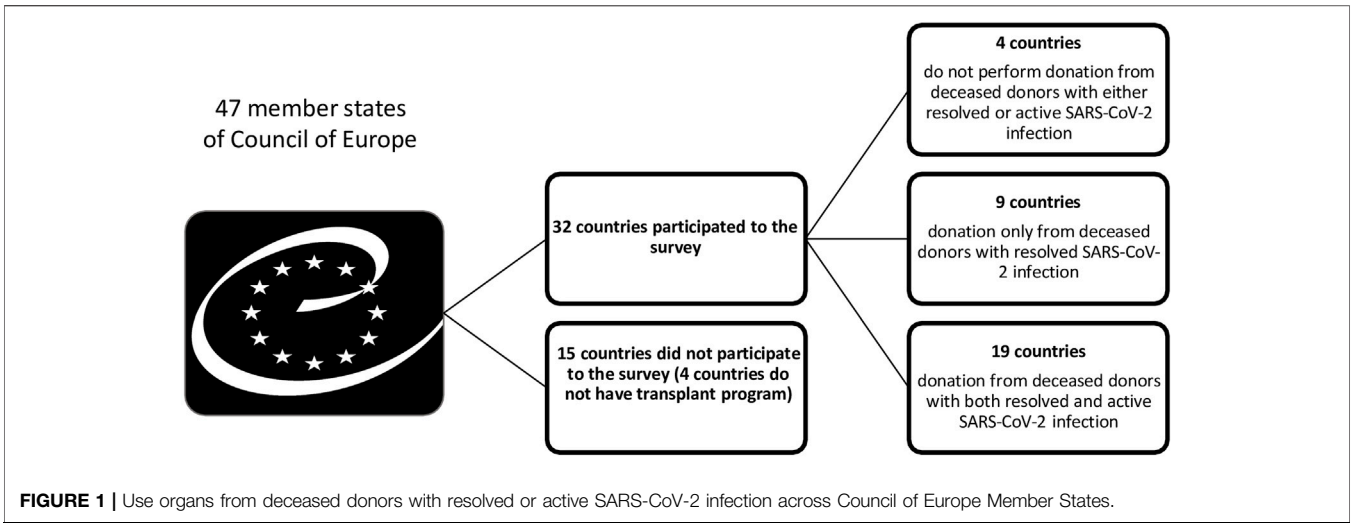


FIGURE 1 | Use organs from deceased donors with resolved or active SARS-CoV-2 infection across Council of Europe Member States.

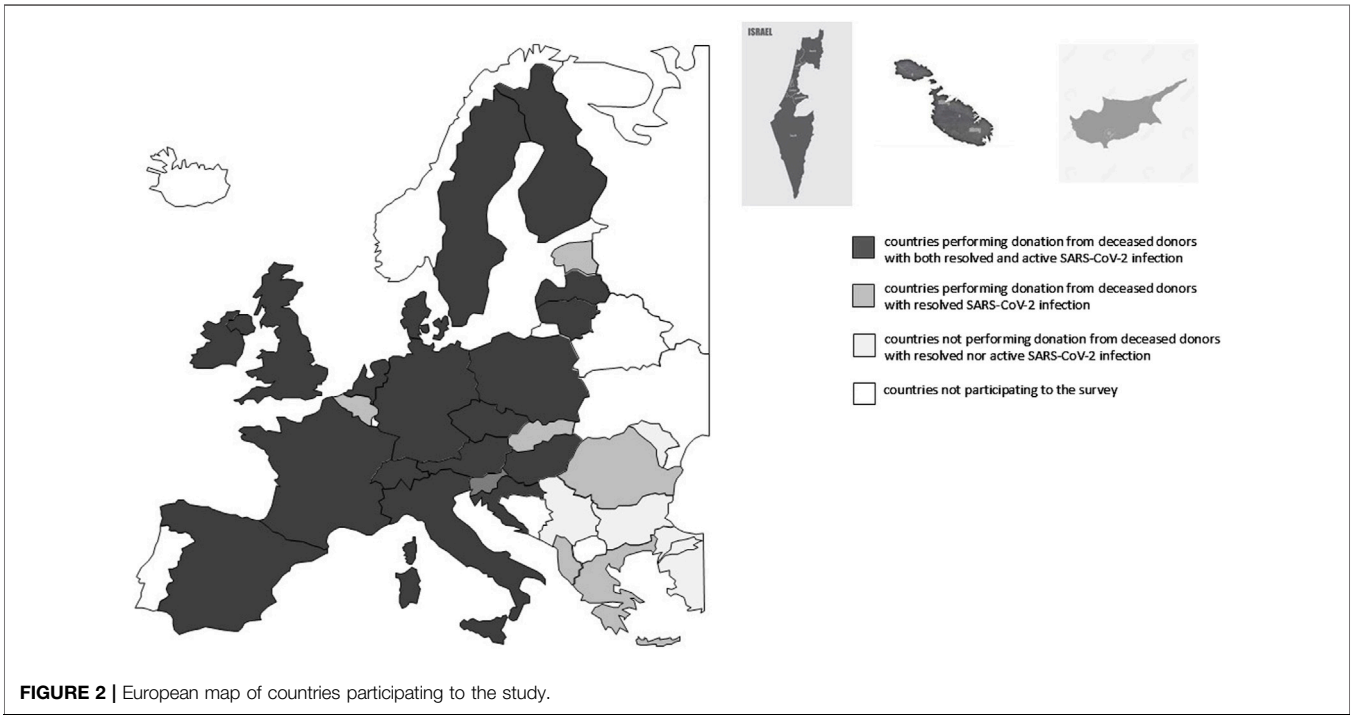


FIGURE 2 | European map of countries participating to the study.

Statistical Analysis

A descriptive analysis was performed. Continuous variables are expressed as the median and range. All proportions were calculated as percentages of patients with available data. Data analyses were performed with Stata 17 software.

RESULTS

General COE Practices

Thirty-two out of 47 Member States of the COE participated in the study. Four countries do not have an active transplant program. Around 4 (12.5%) countries did not use organs from deceased donors with either resolved or active SARS-CoV-2 infection and 8 (25%) countries did not accept organs from deceased donors with active SARS-CoV-2 infection because of uncertainties regarding the risk of SARS-CoV-2 transmissibility (**Figures 1**, **2**).

Use of Organs From Deceased Donors With Resolved SARS-CoV-2 Infection

Overall, 28 (87.5%) countries accepted organs from deceased donors with resolved SARS-CoV-2 infection. This transplantation

TABLE 1 | Reasons for the rejection of donors with resolved or active SARS-CoV-2 infection.

	Donors with active COVID-19	Donors with resolved COVID-19
	n/N, % (n = 18)	n/N, % (n = 21)
Uncertainties on the quality of the organs	9 (50)	14 (66.7)
Concerns about disease transmission	11 (61.1)	5 (23.8)
Severity of COVID-19	8 (44.4)	1 (4.8)
Infectious diseases specialist's decision	5 (27.8)	3 (14.3)
Thrombotic complications	3 (16.7)	2 (9.5)
Inflammatory activity	1 (5.5)	2 (9.5)
Organ damage	2 (11.1)	8 (38.1)

practice was active for a median of 26.2 months (range 17.3–48.8 months), from June 2020 to June 2024. Organs accepted for transplantation from donors with resolved SARS-CoV-2 infection included kidney (28/28, 100%), liver (27/28; 96.4%), heart (25/28; 89.3%), pancreas (21/28; 75%), lung (18/28; 64.3%) and bowel (17/28; 60.7%).

Organ procurement was allowed from deceased donors who died at least an average of 11.4 days (range 0–30 days) after resolution of symptoms for non-lung transplant and an average of 14.5 days (range 10–21 days) for lung transplant. All countries required the donor to be asymptomatic at the time of procurement and to have viral clearance documented by a negative SARS-CoV-2 PCR in nasopharyngeal swab (NPS) and/or lower respiratory tract (LRT) sample. The main reasons for refusal of organs from donors with resolved SARS-CoV-2 infection are listed in **Table 1**.

Use of Organs From Deceased Donors With Active SARS-CoV-2 Infection

Overall, 20 (62.5%) countries accepted organs from deceased donors with active SARS-CoV-2 infection. This policy was active on average for 24.1 months (range 17.1–48.1 months), between November 2020 and June 2024. Organs considered for donation from patients with active SARS-CoV-2 infection included kidney (19/20; 95%), heart (18/20; 90%), liver (19/20; 95%), pancreas (15/20; 75%), lung (2/20; 10%) and bowel (6/20; 30%). All countries required the donor to be asymptomatic or mildly symptomatic for SARS-CoV-2 infection to allow donation. The main reasons for refusal of organs from donors with active SARS-CoV-2 infection are listed in **Table 1**.

Recipients With Active or Resolved SARS-CoV-2 Infection

In general, recipients with active SARS-CoV-2 infection were not allowed to receive organs from donors with active SARS-CoV-2 infection (11/21; 52.4%), except by case-by-case infectious disease evaluation (8/21; 38.1%). Only one country allowed transplantation of individuals with active infection routinely.

Patients with recently resolved SARS-CoV-2 positivity could be re-entered on the transplant waiting lists in most cases after resolution of symptoms and documented virological cure with negative SARS-CoV-2 PCR (19/24; 79.2%). The minimum duration of recipient symptoms from SARS-CoV-2 onset to allow transplantation averaged 19 days (range 0–90).

Screening and Eligibility of Waitlisted Patients

Overall, most national protocols (27/32; 84.4%) recommend waitlisted patients to be vaccinated with a median of 3 doses (range 2–5) of SARS-CoV-2 vaccine. SARS-CoV-2 vaccination was mandatory for waitlisted patients in 5 out of 32 (15.6%) countries. Based on current protocols, SARS-CoV-2 IgG measurement was not routinely performed for most waitlisted patients before transplantation (21/32; 62.6%). SARS-CoV-2 virus-specific cell-mediated immunity was regularly determined before transplantation only in three countries.

Most waitlisted patients were allowed to receive organs from a deceased donor with active (94.5%) or resolved (61.5%) SARS-CoV-2 infection, only if they met specific conditions. Mandatory criteria are listed in **Table 2**.

The majority (17/25; 68%) of national protocols had a specific recipient informed consent required for patients receiving organs from a deceased donor with resolved or active SARS-CoV-2 infection.

Standard Donor SARS-CoV-2 Screening

Current strategies for donor screening for SARS-CoV-2 included evaluation by SARS-CoV-2 PCR. Testing recommendations for non- lung, non-bowel donation included collection of samples from the LRT and NPS (10/29; 34.5%) and from the LRT or NPS (13/29; 44.8%). Most countries performed the assay (18/29; 62.1%) within 1 day before organ recovery.

Regarding lung and bowel donation, routine recommendations were to collect samples from the LRT and NPS (18/26; 69.2%) or only from the LRT (4/26; 15.4%). Most countries performed the assay (17/28; 60.7%) within 1 day before to organ recovery.

Specific Donor SARS-CoV-2 Screening for Deceased Donors With Active or Resolved SARS-CoV-2 Infection

SARS-CoV-2 cycle threshold (Ct) values were required and considered in decision-making processes in 9 countries (11/28; 39.3%) and on a case-by-case basis in 3 countries (3/28; 10.7%).

TABLE 2 | Conditions required by potential recipients to receive organs from donors with resolved or active SARS-CoV-2 infection.

	Donors with active COVID-19	Donors with resolved COVID-19
	n/N, % (n = 18)	n/N, % (n = 26)
History of resolved COVID-19	7 (38.9)	5 (19.2)
Full vaccination (at least 3 doses)	10 (55.5)	10 (38.5)
Documented immunological response (seroconversion and/or virus-specific cell-mediated immunity)	3 (16.7%)	2 (7.7)
Life-threatening organ dysfunction and low probability of a suitable and timely non-infected donor	4 (22.2)	2 (7.7)
None	1 (5.5%)	10 (38.5)
Case-by-case evaluation	5 (27.8%)	3 (11.5)

SARS-CoV-2 IgG measurement and/or SARS-CoV-2 virus-specific cell-mediated immunity was not routinely performed in most donors (27/30; 90%).

SARS-CoV-2 PCR was performed on samples of donor organ biopsy in two countries (2/26; 7.7%) only for deceased donors with active SARS-CoV-2 infection and in one country (1/26; 3.8%) both for active and resolved SARS-CoV-2 donors. SARS-CoV-2 PCR on preservation fluid and analysis of donor organ quality with biopsy was recommended at time of donation only in one country mainly for research purpose.

Chest imaging with computed tomography (CT) scan was routinely performed both for donors with resolved or active SARS-CoV-2 infection (16/28; 57.2%) and only for active (2/28; 7.1%). No routine CT imaging was usually required in 10 out of 28 (35.7%) countries, except in specific settings.

Hospital Setting Preventive Measures for Transplantation

During organ procurement and transplant, infection control measures included the use of filtering facemasks (N95, FFP2 and FFP3) (23/28; 82.1%), eye protection (19/28; 67.6%), dedicated operating theatres (7/28; 25%) and standard gloves and gowns (28/28; 100%).

Recipients of organs from donors with resolved SARS-CoV-2 infection were not placed in isolation and were managed per routine in most countries (20/26; 76.9%). Other countries placed recipients in isolation in an individual room in a non-SARS-CoV-2 area (5/26; 19.2%) or considered hospital-specific procedures (1/26; 3.9%).

Recipients of organs from donors with active SARS-CoV-2 were isolated based on local centre protocols (10/17; 58.8%), isolated in an individual room in a general ward (5/17; 29.6%) or in a SARS-CoV-2 area (1/17; 5.9%). Only one country managed the recipients without specific isolation procedures.

Among countries performing lung transplantation from donors with resolved SARS-CoV-2 infection, 77% (14/18) manage recipients after transplantation routinely, while 11% (2/18) place recipients in isolation in an individual room in a non-COVID-19 area but with isolation procedures. Among the two countries performing lung transplantation from active COVID-19 donors, one manages recipients as routinely and the other one did not specify the isolation procedure.

Vaccination was mandatory for healthcare workers in 37.5% (12/32) countries and was required for family members visiting hospitals in one country.

SARS-COV-2 infections related to the organ procurement or transplantation among healthcare workers were not observed in most countries for which data were available (21/21; 100%).

Treatment Strategies After Transplant

None of the countries recommended routine pre-exposure prophylaxis before transplant for recipients of organs obtained from donors with resolved or active SARS-CoV-2 infection. Post-exposure prophylaxis was not suggested after transplant for recipients of organs with resolved and/or active SARS-CoV-2 infection in most COE countries (22/26; 84.6%). With regards to lung transplantation, most countries performing lung transplantation from donors with resolved SARS-CoV-2 infection (16/18; 88%) do not perform any post-exposure prophylaxis on recipients, as well as one out of the two countries performing lung transplantation from active COVID-19.

Immunosuppression regimens were not routinely changed after transplantation in most countries (19/25; 76%). Within the 10 countries performing transplants for recipients with active SARS-CoV-2 infection, 1 (10%) recommended specific SARS-CoV-2 treatment after transplant and 2 (20%) suggested immunosuppression regimen modification.

Follow-Up After Transplant and Recipient Outcome

After transplantation, all recipients were routinely monitored clinically, about half virologically with periodic SARS-COV-2 PCR in respiratory samples (46.3%, 13/28) and only two immunologically (7.1%, 2/28) with SARS-COV-2 serological testing. No donor derived SARS-CoV-2 infection was described in any country.

Survey results are summarized in **Supplementary Tables S1–S18**.

DISCUSSION

This survey provides an overview of the policies and real-life use of SARS-CoV-2 positive donors for SOT in 32 countries across

Europe. To our knowledge, this is the first international assessment using a standardized questionnaire and providing detailed information about the management practice of organs obtained from deceased donors with resolved or active SARS-CoV-2 infection.

Since the beginning of the pandemic, SARS-CoV-2 infection caused discarding many organs while efforts to maintain SOT activities were being made worldwide [2, 3]. Through our survey, we found increasing support to the acceptance of grafts from deceased donors SARS-CoV-2 positive in Member States of the COE. However, we also identified four countries where organs from deceased donors, either with resolved or active SARS-CoV-2 infection, are not used, as well as eight countries where organs from deceased donors with active SARS-CoV-2 infection are not accepted for SOT.

On the basis of this survey and recent worldwide experience, transplantation of non-lung and non-bowel organs from donors with active SARS-CoV-2 infection is considered safe, without evidence of SARS-CoV-2 transmission. In addition, good short-term outcomes, in terms of graft loss and mortality, have been observed and confirmed in our survey [6–10]. This should prompt more countries to reconsider their policies with regards to the use of organs from SARS-CoV-2 positive donors. However, SARS-CoV-2 infection could potentially lead to adverse outcomes in the long-term, likely due to subclinical endothelial dysfunction, hypercoagulability and organ injury in potential donors; pertinent data on this point are limited from both this COE survey and other available studies [11]. Similar patient and graft survival has been reported among kidney and liver transplant recipients over 1 year after transplantation, regardless of donor SARS-CoV-2 infection status [8, 12–14]. Nonetheless, an Italian study with 1 year follow-up found significantly higher rates of hepatic artery thrombosis among recipients of liver grafts from SARS-CoV-2 positive compared with SARS-CoV-2 negative donors [11, 15]. Moreover, an increase in 6- and 12-month mortality has been observed among recipients of hearts obtained from donors with active SARS-CoV-2 infection compared with recipients of hearts from donors with no SARS-CoV-2 infection or with a history of resolved SARS-CoV-2 infection [14]. Further research is needed to evaluate the long-term evolution of recipients from SARS-CoV-2-positive donors, particularly for vascular complications, and to define a more tailored approach to the donor pool.

The use of lung from COVID-19 positive donors is being explored in two COE countries. Recent series have confirmed that lungs from donors with a positive SARS-CoV-2 PCR might be successfully used with cautious donor selection with comparable early post-transplant outcomes to lung allografts from COVID-19-negative donors [10, 14, 16–18]. Lung donor selection include asymptomatic status, high Ct levels (>30–35) and symptom onset or SARS-CoV-2 test positivity older than 20 days [10, 14, 16–18]. Of note that high Ct levels tend to correlate with culture negativity, but Ct values are not available on many platforms and, when obtained, such values may not be comparable between different platforms and laboratories [2]. Further analysis with longer follow-ups is warranted to determine the safety of utilization of COVID-positive donor lungs.

Current strategies for donor evaluation for SARS-CoV-2 infection include universal microbiologic screening with standard SARS-CoV-2 PCR testing, mostly performed within 24 h prior to organ recovery. In keeping with international recommendations, all but one country considered LRT specimen mandatory for lung donation, on the basis of previous unexpected SARS-CoV-2 donor-derived infection in lung recipients, despite negativity of NPS in the donor [6, 10, 19]. Some experts consider that, due to the impact of SARS-CoV-2 positive testing on organ discard, resource utilization and on the basis of data supporting safety of transplanting SARS-CoV-2 non-lung organs, universal testing of non-lung deceased asymptomatic donors should be reconsidered [20].

SARS CoV-2 PCR testing on grafts was performed in three countries; tissue positivity was recently found to be associated with vascular complications after liver transplantation [15]. Further radiological investigations of donors were performed in ~65% of countries either per protocol or on an individual basis to assess organ damage. Of note, the interpretation of CT scans is challenging, as abnormal CT images are common in SARS-CoV-2 infected patients, even when asymptomatic [21].

In most COE countries, waitlisted patients receiving organs from a deceased donor with active or resolved SARS-CoV-2 infection were preferred to have natural, vaccine-induced or hybrid SARS-CoV-2 associated immunity; documented immunological response was rarely required, so the impact of vaccination is uncertain. Pre-transplant SARS-CoV-2 vaccination should be strongly favored given the expected improvement of immune responses before transplant, the possible decreased risk of complications when infection occurs and the advancement in the use of organs from SARS-CoV-2-positive donors [22]. The exclusion of patients from the transplant waiting list for declining SARS-CoV-2 vaccination was performed in about 15% of COE countries and represents a controversial ethical topic [22].

Wide variability exists in transplant practices for SARS-CoV-2 positive candidates for transplantation. The excellent graft and patient post-transplant outcomes presented in recent series favor an individualized approach [23].

Overall, clinical monitoring of recipients of organs from SARS-CoV-2 positive donors was routine across COE countries. Anti-SARS-CoV-2 pre-exposure or post -exposure prophylaxis was not required, and standard immunosuppression was generally recommended.

Healthcare workers were identified early in the pandemic to be at higher risk of contracting SARS-CoV-2 infection [24]. Optimal infection control measures were recommended in COE countries to perform SOT from SARS-CoV-2 positive donors, with no reported infections related to the organ procurement or transplantation among healthcare professionals.

Our study has several limitations. Firstly, it is a survey, which does not allow to establish any causative link or consensus statement, but may serve as a basis for further studies and protocols. Secondly, almost one-third of the COE countries did not join the survey and some of them did not fulfill all the questions, introducing a potential selection bias and providing an

unbalanced representation of COE activity. Thirdly, data on infections among healthcare workers were frequently not available. Finally, the SARS-CoV-2 pandemic situation is fluid and requires regular updating of practices and policies. Therefore, the description made in this paper is likely to change in the near future.

In conclusion, our survey provides the first international assessment on the use of organs from SARS-CoV-2 positive donors in Europe. Growing evidence on the absence of transmission and good short-term outcomes with organs from deceased donors with resolved or active SARS-CoV-2 infection has led to increasing support for the acceptance of such grafts in Member States of the COE. Similarities and differences in management across countries are significant. Additional standardized protocols and prospective studies are needed to assess the best management and long-term outcomes of recipients of organs from SARS-CoV-2 infected donors and to define a more nuanced approach towards safely maintain the donor pool.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The reference European committee on organ transplantation of the COE (CD-P-TO) approved the study and all procedures were in accordance with established ethical standards (TO129).

AUTHOR CONTRIBUTIONS

MP and PG designed the study and the questionnaire and wrote the paper, EG collected the data, MD and MI performed the statistics, MB, MC, GF, ML-F, and BD-GG reviewed the manuscript and gave their contribution to its improvement. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

MP has the following conflict of interest: speaker for Dia Sorin, Merck, Sharp & Dohme, Menarini, Pfizer, Thermofisher; PG has the following conflict of interest: Consulting fees from Merck, Sharp & Dohme, Gilead Sciences, Takeda, Shionogi, Allovir, Astra-Zeneca, Menarini; member of speakers bureau for Merck, Sharp & Dohme, Gilead Sciences, Takeda, Astra-Zeneca.

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.

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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.2024.13705/full#supplementary-material

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Adjusted Donor Age: A Clinical Score to Support Organ Acceptance Decisions in Deceased-Donor Kidney Transplantation

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As transplant programmes have evolved to allow a wider donor pool, organ acceptance decisions have become increasingly complex and lack transparency and equality. Clinical scoring tools exist but there is limited consensus on their use. From a prospective observation of consecutive deceased-donor kidney offers in a large urban transplant centre, a simple score was developed based on donor age and other risk characteristics, excluding ischemia time and graft histology. The score was validated in subsequent cohorts of consecutive offers in the United Kingdom and Germany. In the development cohort of 389 kidney offers, 110 (28%) were transplanted and 175 (45%) declined. Nine risk factors were incorporated into a score based on age, but adjusted for the number of risk factors present, making an "adjusted donor age," with offers separated into equal quintiles by decade. The score was validated in a UK cohort of 380 subsequent offers, and a German cohort of 431 offers. In both cohorts adjusted donor age discriminated between favourable and poor post-transplant outcomes (C-statistic 0.77 in the United Kingdom, 95% CI 0.65–0.88, and 0.71 in Germany, 95% CI 0.64–0.77). Adjusted donor age is a simple score quantifying deceased donor kidney quality, which is consistent with current practice and predicts post-transplant outcome.

Keywords: kidney transplantation, deceased donor, patient empowerment, acceptance, decision making

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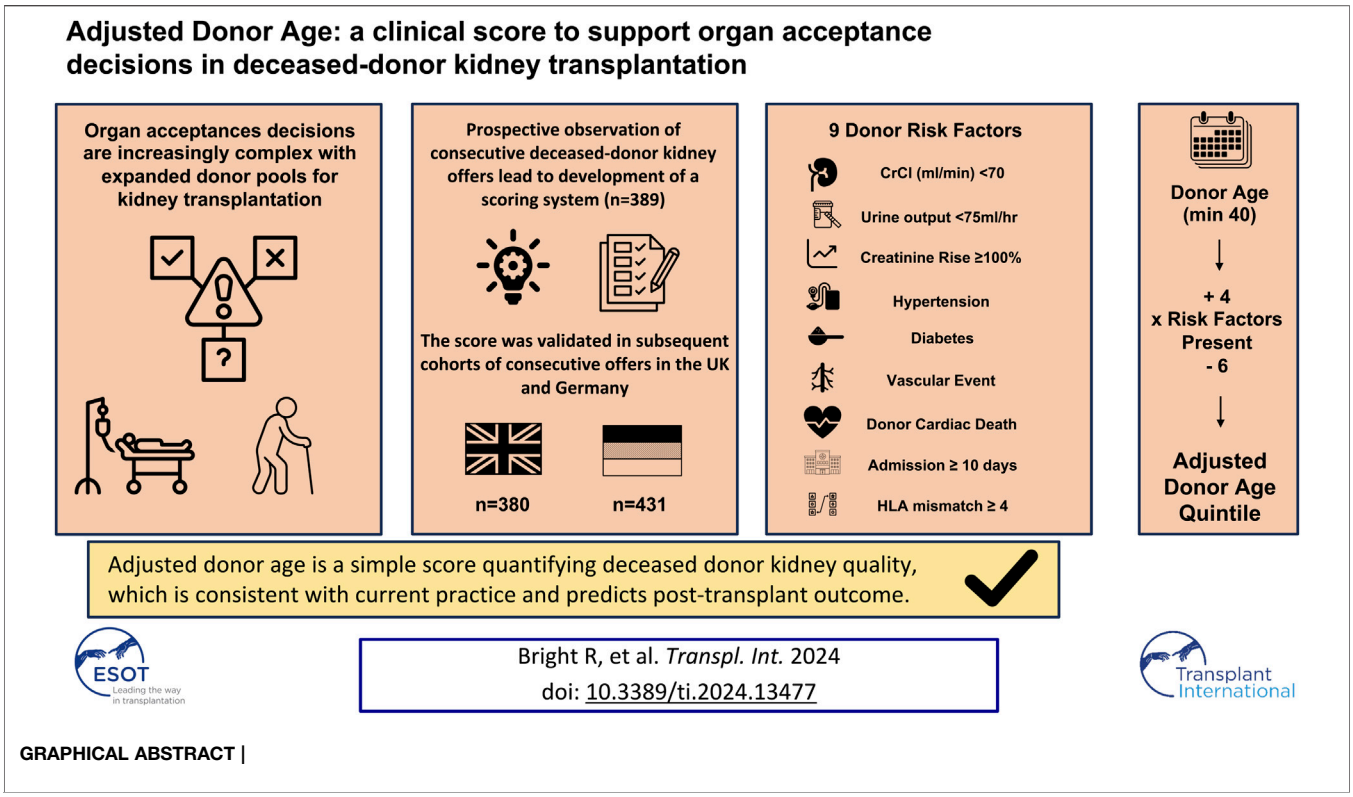
INTRODUCTION

As transplant programmes evolve to tackle the rising burden of end stage kidney disease, the age and comorbidity of those considered as deceased donors has increased [1, 2]. Organ acceptance decisions are therefore increasingly complex, with practices varying significantly between clinicians and institutions, with limited patient involvement in the decision-making process, leading to a system

Abbreviations: CRF, calculated rejection frequency; DCD, donation after circulatory death; GFR, glomerular filtration rate; HLA, anti-human leukocyte antigen; UK, United Kingdom.



GRAPHICAL ABSTRACT |

that lacks transparency and equality [3]. It is a major challenge therefore for clinicians to ensure that the appropriate organ acceptance decisions are made, and that this process is accountable and communicated effectively with patients.

A number of studies have identified donor factors predictive of post-transplant outcome, including donor age and aspects of medical history such as hypertension, stroke, diabetes and kidney function [4]. To support acceptance decisions, clinical tools have been devised which combine several factors into a single numerical score, to indicate quality of the donor kidney, including the Deceased Donor Score [5], Donor Risk Grade [6], Kidney Donor Risk Index [7] and UK Kidney Donor Risk Index [8].

However, scoring systems so far developed have no meaning which is intuitive to patients, and are not calibrated to the donor pool, with over half of all offers placed in the highest category of risk. They provide limited assistance therefore in comparing the current offer to possible future offers, and do little to enhance patient understanding or facilitate a shared decision. There is limited consensus therefore on the use of such tools in the organ acceptance decision process [9]. The hypothesis of this study is that it is possible to develop a valid clinical tool based on age, which can easily be understood by patients, to assist with shared decision making in organ acceptance from deceased kidney donors.

PATIENTS AND METHODS

In two large urban transplant centres, three sequential studies were carried out. The first was a prospective observation of deceased-donor kidney offers in a single UK centre during 2018, collecting donor and recipient characteristics, decisions and clinical outcomes. A simple score was developed based on donor age and other offer characteristics, termed "adjusted donor age," and calibrated to separate all offers into quintiles according to quality. The second was a validation of this score in a subsequent UK cohort of consecutive offers. The third was a further validation in a cohort of consecutive offers in Germany. As a retrospective study of routinely available data, the protocols were approved by the National Research Ethics Service (IRAS Ref 308076) in the United Kingdom, and the local Ethics Committee in Germany (S-187/2022) without requirements for individual consent.

Variables were selected from published donor-scoring literature (age, gender, comorbidities, donor cardiac death, cause of death, length of admission, HLA mismatch) supplemented with additional variables that are commonly considered (smoking, alcohol excess, proteinuria, cardiac arrest duration). Efforts were made to distinguish between acute kidney injury (using urine output and creatinine rise from baseline) and baseline creatinine clearance, which was estimated by the Cockcroft-Gault equation, using a simplified formula for adjusted body weight: (weight+70)/2 (male), (weight+55)/2 (female), and using the average of pre-admission, initial-admission and lowest-during-admission for baseline creatinine. Variables were subsequently excluded from consideration if there was limited or conflicting evidence for outcome prediction in prior literature and the development cohort. Ischaemia times were not considered since they are usually unknown at the time of

the initial decision, and similarly graft histology was not included since it is so rarely available in either institution.

The unit of analysis was the offer of a donor kidney for a particular (named) recipient. Where both kidneys were offered from the same donor (for different recipients), they were considered as separate offers. In a minority of cases no recipient was specified by the allocation system and clinical teams were able to select any suitable recipient. Kidneys declined for one recipient but also thought unsuitable for any recipient were analysed as a single offer for that recipient. Initial acceptance decisions were made by clinicians only, via joint agreement between a nephrologist and a transplant surgeon. Decline decision types were defined as "exclusion" if due to a single qualitative factor occasionally with recipient involvement in the decision (e.g., recent donor cancer) or as "quality" if due to the combination of quantitative variables such as age, creatinine clearance and comorbidities, usually without involvement of the recipient. Decisions were defined as "other" if initially accepted but then not transplanted due to factors outside the control of the clinical team. Most commonly this would be due to a prolonged agonal phase in donation after circulatory death (DCD) donors, leading to withdrawal of the offer, but sometimes recipient factors were involved, for example, if the recipient was unwell or unavailable.

The influence of variables on acceptance decisions was assessed with Fisher's exact test comparing transplanted with declined offers. Outcome prediction was assessed by logistic regression with poor outcome defined as organ failure or GFR below 30 mL/min/1.72 m^2 at 3 months after transplantation, using an average of three consecutive outpatient creatinine measurements. Analyses were performed using Microsoft Excel, JASP (Jeffreys' Amazing Statistics Program, JASP Team, 2020) and RStudio (R Team, 2021).

RESULTS

The development cohort consisted of 389 consecutive kidney offers, from 302 deceased donors (aged 6–84). The majority of offers (93%) were for specified recipients (aged 24–78), with donor and recipient characteristics for all offers provided in **Table 1**. Out of all offers, 110 (28%) were transplanted, 175 (45%) were declined and 104 (27%) initially accepted but then not transplanted due to factors outside the control of the clinical team. Of the 175 offers declined by the clinical team, 43 (11% of all offers) were declined due to an exclusion factor, with the remaining 132 (34% of all offers) declined due to quality concerns.

Several donor characteristics were associated with acceptance decisions including age, creatinine clearance, creatinine rise, urine output, proteinuria, hypertension, diabetes, vascular events and length of the donor's hospital admission (**Table 1**). Decisions had to be made within a short time after receiving the offer, with 39% of decisions made between 21:00 and 06:00.

After 3 months, of the 110 recipients transplanted, 87 (79%) had a favourable clinical outcome, whereas 17 had poor transplant function (GFR below 30 mL/min/1.72 m^2) and 6 kidneys had permanently failed. Donor characteristics predictive of poor transplant outcome (GFR <30 or failure) included age, gender, creatinine clearance, urine output, hypertension, cardiac death, length of admission and HLA mismatch (**Table 1**). Greater recipient weight also predicted poor outcome. Some of the counterintuitive relationships between donor factors and transplant outcome may be explained by collinearity between factors (**Supplementary Table S1**). In a sensitivity analysis, similar prediction characteristics were found using outcome at 12 months post-transplantation (**Supplementary Table S2**).

Donor variables without predictive ability, which were therefore excluded from further analysis, included proteinuria, alcohol excess, smoking, death from stroke and cardiac arrest duration. Gender was also excluded since its effect disappeared after adjustment for creatinine clearance (calculation of which includes gender). The number of kidneys transplanted from donors with diabetes or prior vascular events was small – these factors, which are increasingly prevalent amongst deceased donors, were retained since they exerted a marked influence on acceptance decisions, and would therefore be under-estimated as predictors of post-transplant outcome. Nine risk factors were therefore incorporated into a score based on age, but adjusted for the number of risk factors present, making an "adjusted donor age" (**Figure 1**). Thresholds and coefficients were selected to optimise prediction, with risk factors scaled by 4 years, so that the score remains largely age-dependent (like other scores such as KDRI) with 6 years subtracted to centre the distribution. Since recipient weight was a strong predictor of outcome, weight-dependent thresholds were used for donor creatinine clearance.

As expected, the adjusted donor age was still strongly correlated with donor age with coefficient of determination (R^2) 0.75, suggesting that 75% of the variation in adjusted donor age was explained by donor age. Age was therefore the dominant determinant of the score, as it is with published scoring systems: calculating the Kidney Donor Risk Index [7] and UK Kidney Donor Risk Index [8] in this cohort, gave coefficients of determination of 0.76 and 0.72, respectively for the relationship with donor age. Using published risk thresholds for either score however, over half of all offers from this cohort fell into the highest risk category, whereas offers were separated approximately into quintiles using decade of adjusted donor age (**Table 2**).

The adjusted donor age score was validated in separate cohorts from the UK and Germany. The UK validation cohort consisted of 377 consecutive offers, of which 96 (25%) were transplanted, 176 (47%) declined and 105 (28%) initially accepted but then not transplanted. Three months after transplantation, outcomes in this cohort were similar, with a favourable 3-month clinical outcome (GFR above 30 mL/min/1.72 m^2) was seen in 78 recipients (81%). All risk factors were validated in this cohort by association with acceptance decisions or outcome or both, and greater recipient weight remained marginally predictive of poor outcome (**Table 3**).

TABLE 1 | Offer characteristics, decision and outcome post-transplant in the development cohort (N = 389).

	Acceptance decisions (N = 389)				Post-transplant outcome univariate (N = 110)			Selection[e]
	All offers	Transplant (110)	Declined (175)	*p*-value[a]	OR[b]	95% CI	*p*-value	
Donor								
Age (years)	60 (51–71)	56 (47–67)	68 (59–76)	0.001	1.07	1.03–1.12	0.002	
Male gender	205 (53)	59 (54)	84 (48)	0.330	0.40	0.18–0.91	0.066	Excluded
CrC (mL/min) continuous[c]	86 (70–112)	89 (74–113)	75 (60–92)	0.002	0.96	0.94–0.98	0.000	
<70[d]	102 (26)	24 (22)	67 (38)	0.006	6.61	2.75–15.9	0.000	
Creatinine ≥100% rise	42 (11)	8 (7)	29 (17)	0.029	0.54	0.09–3.31	0.579	
Urine <75 mL/h	143 (37)	36 (33)	77 (44)	0.082	4.02	1.77–9.11	0.005	
Proteinuria (>1+)	189 (49)	41 (37)	113 (65)	0.000	1.11	0.43–2.84	0.836	Excluded
Hypertension	168 (43)	34 (31)	94 (54)	0.000	2.78	1.24–6.24	0.037	
Diabetes	53 (14)	8 (7)	31 (18)	0.014	1.35	0.33–5.50	0.725	
Vascular event	42 (11)	2 (2)	31 (18)	0.000	2.02	0.26–15.8	0.572	
Alcohol excess	69 (18)	18 (16)	31 (18)	0.873	0.40	0.12–1.31	0.204	Excluded
Smoking	158 (41)	40 (36)	68 (39)	0.802	0.77	0.32–1.88	0.631	Excluded
Donor cardiac death	151 (39)	35 (32)	68 (39)	0.310	3.34	1.49–7.51	0.014	
Stroke (cause of death)	167 (43)	46 (42)	78 (45)	0.806	0.74	0.33–1.66	0.536	Excluded
Arrest duration >30 min	67 (17)	21 (19)	32 (18)	0.876	0.99	0.97–1.01	0.444	Excluded
Admission ≥10 days	41 (11)	6 (6)	21 (12)	0.095	4.42	1.08–18.0	0.082	
HLA mismatch ≥4	82 (21)	24 (22)	37 (21)	0.882	1.55	1.01–2.37	0.093	
Recipient (N = 363)								
Age (years)	56 (48–63)	55 (47–65)	56 (47–62)	0.535	1.03	0.99–1.08	0.114	
Weight (kg)	75 (66–85)	74 (64–87)	75 (67–86)	0.639	1.05	1.02–1.09	0.003	
CRF > 50%	80 (22)	23 (20.9)	33 (22)	0.647	0.75	0.23–2.48	0.636	
Wait time (years)	3.4 (2.1–5.0)	2.8 (1.7–4.2)	3.5 (2.2–4.9)	0.009	1.16	0.93–1.45	0.193	

Data provided as number (%) or median (IQR), results shaded if p *< 0.10. HLA, human leukocyte antigen; CRF, calculated reaction frequency.*

[a]p *value comparing transplanted with declined.*

[b]*OR, odds ratio for poor outcome (GFR <30) at 3 months.*

[c]*CrC, creatinine clearance, as continuous variable.*

[d]*CrC: creatinine clearance, as threshold dependent on recipient weight: 60 (<65 kg), 70 (65–85 kg), 80 (>85 kg).*

[e]*Variables excluded as contributors to adjusted donor age are shown (see text).*

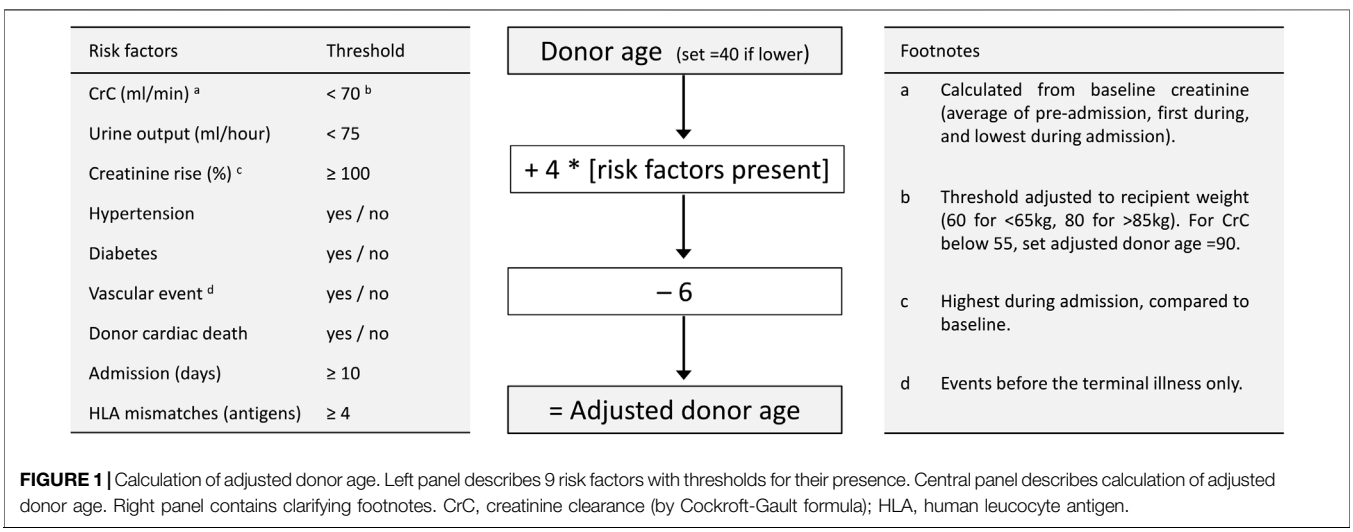


FIGURE 1 | Calculation of adjusted donor age. Left panel describes 9 risk factors with thresholds for their presence. Central panel describes calculation of adjusted donor age. Right panel contains clarifying footnotes. CrC, creatinine clearance (by Cockroft-Gault formula); HLA, human leucocyte antigen.

The German validation cohort included 431 consecutive offers, of which 173 (40%) were transplanted, 224 (52%) declined and 34 (8%) initially accepted but then not transplanted (a smaller category due to the absence of DCD donors). A favourable 3-month clinical outcome (GFR above 30 mL/min/1.72 m^2) was seen in 146 (84%). Apart from vascular events and length of admission, risk factors were also validated in this cohort by association with acceptance decisions or outcome or both (**Table 4**).

The ability of adjusted donor age to predict both acceptance decision and outcome after transplantation was analysed by calibration and discrimination in both validation cohorts. In the UK validation cohort adjusted donor age was well

TABLE 2 | Offer characteristics by adjusted donor age and established risk scores in the development cohort (N = 389).

	Risk category				
	1	**2**	**3**	**4**	**5**
Adjusted donor age	<49	50–59	60–69	70–79	>80
Offers (number)	89 (23)	81 (21)	62 (16)	79 (20)	78 (20)
Age (years)	42 (32–49)	54 (51–57)	61 (59–65)	71 (68–74)	75 (69–78)
Risk factors present[a]	1 (0–2)	2 (1–2.5)	2 (2–3)	3 (2–3)	4 (3–5)
US KDRI[b]	<0.75	0.75–0.91	0.91–1.11	1.11–1.39	>1.39
Offers (number)	28 (7)	25 (6)	51 (13)	67 (17)	218 (56)
Age (years)	28 (20.5–32.5)	39 (35–46)	50 (47–53.5)	54 (51.5–58)	70 (64–75)
Risk factors present[a]	1.5 (0–3)	1 (0–2)	1 (0–3)	2 (1–2.5)	3 (2–5)
UK KDRI[c]	<0.87	0.87–1.02	1.02–1.34	>1.34	
Offers (number)	41 (11)	56 (14)	85 (22)	207 (53)	
Age (years)	28 (23–35)	53.5 (50–56)	52 (47–55)	70 (65–76)	
Risk factors present[a]	1 (0–2)	2 (0–2)	2 (1–3)	3 (2–5)	

Data provided as N (%) or median (IQR). KDRI, kidney donor risk index.
*[a]Risk factors are those given in **Figure 1**.*
[b]Rao, Transplantation, 2009, scaled to median offer and using updated quintile boundaries from 2018 USA cohort.
[c]Watson, Transplantation, 2012, using original quartile boundaries from 2000 to 2007 UK cohort.

TABLE 3 | Offer characteristics, decision and outcome post-transplant in the UK validation cohort.

	Acceptance decisions (N = 377)				Post-transplant outcome univariate (N = 96)			Post-transplant outcome multivariate[a]		
	All offers	**Transplant (96)**	**Declined (176)**	***p*-value[b]**	**OR[c]**	**95% CI**	***p*-value**	**OR[c]**	**95% CI**	***p*-value**
Donor										
Age (years)	61 (51–71)	55 (48–66)	66 (54–72)	0.000	1.06	1.01–1.11	0.016	1.09	1.03–1.16	0.006
CrC (mL/min) < 70[d]	92 (24)	21 (22)	58 (33)	0.069	4.00	1.33–12.1	0.014			
Creatinine ≥100% rise	46 (12)	8 (8)	34 (19)	0.022	0.60	0.07–5.18	0.640			
Urine <75 mL/h	183 (49)	36 (38)	99 (56)	0.004	1.08	0.38–3.08	0.893			
Hypertension	153 (41)	30 (31)	92 (52)	0.001	1.10	0.37–3.29	0.859			
Diabetes	42 (11)	5 (5)	34 (19)	0.001	1.00	0.00–>100	0.993			
Vascular event	42 (11)	3 (3)	30 (17)	0.000	1.00	0.00–>100	0.991			
Donor cardiac death	189 (50)	34 (35)	94 (53)	0.005	2.81	0.99–8.01	0.053			
Admission ≥10 days	31 (8)	3 (3)	16 (9)	0.082	9.50	0.81–>100	0.073			
HLA mismatch ≥4	156 (41)	49 (51)	61 (35)	0.010	6.32	1.33–30.1	0.021			
Risk factors present[e]					1.67	1.08–2.58	0.020	1.72	1.02–2.89	0.042
Recipient (N = 333)										
Weight (kg)	75 (65–88)	78 (65–89)	75 (66–90)	0.729	1.02	1.00–1.04	0.095	1.04	1.01–1.07	0.006

Data provided as number (%) or median [IQR], results shaded if p < 0.10. HLA, human leukocyte antigen.
[a]multivariable model adjusted for variables shown.
[b]p value comparing transplanted with declined.
[c]OR, odds ratio for poor outcome (GFR<30) at 3 months.
[d]CrC, creatinine clearance, as threshold dependent on recipient weight: 60 (<65 kg), 70 (65–85 kg), 80 (>85 kg).
[e]Total number of donor risk factors (from the above list) present.

calibrated to decisions with each quintile increasing the rate of decline (28%, 24%, 37%, 58% and 79%) with OR 1.91 per quintile (95% CI 1.62–2.27, **Figure 2**). In Germany the rate of decline similarly increased with each quintile of adjusted donor age (37%, 44%, 44%, 52% and 68%) with OR 1.36 per quintile (95% CI 1.20–1.54, **Figure 2**). In both cohorts adjusted donor age discriminated between decisions (C-statistic 0.74 in the UK, 95% CI 0.69–0.79, and 0.72 in Germany, 95% CI 0.68–0.76).

In both cohorts adjusted donor age was calibrated to 3-month post-transplant outcome with each quintile increasing the likelihood of a poor outcome post-transplantation: 0%, 12%, 22%, 32% and 57% in the UK with OR 2.29 per quintile (95% CI 1.39–3.77, **Figure 2**) and 4%, 3%, 11%, 28% and 36% in Germany with OR 2.09 (95% CI 1.49–2.92, **Figure 2**). In both cohorts adjusted donor age discriminated between post-transplant outcomes (C-statistic 0.77 in the UK, 95% CI 0.65–0.88, and 0.71 in Germany, 95% CI 0.64–0.77). Receiver operating characteristic curves illustrating the ability of adjusted donor age to discriminate between favourable and poor outcome offers in the combined cohort is shown in **Figure 3**.

DISCUSSION

This paper describes a novel clinical tool for scoring the quality of a donor kidney offer, which is simple to calculate, calibrated

TABLE 4 | Offer characteristics, decision and outcome post-transplant in the German validation cohort.

	Acceptance decisions (N = 431)				Post-transplant outcome univariate (N = 173)			Post-transplant outcome multivariate[a]		
	All offers	Transplant (173)	Declined (224)	*p*-value[b]	OR[c]	95% CI	*p*-value	OR[c]	95% CI	*p*-value
Donor										
Donor age (years)	63 [52–78]	60 [50–72]	68 [56–80]	0.000	1.08	1.04–1.13	0.000	1.07	1.03–1.11	0.001
CrC (mL/min) < 70[d]	123 (29)	66 (32)	57 (25)	0.170	2.12	0.91–4.92	0.080			
Creatinine ≥100% rise	75 (17)	23 (11)	52 (23)	0.001	0.34	0.04–2.65	0.301			
Urine <75 mL/h	109 (25)	39 (19)	70 (31)	0.004	1.87	0.71–4.93	0.205			
Hypertension	225 (52)	104 (50)	121 (54)	0.492	6.24	2.06–18.9	0.001			
Diabetes	62 (14)	21 (10)	41 (18)	0.023	4.29	1.49–12.4	0.007			
Vascular event	53 (12)	23 (11)	30 (13)	0.566	1.09	0.29–4.06	0.896			
Admission ≥10 days	37 (9)	15 (7)	22 (10)	0.435	0.43	0.05–3.45	0.426			
HLA mismatch ≥4	92 (21)	53 (26)	39 (17)	0.050	4.61	1.96–10.9	0.000			
Risk factors present[e]					1.97	1.40–2.78	0.000	1.50	1.01–2.22	0.045
Recipient (N = 305)										
Weight (kg)	75 [64–88]	76 [65–88]	74 [63–87]	0.728	1.00	0.97–1.02	0.906			

Data provided as number (%) or median [IQR], results shaded if p < *0.10. HLA, human leukocyte antigen.*
[a]*multivariable model adjusted for variables shown.*
[b]p *value comparing transplanted with declined.*
[c]*OR, odds ratio for poor outcome (GFR<30) at 3 months.*
[d]*CrC, creatinine clearance, as threshold dependent on recipient weight: 60 mL/min (<65 kg), 70 mL/min (65–85 kg), 80 mL/min (>85 kg).*
[e]*Total number of donor risk factors (from the above list) present.*

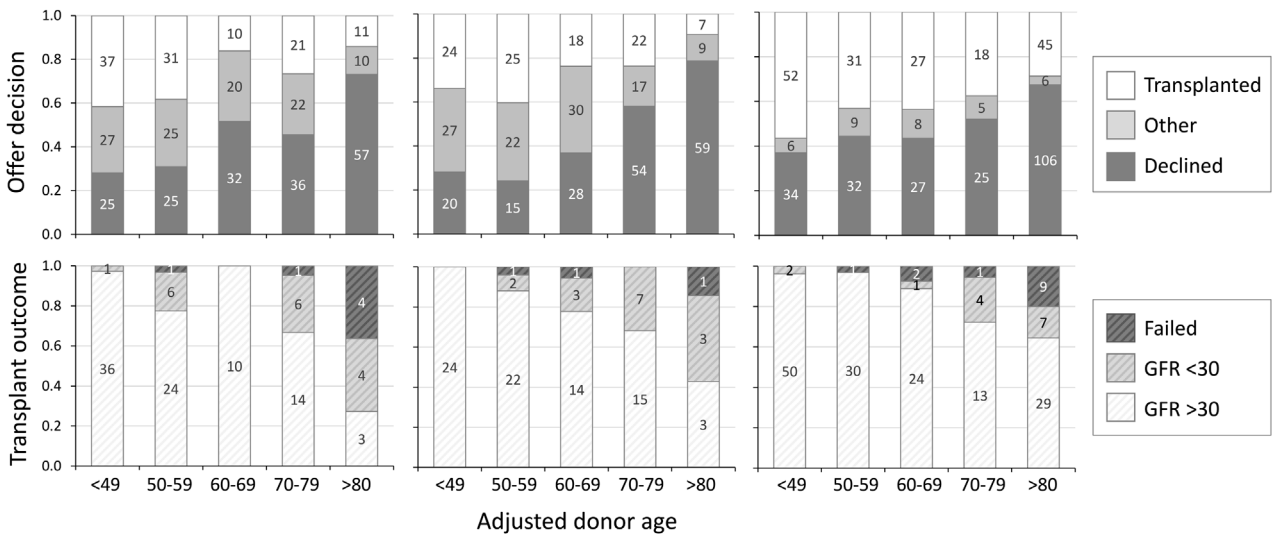


FIGURE 2 | Offer decision and transplant outcome by adjusted donor age. Offer decision (upper panels) and transplant outcome at 3 months (lower panels) by adjusted donor age, in UK development cohort (left), UK validation cohort (centre) and German validation cohort (right). Decision "other" = offers accepted but not transplanted due to non-donor factors. GFR: glomerular filtration rate (ml/min/1.72 m^2).

to current acceptance practice, and predictive of outcome after transplantation. Such tools have appeared increasingly necessary as transplant procurement practices have evolved, from their conservative beginnings to an era of expanded criteria, allowing a much wider pool of potential donors. Studies have confirmed the survival benefit of transplantation from higher risk donors in selected recipients [10–12] but the greater variation in donor quality has made acceptance decisions increasingly complex and recipient-specific [13].

In making decisions about transplant offers, clinicians face a discrete choice within a skewed outcome distribution: kidney transplantation is usually successful, quickly and dramatically improving both quantity and quality of life. But an unsuccessful transplant, though much less common, may be fatal or disabling, or at best provide only a short reprieve from the burden of dialysis, often leaving the patient sensitised with limited prospects for re-transplantation. When to grasp opportunity, and when to play safe, is difficult to determine, not helped by the large number of donor and recipient factors which must be considered, the

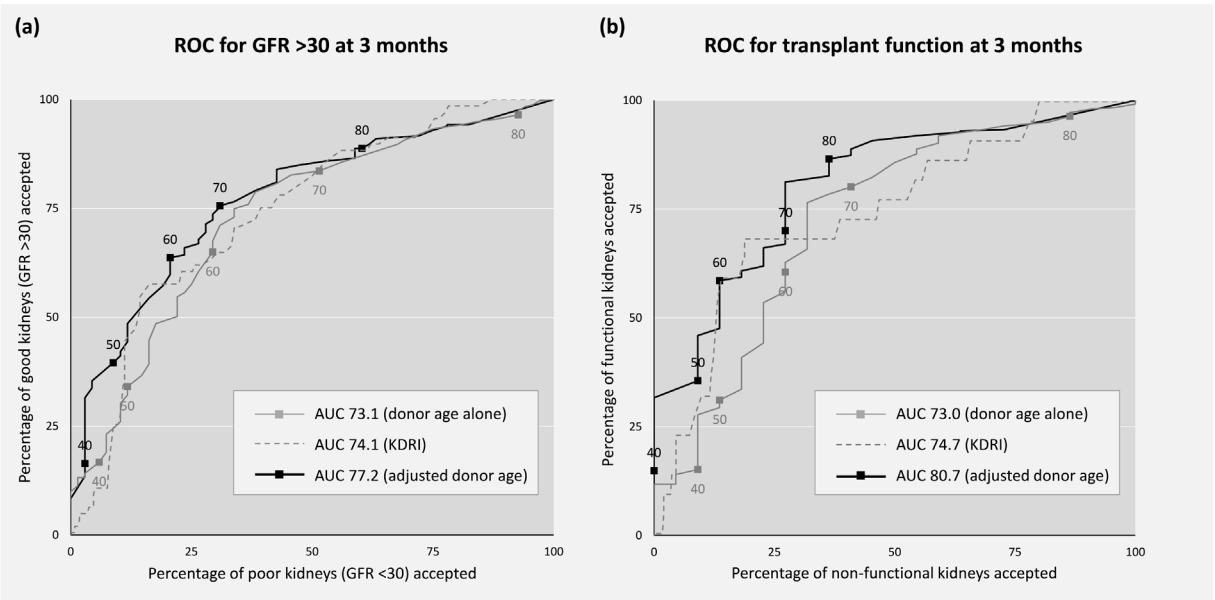


FIGURE 3 | Receiver operating characteristic (ROC) curves. Sensitivity (good kidneys accepted) and 1-specificity (poor kidneys accepted) by threshold of donor age (grey), KDRI (grey dash) and adjusted donor age (black) in the combined cohort. Good outcome is defined as: **(A)** transplant GFR >30 at 3 months vs <30, and **(B)** transplant functioning at 3 months vs non-function. GFR, glomerular filtration rate (ml/min/1.72 m^2), KDRI, kidney donor risk index.

limited evidence base which lacks clear consensus, and the short timeframe within which decisions must be made, frequently at night.

Unsurprisingly marked variation in practice is seen between centres, with many kidneys being sequentially declined before finally being accepted and successfully transplanted – a process which leads to inequality, with marked regional differences in wait-time to transplantation [14]. That clinicians struggle with these decisions is also highlighted by the increased decline rate observed with night-time or weekend decisions [15, 16]. When clinical decisions are difficult due to complexity and time-pressure, yet stereotyped since the same concepts apply to every decision, a numerical tool offers a way to support decision making by framing the information, leading to consistency with less unwarranted variation.

Several clinical tools have previously been published which provide a numerical measure of the quality of a deceased donor kidney offer [5–8]. These have been developed from multivariate analysis of large registries, assessing the ability of donor characteristics to predict post-transplant outcome (either GFR at 6 months or time to transplant failure), leading to a score based heavily on donor age, but also including a small number of other characteristics, which partially overlap between studies. The "adjusted donor age" described in this study is similar to these tools in having age as the dominant contributor, and in predicting post-transplant outcome, but there are a number of important differences which are advantageous.

Firstly, currently available tools have boundaries which classify offers with respect to specific outcomes, rather than against the offer pool. Indeed, by the two most recently published tools [7, 8], over half of the offers in the development cohort would fall into the highest risk category. This is another significant limitation, since the acceptance decision is largely a comparative one, involving the likelihood of receiving a higher quality kidney offer within a short time. Decade boundaries of the adjusted donor age separate offers into quintiles, and although this might require recalibration over time or in different transplant programmes, the concept allows the current offer to be considered against future ones. The need for greater comparative thought in the decision process is obvious when one considers the significant number of offers declined despite belonging to the most favourable quintile, without an exclusion factor (i.e., on grounds of quality). Most often this arises from a failure to appreciate that the presence of several risk factors may be entirely offset by favourable donor age.

Secondly, although kidney function is an accepted predictor of post-transplant outcome, current tools base their estimate of function on terminal creatinine, thus failing to distinguish between chronic and acute kidney dysfunction, with recent studies suggesting the latter has only a much smaller impact on outcome. In contrast, the adjusted donor age is based on creatinine clearance estimated from baseline function, with creatinine rise and urine output as measures of acute dysfunction. Interestingly, whilst baseline creatinine clearance was a strong predictor of both decision and outcome in both cohorts, creatinine rise predicted decisions but not post-transplant outcome, suggesting it may be over-valued by decision makers. As the pool of potential donors expands, the frequency of offers with acute kidney injury will increase, so it will be increasingly important to distinguish between the large effect of chronic kidney disease and the lesser effect of acute kidney injury. Using different creatinine clearance thresholds according

to recipient weight is also helpful in accounting for the negative impact of recipient size on outcome.

Finally, and most importantly, the adjusted donor age has an intuitive meaning: the age of the typical donor (with no unusual risk factors) equivalent in quality to the current offer. Since it can be readily understood, this would facilitate a discussion with the potential recipient and their involvement in the process. Though the value of shared decisions is widely appreciated, in current practice decisions are often made by clinicians only, or with limited patient involvement, in part due to the difficulty in expressing the balance of risk. Such communication is regarded as an essential part of the informed consent process [17] and linking new information to a familiar principal is believed to aid understanding [18]. This intuitive meaning, which also frames the offer within the whole distribution, may therefore facilitate patient understanding and involvement in a shared acceptance decision. Further study would be needed to assess patient feedback on the use of the score, as well as the influence on practice within centres.

There are several important limitations to this study, in particular the dual-centre and relatively small size for this type of study may reduce the ability to assess risk factors reliably. This is partially offset by the advantages of greater data granularity, the ability to understand the decision-making process, and consistency of other aspects of care which may influence outcome. There are clinical practice differences between the two centres, most notably in DCD transplantation which is not performed in Germany, leading, for example, to a much smaller group of accepted offers which did not proceed. Although the tool still validated reasonably well in the German cohort, it is possible that specific optimisation may enhance its utility in this setting.

The adjusted donor age score model does not incorporate graft histology or ischemia time. This is due to graft histology rarely playing a role in organ acceptance decisions in both the UK and Germany. Obtaining optimal pre-implantation graft histology results in the deceased donor setting is also challenging. Likewise, both warm and cold ischemia time are typically not known at the point of the initial offer acceptance decision, when the score is designed to be used. In the UK, a long warm ischaemia time is rare: typically, if there is an excessive delay between withdrawing life-support treatment and circulatory death, the retrieval is cancelled and the offer withdrawn.

As with all such tools, the outcome is only known for those kidneys which are accepted for transplantation, and a characteristic which is highly predictive of a declined offer, will be largely absent from the dataset of transplanted kidneys, and therefore have limited ability to also predict post-transplant outcome. The use of 3-month GFR as the outcome measure overlooks overall transplant survival, which is more meaningful to patients, though it may more easily capture the effect of donor-specific factors, the influence of which becomes diluted over time.

The study is specific to the UK and German deceased-donor transplant programs, and applicability beyond Europe is unknown. Whilst recalibration is likely to be necessary, the concept of an age-adjusted score to assist acceptance decisions and patient involvement should still be widely applicable. One drawback of all clinical tools including this study is outcome evidence restricted to those offers which proceeded to transplantation: the comparator needed is outcomes after not accepting the offer, such as time to the next offer, quality of that offer, and mortality or removal from the waiting list before transplantation is achieved. This outcome has received little research attention, but future studies will hopefully address this important knowledge gap.

The adjusted donor age score provides a transparent method of quantifying deceased donor kidney quality, which is consistent with current practice and predicts post-transplant outcome. Its intuitive meaning, which frames the offer against the donor distribution, may support organ acceptance decision making and facilitate meaningful patient involvement in the process.

DATA AVAILABILITY STATEMENT

The data underlying this article cannot be shared publicly due to the privacy of individuals that were included in the study. The data will be shared on reasonable request to the corresponding author.

ETHICS STATEMENT

As a retrospective study of routinely available data, the protocols were approved by the National Research Ethics Service (IRAS Ref 308076) in the UK, and the local Ethics Committee in Germany (S-187/2022) without requirements for individual consent.

AUTHOR CONTRIBUTIONS

DA, RB, CFM, FD, CM, and FK conceived the study. AA, DD, EM, CFM, FF, CN, CSp, LB, DG, MS, CSo, MM, AM, MZ, CM, and RB collected the data. DA and CFM performed the analysis. DA, RB, CFM, CM, and FK wrote the initial manuscript, which was revised and agreed by all authors. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2024.13477/full#supplementary-material

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Vascular Reconstruction of Multiple Renal Arteries—A Risk Factor for Transplant Renal Artery Stenosis: Insight From a Matched Case-Control Study

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Transplant Renal Artery Stenosis (TRAS) is the leading vascular complication following kidney transplantation (KT), causing premature allograft loss and increased post-KT mortality. While risk factors for TRAS, such as prolonged cold ischemia time and delayed graft function, are well-documented in deceased donor-KT, the risk factors remain less clearly defined in living donor-KT. This matched case-control study, conducted at a leading national transplant center predominantly performing living donor-KT, evaluated risk factors and long-term outcomes of clinical TRAS (cTRAS). cTRAS cases diagnosed from January 2009 to December 2022 were matched with four control kidney transplant recipients (KTRs) in a study powered to assess whether *ex-vivo* arterial vascular reconstruction of multiple renal arteries (VR-MRA) increases the risk of cTRAS. Among 2,454 KTs, 28 KTRs (1.14%) were diagnosed with cTRAS around 3.62 ± 1.04 months post-KT, with renal allograft dysfunction (92.86%) as the most common presenting feature. Notably, 27 cTRAS cases were successfully treated with endovascular intervention, yielding favorable outcomes over a 6–180 months follow-up period. The study identified *ex-vivo* VR-MRA as an independent risk factor for cTRAS ($P < 0.001$).

Abbreviations: ATN, Acute tubular necrosis; BMS, Bare metal stents; CDU, Color Doppler ultrasound; CIT, Cold ischemia time; cTRAS, Clinical transplant renal artery stenosis; DES, Drug-eluting stents; DGF- Delayed graft function; DM, Diabetes mellitus; ECD, Expanded criteria donors; EIA, External iliac artery; ES, end-to-side anastomosis; ESRD, End-stage renal disease; EVI, Endovascular interventions; eGFR, Estimated glomerular filtration rate; IH, Neointimal Hyperplasia; IIA, Internal iliac artery; ISR, In-stent restenosis; KT, Kidney transplantation; KTRs, Kidney transplant recipients; MRA, Multiple renal arteries; PTA, Percutaneous transluminal angioplasty; RA, Renal Artery; RBF, Renal blood flow; Scr, Serum creatinine; SGF, Slow graft function; SRA, Single renal artery; STS, Senior transplant surgeon; TRAS, Transplant renal artery stenosis; VR-MRA, *Ex-vivo* back-table vascular reconstruction of multiple renal arteries.

cTRAS cases receiving timely treatment exhibited long-term outcomes in graft and patient survival similar to control KTRs. Early screening and timely intervention for cTRAS post-KT may improve graft and patient outcomes.

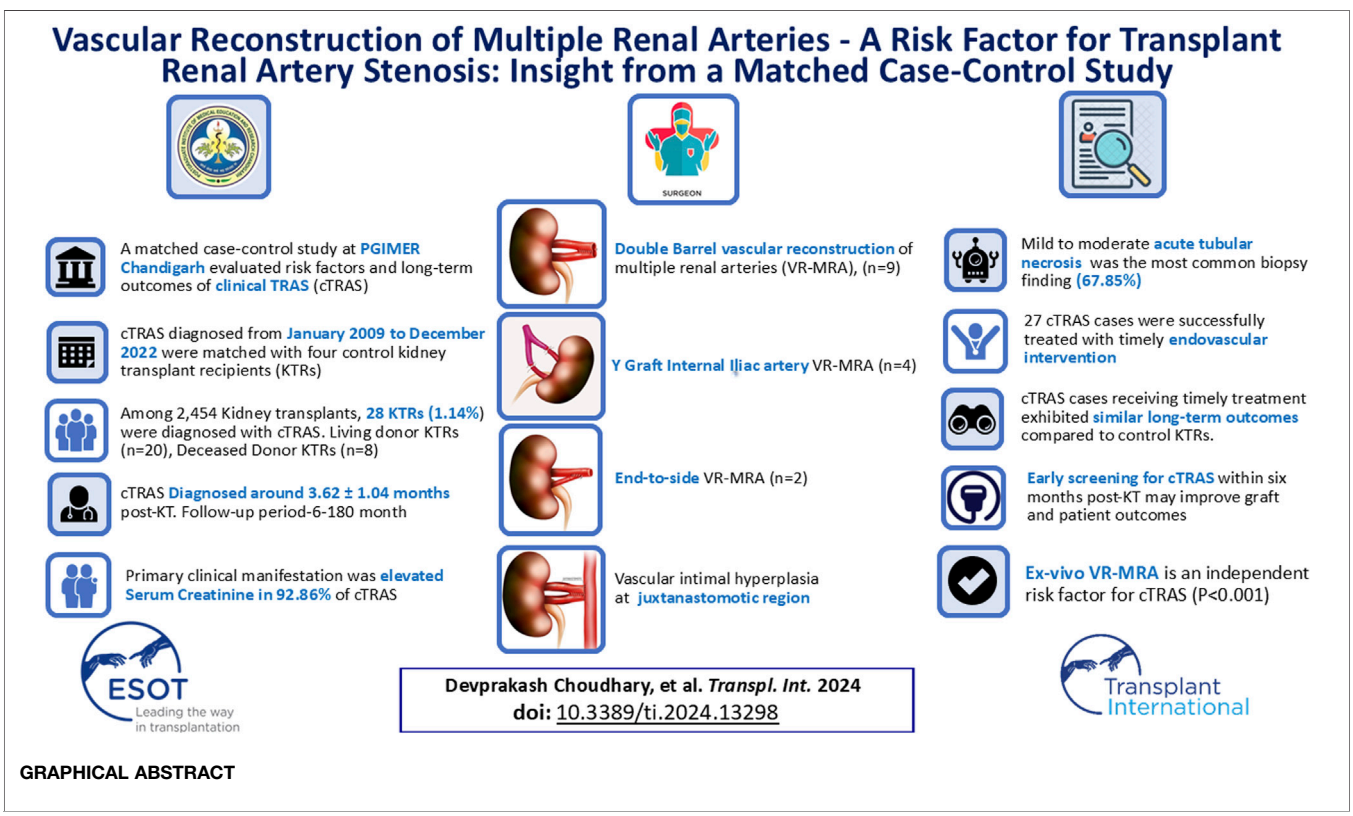
Keywords: transplant renal artery stenosis, vascular reconstruction, multiple renal arteries, ex-vivo back-table reconstruction, endovascular intervention

INTRODUCTION

Kidney transplantation (KT) is the optimal therapy for individuals with end-stage renal disease (ESRD). With advancements in immunosuppression, non-immunological elements have emerged as the primary cause of allograft loss and mortality among kidney transplant recipients (KTRs). During the initial 6 months post-KT, surgical complications present a higher risk of allograft loss compared to allograft rejection [1]. Transplant Renal Artery Stenosis (TRAS) is the predominant vascular complication following KT, accounting for 75% of such issues. TRAS significantly contributes to allograft dysfunction, allograft loss, and premature death amongst KTRs [2]. The reversible nature of TRAS emphasizes the importance of prompt diagnosis and timely intervention to prevent irreversible allograft damage caused by TRAS, thereby reducing allograft loss and improving patient survival [3].

Since its first identification in 1973, varying diagnostic criteria and improved screening techniques have resulted in a reported increase in the incidence of TRAS from 1% to 23% post-KT [3–5]. However the majority of risk factors associated with TRAS were described concerning deceased donor-KT, like expanded criteria donors (ECD), older donors and recipients, prolonged cold ischemia time (CIT), delayed graft function (DGF), allograft-rejection, diabetes-mellitus (DM), and atherosclerotic vessels [3]. Nevertheless, these risk factors are much less prevalent in living donor-KT.

In a series of clinical-TRAS (cTRAS) following living donor-KT, the utilization of internal iliac artery Y-graft for allografts with multiple renal arteries (MRA) has been suggested as a potential risk factor for cTRAS, indicating a complex interaction of anatomical variations and vascular reconstruction surgical techniques affecting the risk of cTRAS in living donor-KT [6]. This matched case-control study, conducted at a leading national transplant center known for primarily performing living donor-KT, was designed to identify various risk factors and outcomes associated with cTRAS. The present study hypothesized that *ex-vivo* back-table vascular reconstruction of multiple renal arteries (VR-MRA) could significantly contribute to the development of cTRAS by inducing vascular intimal hyperplasia (IH) at the juxtanastomotic region. This IH could disproportionately



GRAPHICAL ABSTRACT

affect the luminal diameter, particularly in the reconstructed smaller vessels of multiple renal arteries, thereby providing a biological rationale for the occurrence of cTRAS.

PATIENTS AND METHODS

Study Population

This study involving data from human participants was approved by the Institutional Ethical Committee of the Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, INDIA (NK/7617/study/710). The research adhered to the ethical standards outlined in the 1964 Declaration of Helsinki and its subsequent amendments or comparable ethical standards. Considering the retrospective nature of the study, the informed consent was waived by the PGIMER ethics committee. The study included KT performed from January 2009 to December 2022. The follow-up duration extended from the date of KT to December 2023.

Cases and Controls

Cases and controls were selected from the study center's prospectively maintained electronic database. The cases comprised KTRs diagnosed with cTRAS. The matched control encompassed KTRs who underwent KT within the same calendar year but did not develop cTRAS.

Matching Criteria and Control Allocation

Each TRAS case was matched with four control KTRs using nearest neighbor matching to control for confounding factors. Subject in the cTRAS group was matched with the nearest control subject (KTRs) transplanted by the same surgeon in the same year the cTRAS case was diagnosed based on observed characteristics. The matching criteria included time from KT, senior operating surgeon, KTR age (within ±5 years), KTR gender, KT timing, and type of transplant (living vs. deceased donor). By maintaining consistency in the operating surgeon and these various parameters, we aimed to minimize bias and ensure comparability between the groups.

cTRAS Definition

cTRAS observed post-KT was identified following an elevation in serum creatinine (Scr-mg/dL) by over 20% from baseline or the presence of symptoms, including reduced urine output, fluid retention, weight gain, or worsening uncontrolled hypertension requiring more than one antihypertensive medication after ruling out other causes of allograft dysfunction, such as allograft-rejection, infection, drug-toxicity, acute kidney injury, or recurrence of primary disease. The diagnosis of cTRAS is then confirmed through selective renal angiography following supportive color Doppler ultrasound (CDU) findings.

Positive CDU criteria included a renal artery peak systolic velocity of ≥200 cm/s and/or distal spectral broadening or a parvus tardus waveform with a low resistive index (<0.5) in post-stenotic intrarenal arteries.

Confirmatory angiographic evidence of cTRAS included renal artery stenosis >50% of the renal artery (RA) internal diameter with successful stenosis correction leading to an improved renal-allograft function and/or blood pressure regulation.

Hypertension (HTN) post-KT in KTRs was defined as blood pressure readings exceeding 130/80 mm Hg on more than two separate occasions. First-line agents included calcium channel blockers; second-line were thiazide diuretics and/or Beta-blockers or (ACE inhibitors or ARBs).

Exclusion Criteria

KTRs who experienced immediate postoperative technical complications, such as RA dissection or kinking, requiring intervention within 1 month post-KT for renal artery stenosis, were excluded.

Study Aim

To investigate the risk factors and outcomes associated with cTRAS amongst KTRs.

Study Objective

To evaluate the association between VR-MRA and the heightened risk of cTRAS.

Study Hypothesis

Performing VR-MRA to create a common channel for vascular implantation is associated with an increased risk of cTRAS.

Study Parameters: Baseline Characteristics

Type of transplant (living donor-KT or deceased donor-KT), baseline donor and recipient demographics, pre-transplant hemodialysis duration, and HLA mismatch.

Intraoperative Variables

Donor's kidney side, warm ischemia time (WIT) (time from intraoperative renal-artery clamping until cold organ flush), CIT (from cold organ flush until the kidney was removed from ice for KT), back-table vascular reconstruction (illustrated as pantaloon/double-barrels **Figure 1A**, recipient internal iliac artery Y-graft **Figure 1B**, or end-to-side anastomosis of a small artery to the main artery **Figure 1C**, vascular anastomosis time, graft-kidney weight, main renal-allograft vessel anastomosis method (either end-to-end anastomosis {EE} to the internal iliac vessel or end-to-side anastomosis {ES} to the external iliac vessel), and anti-thymocyte globulin induction (ATG).

Immediate Post-Transplant Indicators

DGF (dialysis requirement in the first week post-KT), slow graft function (SGF) (SCr >1.5 mg/dL for more than 10 days following KT), biopsy-proven acute rejection (BPAR), and hospital stay duration.

Post-KT Discharge Metrics

CMV infection, baseline graft function (mean of last five SCr (mg/dL) following stabilization of allograft function past 1-month post-KT), time to TRAS (days), which was defined as the time from KT till clinical manifestation as per the cTRAS definition, and the number of antihypertensive medications used to control HTN pre- and post-TRAS intervention.

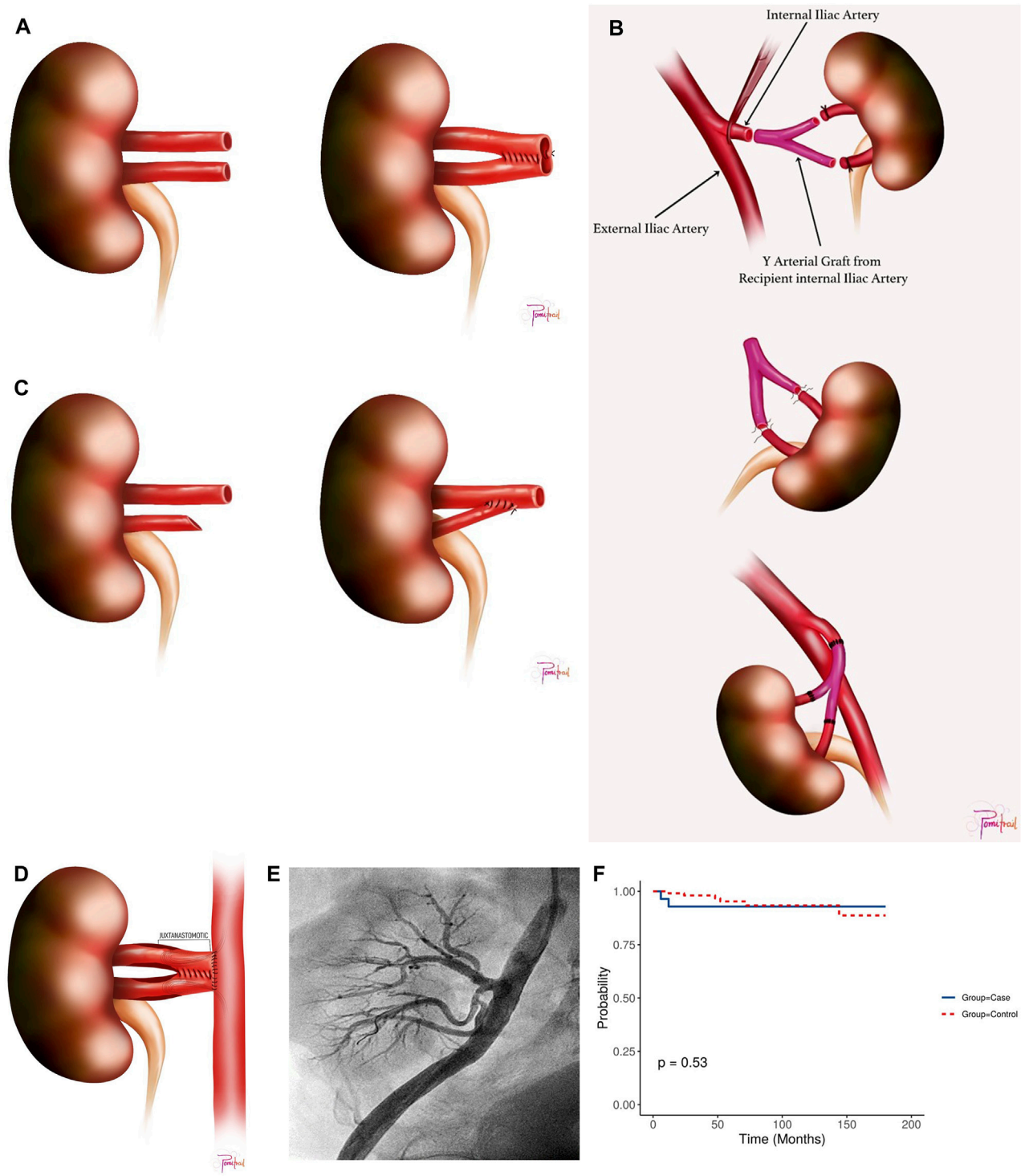


FIGURE 1 | (A) Double Barrel/Pantaloon Technique. **(B)** Y Graft Internal Iliac Artery Technique. **(C)** End-to-side anastomosis of the smaller artery to the main artery. **(D)** Juxta-anastomosis Region with Pantaloon Technique. **(E)** Post-intervention angiography following stenting of one upper renal artery branch and percutaneous transluminal angioplasty of the remaining two branches, showing restored flow with no residual stenosis. **(F)** Kaplan Meier Survival Plot for the study cohort.

Graft function pre and post-intervention for TRAS (Scr and eGFR calculated using a modified modification of diet in renal disease equation), reduction in number of antihypertensive medications, graft, and patient survival, and graft function at follow-up. Graft failure was labeled when a KTR required maintenance hemodialysis.

Immunosuppression Protocol

All living KTRs with HLA mismatch >3 and deceased donor KTRs received ATG induction (1 mg/kg body weight for 3 days). For living KTRs with HLA mismatch <3, Simulect induction was administered, while in selective cases with a full HLA match, no induction agent was utilized. All KTRs then received a center-specific triple-drug immunosuppression regimen (tacrolimus 0.2 mg/kg/day in two divided doses, mycophenolate mofetil 1 g BD, and prednisolone 0.4 mg/kg OD), along with concomitant antimicrobial and anti-CMV prophylaxis, with steroids tapered to 5 mg at 3 months.

CDU Protocol

Study centers KTRs undergo standard CDU before discharge to assess graft vascularity, renal artery peak systolic velocity, and detect any fluid collections. Those with abnormal findings are subjected to sequential monitoring of graft function, incorporating additional imaging tests based on the initial CDU results.

Surgical Protocols and Techniques: Donor Kidney Selection

The study center's protocol for living-KT includes laparoscopic procurement of kidneys with a single renal artery (SRA) or the left kidney in cases of bilateral MRA, aiming to maintain a split differential glomerular filtration rate (GFR) discrepancy under 10%. Standard laparoscopic living-donor nephrectomies, KTR-surgeries, and an in-house deceased-organ retrieval were performed according to the established procedures [7, 8].

Operating Coordination and Anastomosis Strategy

Two senior transplant surgeons (STS) meticulously performed the *ex-vivo* VR-MRA during back-table bench surgery, using 3.5-4X magnification surgical loupes. The VR-MRA was performed over atraumatic silastic catheters using double-armed 7-0 monofilaments in an interrupted fashion.

In cases of live-KT, donor and recipient surgeries were performed in adjacent operating rooms, led by two senior transplant surgeons (STS) with combined experience of over 2000 KT, and assisted by two transplant surgery fellows. Post-retrieval, kidneys were flushed with cold Histidine-Tryptophan-Ketoglutarate solution and subsequently preserved in ice until KT. For living donor-KT, VR-MRA was performed when MRAs were present to ensure optimal perfusion of the graft kidney. However, in a minority of cases where VR-MRA could not be safely performed without kinking the MRA, the additional arteries were implanted separately. In contrast, for deceased donor-KT, MRAs were always implanted with a Carrel's patch. VR-MRA was required only when the MRAs were too far apart to be included in a single patch or when the MRA were injured during kidney retrieval.

For both live and deceased-KT, the SRA and MRA (after VR-MRA) were preferably anastomosed end-to-end to the Internal Iliac Artery (IIA) when its patency was confirmed. In cases where the IIA was not suitable for anastomosis, such as lumen size discrepancy or atherosclerotic IIA, the SRA or MRA was attached to the External Iliac Artery (EIA) using a punch arteriotomy in an end-to-side (ES) fashion. In deceased-KT, MRAs without VR-MRA were preferably anastomosed on the Carrel patch in ES fashion to the EIA.

Surgical VR-MRA Techniques

- For two RA with a lumen discrepancy of up to 70:30 and aligned ostial axes, a side-to-side double-barrel/pantaloon technique was employed (**Figure 1A**).
- In cases where two RA were distantly separated for pantaloon-anastomosis without undue tension, an internal iliac artery (IIA) Y-graft reconstruction was performed using the recipient's IIA (**Figure 1B**).
- If the lumen discrepancy falls short of 70:30, the smaller RA was anastomosed in an end-to-side (ES) fashion to the larger RA or anastomosed separately as end-to-end (EE) or ES to the IIA or external iliac artery (EIA), respectively (**Figure 1C**).
- The senior transplant surgeon (STS) employed customized approaches for complex scenarios involving three or more RA, selecting from or combining the aforementioned techniques (**Supplementary Figure S1**).
- The renal vein was consistently anastomosed ES to the external iliac vein.

Management Strategies for cTRAS

All endovascular interventions (EVI) for cTRAS were performed by a single senior interventional cardiologist experienced in about ten thousand percutaneous coronary interventional procedures. Stents were deployed in cases where post-angioplasty residual stenosis exceeded 30%. Drug-eluting stents (DES) were preferred for smaller renal arteries (≤5 mm), while bare metal stents (BMS) were chosen for larger ones (≥5 mm). Success following EVI for cTRAS was defined both clinically and technically. Technical success was indicated by a minimal systolic pressure gradient or clear fluoroscopic evidence of no residual stenosis. Clinically, success was defined as a reduction in SCr by more than 20% or a decrease in atleast one antihypertensive medication within 2 weeks post-intervention. All KTRs diagnosed with cTRAS underwent an initial CDU at 4 weeks post-EVI, followed by surveillance scans every 6 months, and then annually after that. Post-EVI, all KTRs were prescribed dual-antiplatelet therapy (aspirin 75 mg and clopidogrel 75 mg daily) for 1 year, and those experiencing recurrent cTRAS continued the therapy for life.

Sample Size and Hypothesis

The sample size was determined with the null hypothesis that *ex-vivo* VR-MRA to create a common channel for anastomosis does not increase the risk of cTRAS [9]. With a power of 80%, an alpha level of 5%, and a ratio of four matched controls per cTRAS case, the calculation factored in a 10% probability of exposure in the control group (reflecting the incidence of bilateral MRA in the donor) [10]. A correlation coefficient of 0.2 was chosen to account for a small anticipated effect size and to minimize the risk of Type-II errors, ensuring an adequately powered study. Drawing from a previous study where Y-graft was identified as an independent risk factor for cTRAS (odds ratio = 4.957) [6]. Similar odds were assumed for other VR-MRA techniques involving creating a common channel for anastomosis (e.g., pantaloon technique, end-to-side anastomosis of smaller RA **Figure 1A–C**). These calculations determined a minimum sample size of 22 cTRAS cases and 88 controls.

Statistical Analysis

Univariable comparisons of continuous data were conducted using Student's t-test or the Mann–Whitney U test based on data distribution. Categorical data were analyzed using the $\chi 2$ test or Fisher's exact test. The Wilcoxon Signed-Rank and Stuart-Maxwell tests were applied to pre- and post-intervention analyses. Univariable logistic regression was employed for each significant variable to evaluate its association with the outcome, followed by multivariable logistic regression to adjust for potential confounders, thereby generating multivariable odds ratios. The study analysis employed a dual-method analytical framework, integrating multivariable regression analysis with all predictor variables and a bidirectional stepwise selection methodology to validate the significance of VR-MRA as an independent risk factor for cTRAS. The robustness of the logistic regression model was then assessed using the Chi-Square statistic, Pseudo R^2, Akaike Information Criterion, C-statistic, and Hosmer-Lemeshow test to ensure a reliable statistical assessment of cTRAS predictors. A P-value of <0.050 was considered statistically significant.

RESULTS

Of the 2,454 KT performed during the study period, 28 KTRs (1.14%) were diagnosed with cTRAS. The average time for the presentation of cTRAS was around 110.07 ± 31.78 days post-KT, with most cases (78.57%) exhibiting stenosis in the juxta-anastomotic region (**Figure 1D**). This juxta-anastomotic region narrowing was observed in all cases involving VR-MRA and in 45.45% of cases with SRA. (**Supplementary Figures S2, S3A, B**). Renal allograft dysfunction, marked by elevated SCr, was the primary clinical manifestation in 92.86% of cTRAS cases. Furthermore, over half of cTRAS cases (57.14%) necessitated the usage of ≥2 antihypertensive medications. Clinical features of fluid overload, such as weight gain and pulmonary edema, were present in two KTRs. Beyond the 28 cTRAS cases, three KTRs not included in this study were identified with early-stage TRAS attributed to dissection of EIA.

The etiology of ESRD in cTRAS cases were diabetic nephropathy (28.57%), IgA nephropathy (21.42%), obstructive nephropathy (10.71%), hypertensive nephropathy (7.14%), and autosomal dominant polycystic kidney disease (7.14%). The underlying etiologies remained unidentified in 25% of cTRAS cases.

No significant differences were observed between cTRAS cases and controls in baseline pretransplant and intraoperative parameters, except for the higher occurrence of VR-MRA (53.57%) ($p < 0.001$) and MRA (60.7%) ($p < 0.001$) in cTRAS cases. All MRA allografts in the cTRAS cohort underwent VR-MRA, except for two cases from living donor-KT, where the MRAs were implanted separately into the IIA and EIA (**Table 1**). VR-MRA was performed in 55% of living-KTR and 50% of deceased donor-KTR diagnosed with cTRAS. Postoperatively, slow graft function (SGF) was more prevalent in cTRAS cases (64.28%) compared to controls (36.60%) $p = 0.013$. Despite a significantly higher rate of SGF in cTRAS cases, both cases and controls recorded a similar baseline line Scr (mg/dL) and eGFR 1-month post-KT. Furthermore, all cTRAS cases exhibited normal CDU results upon discharge following the KT. However, before cTRAS was diagnosed, frequent allograft biopsies were performed in cTRAS cases for prevalent allograft dysfunction. Mild to moderate acute tubular necrosis (ATN) was the most common biopsy finding in 67.85% of cTRAS cases, and 10.71% of cTRAS cases had biopsy-proven acute rejection (**Tables 1–3**).

The diagnosis of cTRAS was confirmed through angiography in all cases except for three KTRs (**Figure 1E**), where magnetic-resonance angiography was employed to diagnose cTRAS following inconclusive CDU findings with a high index of suspicion for cTRAS with graft-dysfunction. N = 27 cases of cTRAS were successfully managed with EVI. EVI predominantly comprised percutaneous transluminal angioplasty (PTA) with stenting in 89.28% of cases. PTA alone was performed in three KTRs. Intravascular imaging using optical-coherence tomography was employed in seven cTRAS cases to optimize EVI. Notably, all EVIs were accomplished without any procedural complications. Recurrent cTRAS, manifesting as in-stent restenosis (ISR), occurred in two KTRs at one and 4 years post-EVI procedures, leading to a reintervention rate of 7.14%. Both these cases were successfully treated with cutting balloon angioplasty and DES. In cTRAS cases, the patency rates following EVI were 92% for PTA with stenting and 100% for PTA alone. Following EVI, significant clinical improvements were observed, including decreased SCr levels and reduced requirement for antihypertensive medications (**Table 3**). Within the cTRAS-cohort, one fatality was attributed to cTRAS in a KTR who presented with severe graft dysfunction (Scr = 5.2 mg/dL) in a hypertensive crisis, volume-overload, and pulmonary edema, a clinical scenario known as Pickering syndrome. Another fatality in the cTRAS group resulted from COVID-19 infection at 1 year post-EVI. Seven KTRs with cTRAS experienced a diabetes-insipidus-like state following EVI and required conservative therapy, consequently prolonging their hospital stay by 1 week.

TABLE 1 | Baseline pretransplant, intraoperative, and postoperative characteristics.

KTR characteristics	TRAS-KTR (n = 28)	Non-TRAS-KTR (n = 112)	P-value
Baseline Pretransplant Parameters			
Type of Transplant (Live/Deceased KT)	(20/8) (71.4%/28.6%)	(80/32) (71.4%/28.6%)	1.000
Blood Group A	10 (35.7%)	31 (27.7%)	0.842
B	10 (35.7%)	41 (36.6%)	
AB	3 (10.7%)	15 (13.4%)	
O	5 (17.9%)	25 (22.3%)	
Donor age (yrs) (mean ± SD) (median)	44.86 ± 12.10 (43)	41.68 ± 12.53 (43.50)	0.224
Donor BMI (kg/m^2) (mean ± SD) (median)	23.07 ± 2.97 (22.25)	24.48 ± 4.68 (24.00)	0.127
Donor Sex (Female/Male)	(20/8) (71.4%/28.6%)	(66/46) (58.9%/41.1%)	0.281
KTR age (yrs) (mean ± SD) (median)	37.64 ± 13.71 (37.50)	36.28 ± 11.65 (35.00)	0.630
KTR BMI (kg/m^2) (mean ± SD) (median)	22.07 ± 3.34 (22.04)	22.61 ± 4.12 (22.50)	0.794
KTR Sex (Female vs. Male)	(5/23) (17.9%/82.1%)	(20/92) (17.8%/82.1%)	1
Pre-KT Haemodialysis Duration (months) (mean ± SD) (median)	25.89 ± 22.77 (12.00)	23.57 ± 26.06 (12.00)	0.661
HLA Mismatch (≤3 vs> 3)	(11/17) (39.3%/60.7%)	(49/63) (43.8%/56.2%)	0.669
Diabetes Mellitus	(8) (28.6%)	(36) (32.1%)	0.716
Intraoperative Parameters			
Donor Kidney Side (Left/Right)	(22/6) (78.6%/21.4%)	(99/13) (88.4%/11.6%)	0.216
Warm Ischemia Time (minutes) (mean ± SD) (median)	3.54 ± 2.36 (5.00)	3.69 ± 2.61 (5.00)	0.793
Cold ischemia Time (minutes) (mean ± SD) (median)	180.18 ± 116.50 (125.50)	180.42 ± 196.42 (100.00)	0.047
Anastomosis Time (minutes) (mean ± SD) (median)	30.93 ± 3.68 (30.00)	31.10 ± 2.07 (30.00)	0.623
Living Donor Surgery Operating Time (minutes) (mean ± SD) (median)	194.5 ± 31.37 (190.00)	198.62 ± 28.41 (180.00)	0.768
Donor Kidney weight (grams) (mean ± SD) (median)	141.64 ± 36.98 (138.50)	149.45 ± 34.89 (144.00)	0.302
Multiple Renal Arteries {double RA, triple RA}	(17){12 + 5} (60.7%)	(16){15 + 1} (14.3%)	<0.001
Vascular Reconstruction for Multiple renal arteries (VR-MRA) (a+b + c)	(15) (53.6%)	(9) (8.0%)	<0.001
a. Double Barrel (VR-MRA) (**Figure 1A**)	9 (32.1%)	5 (4.5%)	<0.001
b. Y-Graft (VR-MRA) (**Figure 1B**)	4 (14.3%)	2 (1.8%)	0.015
c. End-to-side (VR-MRA) (**Figure 1C**)	2 (7.14%)	2 (1.78%)	0.18
End-to-end Anastomosis (Graft Implantation to Internal Iliac Artery)	(11) (39.3%)	(67) (60.7%)	0.073
End-to-side Anastomosis (Graft Implantation to External Iliac Artery)	(17) (60.7%)	(45) (40.2%)	0.050
Postoperative Parameters			
Antithymocyte Globulin Induction	(18) (64.3%)	(64) (57.11%)	0.493
Slow Graft Function	(18) (64.23%)	(41) (36.6%)	0.008
Delayed Graft Function	(5) (17.9%)	(10) (8.9%)	0.181
Renal Allograft Biopsy	(25) (89.3%)	(45) (40.2%)	<.001
Duration of Post-Transplant Hospital Stay (Days) (mean ± SD) (median)	14.32 ± 6.8 (12)	12.26 ± 6.28 (10)	0.074
Biopsy-proven acute rejection	(3) (10.7%)	(17) (15.2%)	0.764

(Continued on following page)

TABLE 1 | (*Continued*) Baseline pretransplant, intraoperative, and postoperative characteristics.

KTR characteristics	TRAS-KTR (n = 28)	Non-TRAS-KTR (n = 112)	P-value
Baseline SCr (mg/dL) (mean ± SD) (median)	1.30 ± 0.38 (1.30)	1.42 ± 0.49 (1.30)	0.458
Baseline eGFR (mean ± SD) (mL/min/1.73 m^2) (median)	71.95 ± 27.85 (67.50)	70.80 ± 25.76 (67.50)	0.845
Follow-up SCr (mg/dL) (mean ± SD) (median)	1.35 ± 0.40 (1.35)	1.82 ± 1.49 (1.40)	0.567
Follow-up eGFR (mean ± SD) (mL/min/1.73 m^2) (median)	70.43 ± 21.29 (69.50)	66.55 ± 32.34 (61.00)	0.446
Patient Survival	(26) (92.9%)	(106) (94.6%)	0.660
Renal allograft Survival	(27) (96.4%)	(102) (91.1%)	0.694

Follow-up duration for cTRAS cases varied from 6 to 180 months (mean-58.89 months, median-43 months). Notably, both the cTRAS cases and control groups demonstrated comparable graft and patient survival rates (Kaplan-Meier survival **Figure 1F**). The study analysis confirms and validates the significance of VR-MRA as an independent risk factor for cTRAS ($p < 0.001$) in the study cohort (**Table 2**).

DISCUSSION

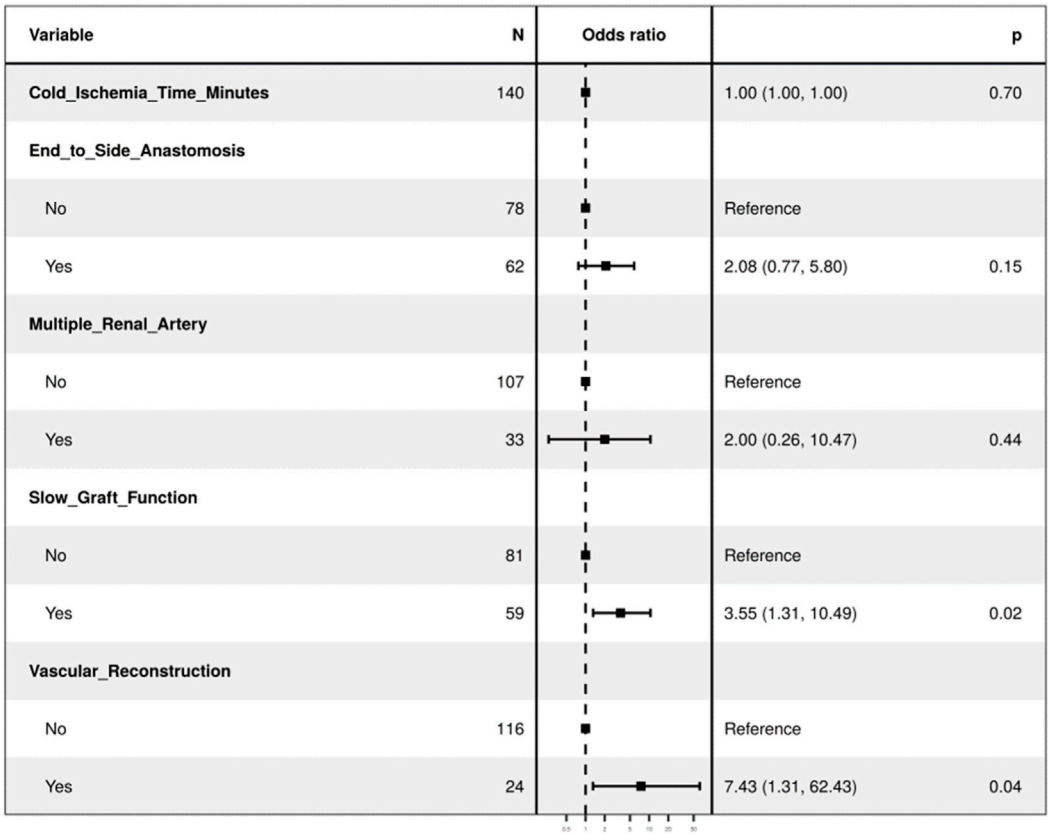
The occurrence of cTRAS significantly increases the risk of allograft loss and mortality among KTRs. However, prompt diagnosis and management of cTRAS can potentially improve patient and graft survival [2, 3, 11]. The risk factors inciting cTRAS in living-KT remain inadequately defined. This study represents the largest single-center experience from Asia, highlighting VR-MRA as a significant independent risk factor for cTRAS in a predominantly living KT program. cTRAS mainly occurred in the juxtanastomotic area, typically around 3.62 ± 1.04 months post-KT, with timely management of cTRAS resulting in graft outcomes similar to those in KTRs without cTRAS.

VR-MRA, as a significant predictor for cTRAS, holds particular importance in the context of evolving transplant practices across the globe and underscores the critical relevance of index study in informing surgical decisions and patient outcomes in Kidney transplantation. With the advancement of laparoscopic kidney retrieval, many transplant surgeons have shown a growing preference for using left kidneys from living donors for KT despite the presence of MRAs, which often necessitates VR-MRA. In the USA, left laparoscopic donor-nephrectomy is the preferred method for KT, with an adoption rate of 86.1%, regardless of the presence of MRA [12]. In contrast, practices in the UK vary; some centers exclusively opt for left laparoscopic donor-nephrectomy, while others prefer kidneys with SRA, as is the current practice at the index center [13]. A few small series have reported a heightened risk of cTRAS in KTRs who received allografts with MRA in living donor-KT [6, 14]. Additionally, studies on TRAS outcomes in both living and deceased donor KT have noted a higher prevalence of allografts with MRA in their TRAS groups. [15, 16]. Furthermore, a small subgroup analysis suggested an elevated risk of cTRAS for both living donor-KT (cTRAS, n = 13) and deceased donor-KT (cTRAS, n = 20) involving MRA that underwent VR-MRA [6, 17]. Meta-analysis amongst KTRs receiving allografts with MRA has also revealed that KTRs receiving allografts with MRAs face significantly higher immediate vascular complications like bleeding and vascular thrombosis, increased DGF, elevated SCr at one/5 years, and decreased 1-year graft survival when compared to KTRs receiving allografts with SRA regardless of donor type (living or deceased-donor) [18, 19]. However, these meta-analyses did not explicitly investigate the occurrence of cTRAS in SRA versus MRA groups and their impact on graft outcomes.

The incidence ratio of cTRAS (1.14%) observed in the index study potentially reflects a falsely low estimate compared to the broader reported range of 1%–23% [3–5, 11]. This discrepancy can be primarily attributed to the absence of routine imaging screening methods for cTRAS at the study center. The existing literature indicates that cTRAS typically manifests within the initial 3–6 months post-KT, with as many as 78% of cTRAS cases exhibiting stenosis primarily in the juxta-anastomotic region of the donor-renal artery [3, 6, 11, 15, 20–25]. The findings of the index study affirm this trend. The juxta-anastomotic region may be prone to altered shear stress-induced endothelial damage due to turbulent renal blood flow (RBF), particularly when the RBF transitions from an SRA to reconstructed MRAs implanted with a single common channel. This juxta-anastomotic region might also be affected by stretching or redundancy in the reconstructed arteries after the final placement of the renal allograft in the KTR, potentially leading to localized endothelial injury and the development of neointimal-hyperplasia (IH) in the juxta-anastomotic region [26, 27]. Immunological factors like allograft rejection and Class-II de-novo donor-specific antibodies (cutoff mean fluorescence intensity of over 300) have been proposed as potential risk factors for TRAS [11, 22, 28]. The predominant localization of cTRAS to the juxta-anastomotic region, as observed in numerous studies, including ours, strongly suggests that the primary etiological factor is altered hemodynamics rather than an immunological response [29, 30]. Typically, immunological factors would be expected to cause more widespread endothelial damage than a focal endothelial injury. Although the study center did not routinely screen for de-

TABLE 2 | Multivariable regression model.

Regression with all variables in the model	OR (univariate)	OR (multivariate)
Cold Ischemia Time (Minutes)	1.00 (1.00–1.00, p = 0.995)	1.00 (1.00–1.00, p = 0.700)
End-to-side anastomosis	2.30 (1.00–5.50, p = 0.054)	2.08 (0.77–5.80, p = 0.150)
Multiple Renal Arteries	9.27 (3.75–24.11, p< 0.001)	2.00 (0.26–10.47, p = 0.440)
Slow Graft Function	3.12 (1.34–7.63, p = 0.010)	3.55 (1.31–10.49, p = 0.015)
Vascular reconstruction of multiple renal arteries (VR-MRA)	13.21 (4.95–37.69, p < 0.001)	7.43 (1.31–62.43, p = 0.035)



Regression with selected variables in the model (Bidirectional Stepwise Selection)	OR (univariate)	OR (multivariate)
Cold Ischemia Time (Minutes)	1.00 (1.00–1.00, p = 0.995)	-
End to side- Anastomosis (ES)	2.30 (0.99–5.37, p = 0.054)	2.09 (0.78–5.61, p = 0.145)
Multiple Renal Arteries	9.27 (3.68–23.38, p < 0.001)	-
Slow Graft Function (SGF)	3.12 (1.31–7.39, p = 0.010)	3.66 (1.32–10.12, p = 0.013)
Vascular reconstruction of multiple renal arteries (VR-MRA)	13.21 (4.82–36.18, p < 0.001)	13.51 (4.58–39.88, p < 0.001)

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novo DSA in all KTRs, the rates of allograft rejection and HLA mismatches were non-significant (**Table 1**).

Poiseuille's law underscores the exponential influence of vascular-radius on the RBF rate ($Q = \Delta P\pi r^4/8\eta l$), where even modest luminal reductions due to IH (5%–15%) can significantly decrease RBF by 18.5%–47.8% (**Supplementary Figures S2A, B, S4, S5**). The impact of IH causing luminal reduction is more pronounced in allograft implanted with VR-MRA, particularly when the same thickness of IH extends from larger SRA to smaller MRAs in the juxtanastomotic region, leading to a substantial reduction in luminal diameter, thereby significantly reducing RBF. Elevated blood pressure is necessary to maintain RBF in such circumstances of reduced luminal diameter, ultimately leading to a vicious cycle of increased turbulence and low shear stress on endothelial cells in the juxta-anastomotic region, exacerbating endothelial damage by promoting the release of prothrombotic factors (**Supplementary Figures S2A**) [2, 26, 27, 30]. A recent randomized clinical trial reinforces this mechanistic understanding by demonstrating that low-dose aspirin (100 mg) effectively reduces cTRAS development amongst KTRs [31]. Aspirin prevents microthrombi formation by inhibiting platelet aggregation in areas of abnormal shear stress, underscoring the critical role of platelets in the pathogenesis of cTRAS [32].

TABLE 2 | (*Continued*) Multivariable regression model.

Regression with selected variables in the model (Bidirectional Stepwise Selection)	OR (univariate)	OR (multivariate)

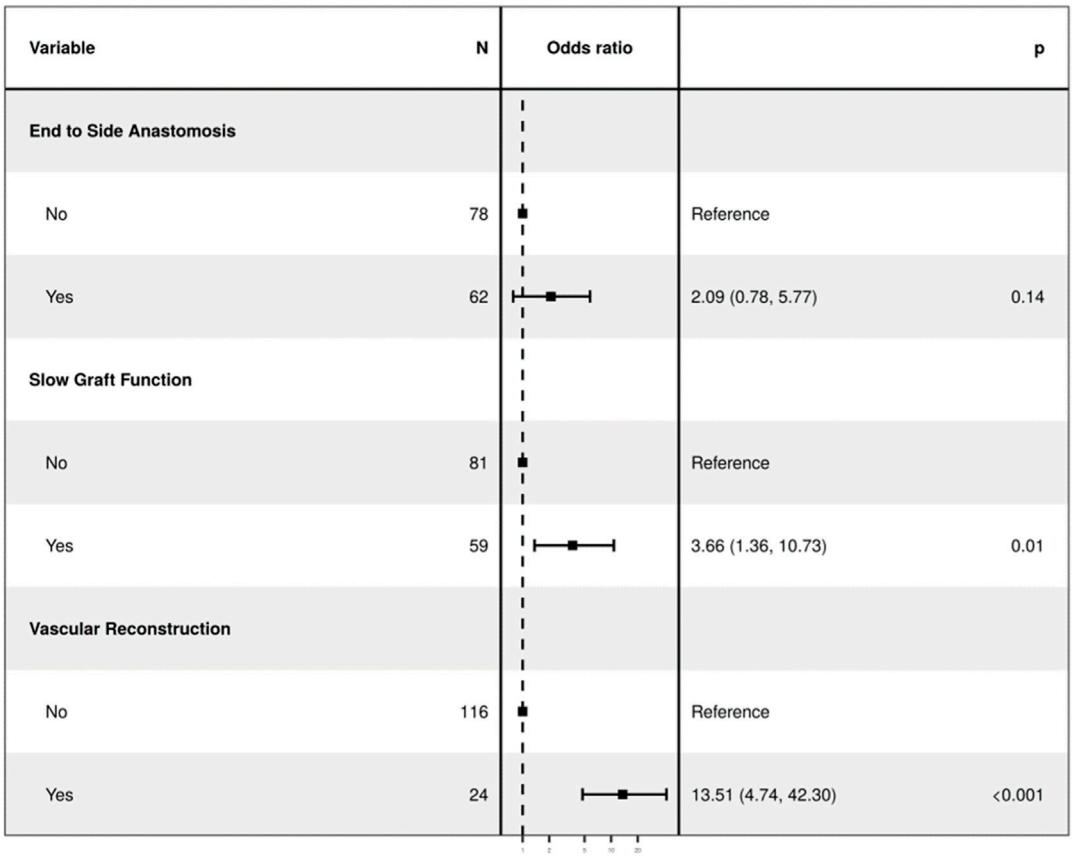

TABLE 3 | cTRAS Cases baseline parameters.

TRAS cases parameters	
Time to TRAS (Days) (mean ± SD) (median) (Interquartile Q1-Q3)	110.07 ± 31.78 (101.00) (Q1 90.75-Q3 130 days)
Follow-up Duration in months {Inter quartile range-IQR}	6–180 months (mean-58.89 months median {IQR} = 43{24–67} months)
Number of Antihypertensive medications at TRAS Diagnosis (mean ± SD)	2.46 ± 0.92
Number of Antihypertensive medications at 1 month Post Intervention (mean ± SD)	1.61 ± .057 χ^2 = 18.237, p = 0.001
SCr (mg/dL) at TRAS Diagnosis (mean ± SD)	2.06 ± 0.85
SCr (mg/dL) 2-week Post TRAS Intervention (mean ± SD)	1.33 ± 0.36 (p < 0.001)
eGFR at (mL/min/1.73 m^2) at TRAS Diagnosis (mean ± SD)	48.50 ± 21.15
eGFR at (mL/min/1.73 m^2) 2-week Post TRAS Intervention (mean ± SD)	69.13 ± 21.87 (p< 0.001)
Biopsy feature • Acute tubular necrosis • BPAR	• Mild (n = 17) • Moderate (n = 5) • (n = 3) KTRS
VR-MRA- Double Barrel/Y-graft/ES to main RA	9/4/2 = 15
Only Angioplasty	n = 3
Angioplasty + Stenting (BMS)	n = 10
Angioplasty + Stenting (DES)	n = 15
Restenosis in KTR	n = 2

The high procedural success rate of EVI at the study center reinforces its established efficacy as the preferred therapeutic method for treating cTRAS [33, 34]. KTRs who underwent EVI at the index study center demonstrated significant improvements in SCr and reduced reliance on antihypertensive medications, paralleling the long-term graft and patient survival observed in

KTRs without cTRAS (**Table 3**). The efficacy of EVI in managing cTRAS largely stems from the early detection of cTRAS and the expertise of the interventional team. Moreover, the study center's adoption of optical coherence tomography for guiding EVI has refined the therapeutic approach, contributing to advancements in this domain [35].

The index study has certain limitations. Firstly, the study's design does not allow for definitive causality establishment, a limitation of case-control studies. The limited cohort size presented a constraint in conducting extensive subgroup analyses between living donor-KT and deceased donor-KT. The study's emphasis on VR-MRA within a small sample size may have reduced its power to evaluate other risk factors for cTRAS. While VR-MRA emerged as an independent risk factor for cTRAS in our study, we also recognize that a smaller luminal diameter at the graft implantation site, irrespective of VR-MRA, may contribute to the risk of cTRAS. However, we could not perform a subgroup analysis due to the limited number of cTRAS cases involving MRAs implanted separately without VR-MRA (n = 2). To definitively determine whether the primary factor driving turbulence and the subsequent occurrence of cTRAS is the luminal diameter or the presence of VR-MRA, a larger study that includes measurements of the minimum diameter at the arterial anastomosis across MRAs undergoing VR-MRA versus those implanted separately would be essential. Such a study would clarify the specific contributions of smaller luminal diameter and VR-MRA to the risk of cTRAS. Additionally, using retrospective odds ratios for sample size calculation may have limited the precision in capturing a full spectrum of effect sizes. Moreover, the predominance of data from living donor-KT in the index study could limit the applicability of the findings to deceased donor-KT, which often involves MRA allografts implanted on a Carrel patch without VR-MRA. Lastly, variability in CDU techniques due to operator differences could have led to inconsistent cTRAS detection, especially in less obvious clinical cases. Considering all these factors, the study's findings should be interpreted with caution. The study's strength is evidenced by enhanced validity achieved through a meticulous study design that includes precise power estimation. By meticulously matching cases to controls, the study controlled for confounding factors, reducing selection bias and biases due to surgical variations. Thereby enhancing the representativeness and applicability of the findings, particularly in the context of living donor-KT.

The predominance of cTRAS, particularly in the juxta anastomotic region within the first 6 months after KT, underscores the need for early intervention. We recommend routine CDU screenings during this critical period, especially for KTRs with VR-MRA, to enhance graft and patient survival, enabling early identification and treatment of cTRAS.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

This study involving data from human participants was approved by the Institutional Ethical Committee of the Post Graduate Institute of Medical Education & Research (PGIMER), Chandigarh, INDIA (NK/7617/study/710). The research adheres to the ethical standards of the 1964 Declaration of Helsinki and its subsequent amendments or comparable ethical guidelines. Given the retrospective nature of the study, informed consent was waived by the PGIMER ethics committee.

AUTHOR CONTRIBUTIONS

DC: Participated in research design, drafting the paper and approval of the submitted and final versions, Participated in the writing of the paper, Participated in the performance of the research, Contributed in analytic tools, Participated in data analysis; RV: Participated in the writing of the paper, Participated in the performance of the research, Contributed in analytic tools, Participated in data analysis; KK: Participated in the performance of the research, Contributed in analytic tools, Participated in data analysis; VS: Participated in research design, drafting the paper and approval of the submitted and final versions; AP: Participated in the performance of the research; MM: Participated in the performance of the research; MB: Participated in the performance of the research; BB: Participated in the performance of the research; SR: Participated in data analysis; PG: Participated in data analysis; SP: Participated in the performance of the research; JS: Contributed in analytic tools; UG: Contributed in analytic tools, Participated in data analysis, SS: Participated in research design, drafting the paper and approval of the submitted and final versions. Participated in the writing of the paper, Participated in the performance of the research; DK: Participated in the performance of the research, Contributed in analytic tools; AS: Participated in research design, drafting the paper and approval of the submitted and final versions. Participated in the writing of the paper, Participated in the performance of the research, Contributed in analytic tools, Participated in data analysis.

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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.2024.13298/full#supplementary-material

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Continuous Glucose Monitoring for the Diagnosis of Post-Transplantation Diabetes Mellitus and Impaired Glucose Tolerance From Years One to Five After Kidney Transplantation—A Prospective Pilot Study

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Post-transplantation diabetes mellitus (PTDM) and prediabetes are associated with increased cardiovascular morbidity and mortality in kidney transplant recipients (KTR), when diagnosed by an oral glucose tolerance test (oGTT). Hemoglobin A1c (HbA1c) and fasting plasma glucose (FPG) display low concordance with the oGTT in the early phase posttransplant. For this prospective cross-sectional pilot study, 41 KTR from years one to five after transplantation without known preexisting PTDM (defined by HbA1c ≥ 6.5% (NGSP) or 48 mmol/mol (IFCC) at last visit or glucose-lowering therapy) were recruited at the Charité Transplant Outpatient Clinic. For each study participant HbA1c, FPG and an oGTT were followed by CGM. 38 of the 41 patients recruited had sufficient CGM-recordings (≥10 days). PTDM and impaired glucose tolerance (IGT), as defined by the gold standard oral glucose tolerance test (oGTT)-derived 2-h plasma glucose (2hPG), were diagnosed in one (3%) and twelve (32%) patients, respectively. HbA1c exhibited good test characteristics regarding IGT (ROC-AUC: 0.87); sensitivity/specificity of HbA1c-threshold 5.7% (NGSP) or 39 mmol/mol (IFCC) were 1.0/0.64, respectively. Best performing CGM-readouts mean sensor glucose and percent of time >140 mg/dL (%TAR (140 mg/dL)) displayed acceptable diagnostic performance (ROC-AUC: 0.78 for both). Thus, HbA1c can aid in timely diagnosis of IGT in the stable phase after kidney transplantation.

Keywords: kidney transplantation, cardiovascular disease, Post-transplantation diabetes mellitus, prediabetes, continuous glucose monitoring

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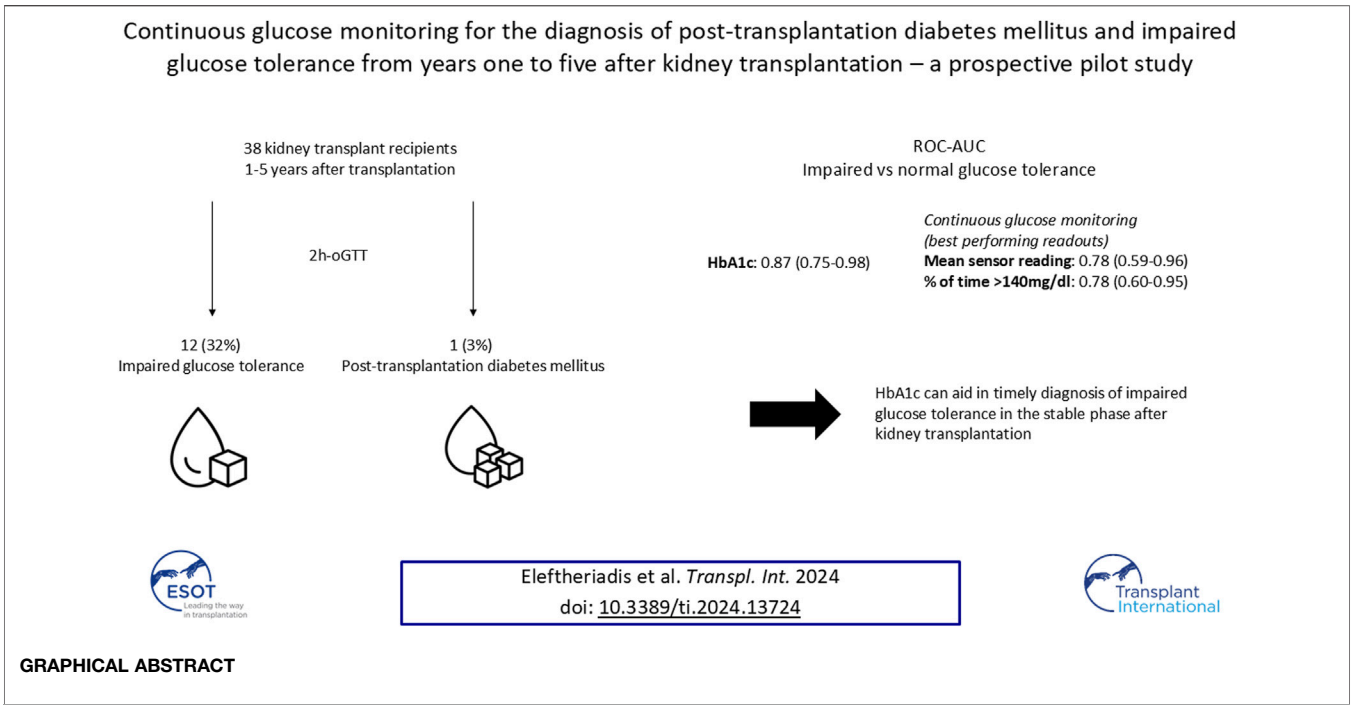
INTRODUCTION

Post-transplantation diabetes mellitus (PTDM) and prediabetes affect 20%–30% of kidney transplant recipients (KTR) and are associated with increased cardiovascular morbidity and mortality, when diagnosed by an oral glucose tolerance test (oGTT) [1–3]. Though widely regarded as the gold standard for the diagnosis of PTDM and prediabetes [4, 5], routine implementation of the oGTT is impeded by its time consuming and impractical nature in most large transplant programs [4]. Pathophysiologic



GRAPHICAL ABSTRACT

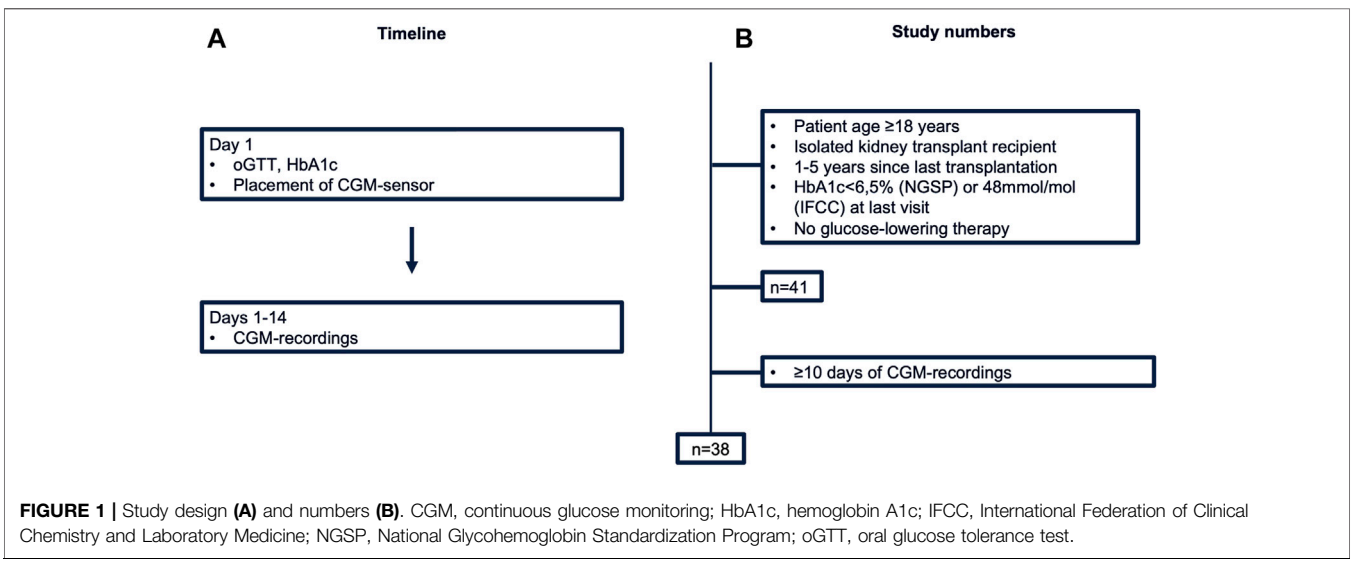


FIGURE 1 | Study design **(A)** and numbers **(B)**. CGM, continuous glucose monitoring; HbA1c, hemoglobin A1c; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; NGSP, National Glycohemoglobin Standardization Program; oGTT, oral glucose tolerance test.

alterations in the early stage posttransplant, in particular increased rates of red blood cell turnover, immunosuppressive effects on erythrocyte proliferation in the bone marrow and steroid-induced glucose maxima in the early afternoon and evening, contribute to a severely compromised validity of hemoglobin A1c (HbA1c) and fasting plasma glucose (FPG) during this stage [6–8]. In fact, neither HbA1c nor FPG in the first year after kidney transplantation show a robust association with patient survival or cardiovascular events [2, 3, 9]. Test characteristics of HbA1c and FPG have been shown to improve in the second year after kidney transplantation compared to the gold standard oGTT, though still remaining suboptimal [10, 11]. Concordance of glycemic parameters >2 years after kidney transplantation has not been extensively studied.

Continuous glucose monitoring (CGM) has transformed diabetes care for patients with diabetes mellitus type 1 and 2, improving glycemic management and lowering the risk of acute diabetic complications and hospital admissions [12, 13]. Experience of CGM-utilization after kidney transplantation has been limited [8, 14–17], especially with regards to the stable phase (>1 year) after transplantation [16].

The aim of this prospective cross-sectional pilot study was to assess feasibility of CGM and investigate its potential for the diagnosis of

TABLE 1 | Baseline characteristics of study participants.

Characteristic	N = 38[a]
Demographics	
Age (years)	57 (52, 63)
Female/Male	11/27 (29%/71%)
BMI (kg/m2)	25.4 (22.6, 29.2)
Metabolic parameters	
LDL (mg/dL)	106 (75, 132)
HDL (mg/dL)	51 (43, 66)
Total Cholesterol (mg/dL)	183 (148, 224)
Triglycerides (mg/dL)	129 (105, 188)
Kidney parameters	
eGFR (by CKD-EPI, mL/min)	55 (49, 67)
UPCR (mg/g)	87 (68, 110)
UACR (mg/g)	13 (4, 27)
Kidney history	
Number of kidney transplants	
1/2/3	35/2/1 (92%/5%/3%)
Time since last transplantation (years)	3.2 (1.3, 4.1)
DD/LD	22/16 (58%/42%)
Primary cause of ESKD	
Glomerulonephritis	18 (47%)
ADPKD	6 (16%)
Other	3 (7.9%)
Unknown	11 (29%)
Immunosuppression	
Tacrolimus	37 (97%)
Ciclosporin	1 (2.6%)
Mycophenolate	36 (95%)
Systemic steroid	34 (89%)

ADPKD, autosomal dominant polycystic kidney disease; BMI, body mass index; DD, deceased donor; ESKD, End-Stage Kidney Disease; LD, living donor.
[a]*Median (IQR); n (%).*

TABLE 2 | Characteristics of study participants, grouped by 2hPG.

Characteristic	NGT, N = 25[a]	IGT, N = 12[a]
Demographics		
Age (years)	55 (47, 58)	65 (61, 67)
Female/Male	3/22 (12%/88%)	7/5 (58%/42%)
BMI (kg/m2)	25.0 (22.4, 28.1)	25.7 (22.9, 31.0)
Metabolic parameters		
LDL (mg/dL)	103 (76, 131)	103 (63, 134)
HDL (mg/dL)	48 (41, 57)	65 (51, 71)
Total Cholesterol (mg/dL)	191 (147, 225)	181 (147, 222)
Triglycerides (mg/dL)	119 (99, 214)	148 (111, 171)
Kidney parameters		
eGFR (by CKD-EPI, mL/min)	55 (50, 64)	59 (46, 70)
UPCR (mg/g)	84 (59, 109)	97 (81, 192)
UACR (mg/g)	10 (4, 27)	19 (11, 35)
Kidney history		
Number of kidney transplants		
1/2/3	22/2/1 (88%/ 8%/4%)	12/0/0 (100%/ 0/0)
Time since last transplantation (years)	3.1 (1.3, 4.0)	4.2 (1.2, 4.8)
DD/LD	13/12 (52%/48%)	8/4 (67%/33%)
Primary cause of ESKD		
Glomerulonephritis	10 (40%)	7 (58%)
ADPKD	4 (16%)	2 (17%)
Other	3 (12%)	0 (0%)
Unknown	8 (32%)	3 (25%)
Immunosuppression		
Tacrolimus	24 (96%)	12 (100%)
Ciclosporin	1 (4.0%)	0 (0%)
Mycophenolate	24 (96%)	11 (92%)
Systemic steroid	22 (88%)	11 (92%)

2hPG, oral glucose tolerance test (oGTT)-derived 2-h plasma glucose; ADPKD, autosomal dominant polycystic kidney disease; BMI, body mass index; DD, deceased donor; ESKD, End-Stage Kidney Disease; IGT, impaired glucose tolerance; LD, living donor; NGT, normal glucose tolerance.
[a]*Median (IQR); n (%).*

PTDM and IGT based on the gold standard oral glucose tolerance test (oGTT)-derived 2-h plasma glucose (2hPG) in patients without known preexisting PTDM one to 5 years after kidney transplantation.

PATIENTS AND METHODS

Study Design

This prospective cross-sectional pilot study was conducted between September 2022 and May 2023 at our Transplant Center at the Department of Nephrology and Medical Intensive Care, Charité – Universitätsmedizin Berlin. Study design and study numbers are shown in **Figure 1**. Inclusion criteria were: (i) age ≥18 years (ii) isolated kidney transplant recipient (iii) one to 5 years since last transplantation. Patients with known PTDM (diagnosed through HbA1c ≥ 6.5% (NGSP) or 48 mmol/mol (IFCC) at last visit or glucose-lowering therapy) were excluded from the study. The study protocol was approved by the Ethics Committee of Charité – Universitätsmedizin Berlin (EA4/110/22). All evaluations were performed according to the Declaration of Helsinki (2013 Amendment). Written informed consent was obtained from each participant.

Laboratory Measurements

Blood tubes were sent to the laboratory for analysis directly after blood drawing. HbA1c (ethylenediamine tetraacetic acid tube) was measured by highperformance liquid chromatography separation of hemoglobin fractions. An oral glucose tolerance test (oGTT), consisting of a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water as described by the WHO, was performed with blood drawings at timepoints 0, 1 h and 2 h [4]. FPG was obtained as part of the oGTT. Plasma glucose (sodium fluoride tube) was assessed by the hexokinase method.

Diagnostic Criteria for PTDM and IGT

Diagnosis of PTDM and IGT was based on the 2hPG-criterion of the American Diabetes Association (ADA) (**Supplementary Table S1**) [18]. PTDM was defined by oral glucose tolerance test-derived 2-h plasma glucose (2hPG) ≥200 mg/dL, IGT by 2hPG ≥ 140 mg/dL in the absence of PTDM and normal glucose tolerance (NGT) by 2hPG < 140 mg/dL. Index test results were not available to the assessors of the reference standard.

CGM Recordings

Continuous Glucose Monitoring (CGM) was performed with the "FreeStyle Libre Pro IQ Sensor" (Abbott GmbH, Wiesbaden, Germany). Sensors were placed on the back of the upper arm, with glucose readings blinded for participants and staff. Each

TABLE 3 | Results of glycemic tests.

Glycemic Test	Normoglycemia	Prediabetes	PTDM
2hPG	25	12	1
HbA1c	16	20	2
FPG	31	6	1

Results are shown for patients with all three diagnostic tests.
FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; 2hPG, oral glucose tolerance test (oGTT)-derived 2-h plasma glucose; PTDM, posttransplant diabetes mellitus.

sensor was worn for the duration of 14 days and interstitial glucose levels were measured in 15-min intervals. Sensors with ≥10 days recording duration were considered for further analysis [19].

Sensor data were extracted using the "FreeStyle Libre Pro IQ Reader" (Abbott GmbH, Wiesbaden, Germany). CGM files were cleaned and analyzed using the R-package "cgmanalysis" (Version 2.7.7) [20]. The endings of the CGM raw files were trimmed to ensure discrete 24-h chunks. Selection of CGM-readouts was based on the "Recommendations from the International Consensus on Time in Range" [19]. CGM-readouts consisted of: mean sensor readings, percent of time >140 mg/dL [%TAR (140 mg/dL)], percent of time >180 mg/dL [%TAR (180 mg/dL)], percent of time <70 mg/dL [%TBR (70 mg/dL)], estimated A1c, glucose management indicator (GMI), standard deviation (SD), coefficient of variation (CV), low blood glucose index (LBGI), high blood glucose index (HBGI), mean amplitude of glycemic excursions (MAGE) and continuous overall net glycemic action (CONGA) [19]. Reference standard results were not available to the readers of the index test.

Statistical Analyses

Categorical outcomes were described using frequencies and proportions, while continuous variables were described using means ± standard deviations (SD) or medians and interquartile ranges (IQR) when appropriate. Receiver operating characteristic (ROC) curves for IGT vs. NGT based on the gold standard 2hPG were plotted and the area under the curve (AUC) with respective 95% confidence intervals (CI) calculated. Exploratory screening thresholds for CGM-readouts were based on a sensitivity of around 90% for IGT vs. NGT. Sensitivity, specificity, positive and negative predictive values with 95% CIs, as well as true positives/false negatives and true negatives/false positives for respective IGT thresholds, were calculated. A formal sample size calculation was not performed due to the exploratory design of the study. Patient information was retrieved from our electronic health record and research database for KTR "TBase" [21]. Statistical analysis was performed with "R" version 4.3.1.

We used the Standards for the Reporting of Diagnostic Accuracy Studies (STARD) statement to ensure completeness of reporting [22].

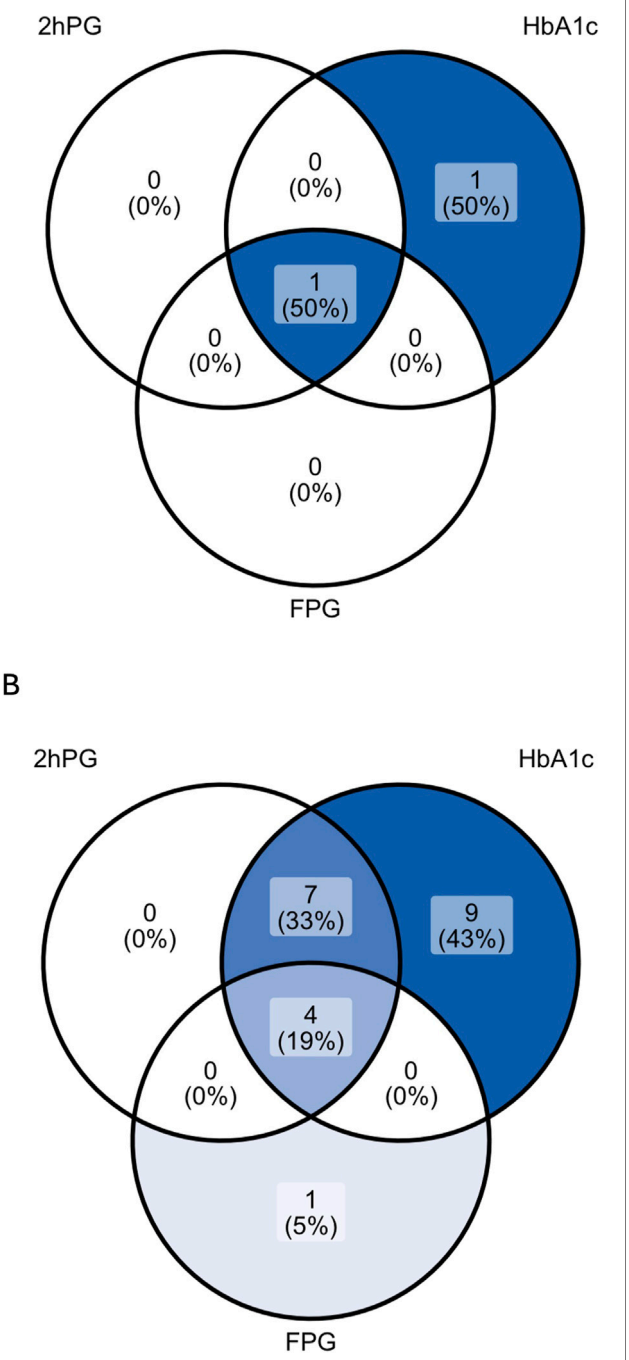


FIGURE 2 | Venn diagrams showing the rate of patients with PTDM **(A)** and prediabetes **(B)** as diagnosed by 2hPG, HbA1c and FPG in patients with complete diagnostic test data. For the analysis of prediabetes **(B)**, patients diagnosed with PTDM by any glycemic test were excluded (n = 2). 2hPG, oGTT-derived 2-h plasma glucose; HbA1c, hemoglobin A1c; FPG, fasting plasma glucose.

TABLE 4 | HbA1c and FPG, grouped by 2hPG. Median [IQR].

Group	HbA1c (% - NGSP mmol/mol - IFCC)	FPG (mg/dL)
Overall	5.7 [5.5;6.0] 39 [37;42]	90 [87;96]
NGT	5.5 [5.4; 5.9] 37 [36; 41]	89 [86; 91]
IGT	6.0 [5.9; 6.2] 42 [41; 44]	96 [93; 114]

2hPG, oral glucose tolerance test (oGTT)-derived 2-h plasma glucose; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; IFCC, international federation of clinical chemistry and laboratory medicine; IGT, impaired glucose tolerance; NGSP, national glycohemoglobin standardization program; NGT, normal glucose tolerance.

RESULTS

Patient Characteristics

41 KTR fulfilled the inclusion criteria and consented to participate. Of these, three patients were excluded from the final analysis due to insufficient CGM-recordings (<10 days). Thus, a total of 38 patients represented the final study population (**Table 1**). In brief, median age of study participants was 57 years [52–63 years] and 71% (27/38) were male. Median time since last transplant was 3.2 years [1.3 years–4.1 years]. Median eGFR (by CKD-EPI) was 55 mL/min [49–67 mL/min] and urine protein creatinine ratio 87 mg/g [68–110 mg/g]. Primary cause of end stage kidney disease (ESKD) was glomerulonephritis (47%, 18/38), followed by autosomal dominant polycystic kidney disease (ADPKD) (16%, 6/38), while 29% of patients (11/38) reached ESKD without defined underlying cause. 92% (35/38) had one kidney transplant, 42% (16/38) from a living donor. All patients were on calcineurin inhibitor therapy (37/38 tacrolimus, 1/38 ciclosporin), 95% (36/38) received mycophenolate and 89% (34/38) systemic steroid. Patients diagnosed with IGT were older [65 (61,67) vs. 55 (47, 58) years for NGT-patients]. Metabolic (LDL, HDL, total cholesterol, triglycerides) and kidney laboratory parameters (eGFR, UPCR, and UACR) showed overlapping interquartile ranges between groups (**Table 2**).

Prevalence of PTDM and IGT

Among 38 patients with an oGTT, 3% (1/38) fulfilled the diagnostic criterion of PTDM and 32% (12/38) of IGT by 2hPG. Results of each glycemic test are depicted in **Table 3**; **Figure 2**.

HbA1c, FPG, and CGM-Readouts

Median HbA1c was 6.0% (NGSP) or 42 mmol/mol (IFCC) [5.9%–6.2% or 41–44 mmol/mol] for IGT-patients and 5.5% (NGSP) or 37 mmol/mol (IFCC) [5.4%–5.9% or 36–41 mmol/mol] for NGT-patients. Median FPG was 96 mg/dL [93–114 mg/dL] for IGT-patients and 89 mg/dL [86–91 mg/dL] for NGT-patients (**Table 4**; **Figure 3**). Boxplots and median [IQR] of CGM-readouts, grouped by 2hPG are depicted in **Figure 4**; **Table 5**.

Test Characteristics of HbA1c, FPG, and CGM-Readouts

ROC curves of HbA1c, FPG and CGM-readouts for the diagnosis of IGT vs. NGT based on the gold standard 2hPG were plotted (**Figures 5**, **6**) Diagnostic test characteristics were good for HbA1c (ROC-AUC 0.87). FPG and CGM-readouts mean sensor readings, %TAR (140 mg/dL), %TAR (180 mg/dL), estimated A1c, GMI, SD, CV, HBGI, MAGE and CONGA displayed acceptable test characteristics (ROC-AUC 0.74 and 0.78, 0.78, 0.73, 0.77, 0.75, 0.75, 0.74, 0.76, 0.76, and 0.74) while %TBR (70 mg/dL) and LBGI performed poorly (ROC-AUC 0.53 and 0.49) (**Table 6**).

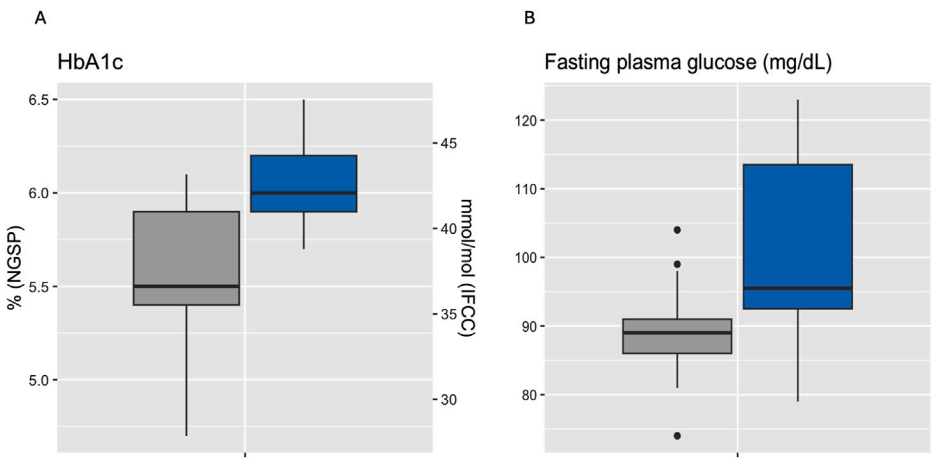


FIGURE 3 | Boxplots of HbA1c **(A)** and fasting plasma glucose **(B)** grouped by 2hPG (*NGT, gray, IGT, blue*). NGT (n = 25), IGT (n = 12). 2hPG, oGTT-derived 2-h plasma glucose; HbA1c, hemoglobin A1c; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; IGT, impaired glucose tolerance; NGSP, National Glycohemoglobin Standardization Program; NGT, normal glucose tolerance.

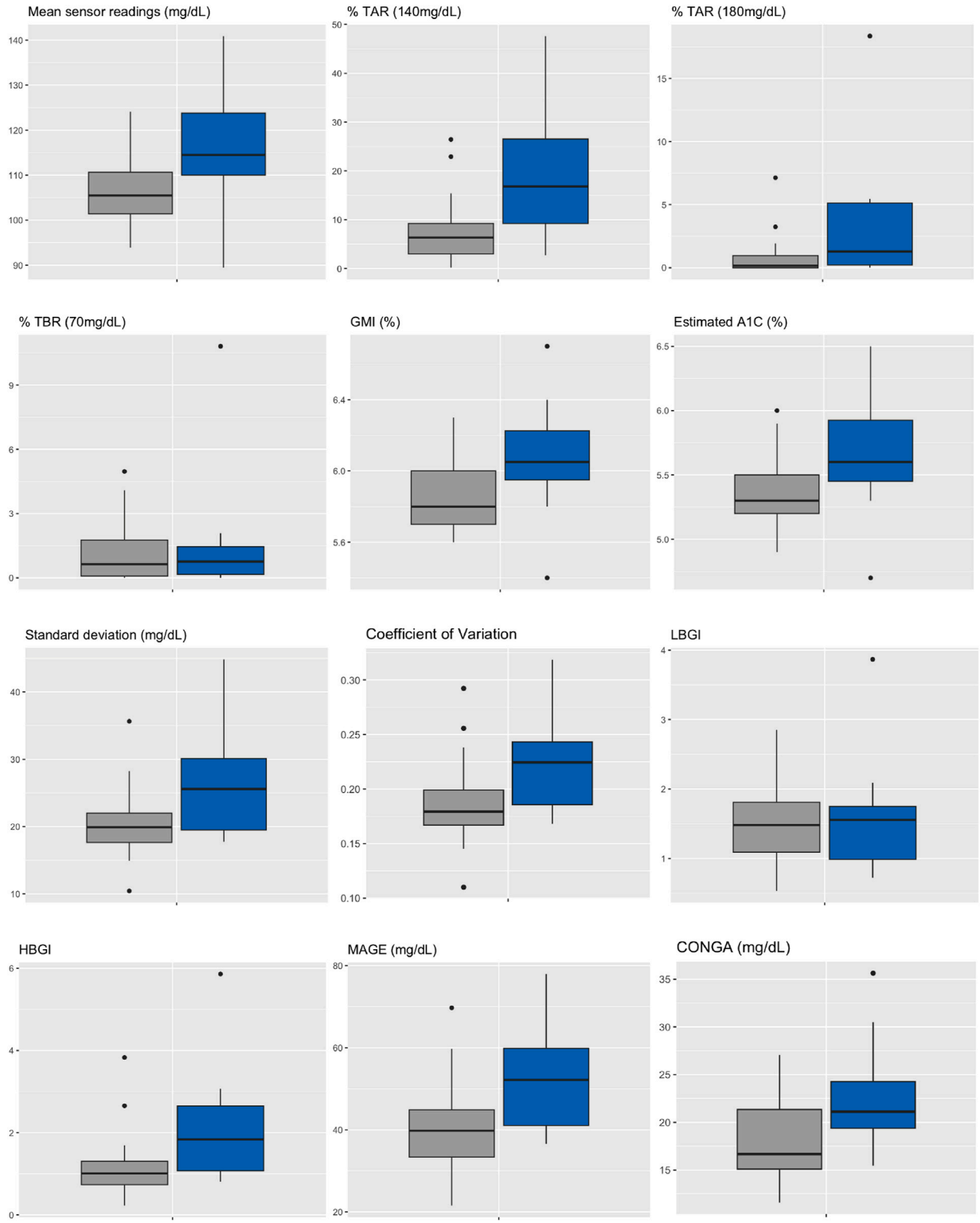


FIGURE 4 | Boxplots of CGM-readouts, grouped by 2hPG (*NGT, gray, IGT, blue*). NGT(n = 25), IGT (n = 12). %TAR, percent of time above range; %TBR, percent of time below range; 2hPG, oral glucose tolerance test (oGTT)-derived 2-h plasma glucose; CONGA, continuous overall net glycemic action; CV, coefficient of variation; GMI, glucose management indicator; HBGI, high blood glucose index; IGT, impaired glucose tolerance; LBGI, low blood glucose index; MAGE, mean amplitude of glycemic excursions; NGT, normal glucose tolerance; SD, standard deviation.

TABLE 5 | CGM-readouts, grouped by 2hPG.

CGM-readouts, grouped by 2hPG. Median [IQR]							
	n()	Mean sensor readings (mg/dL)	% TAR (140mg/dL)	% TAR (180mg/dL)	% TBR (70mg/dL)	Estimated A1C (%)	GMI (%)
Overall	37	108 [103;114]	8.6 [4.2;13.7]	0.4 [0;1.3]	0.6 [0.1;1.8]	5.4 [5.2;5.6]	5.9 [5.8;6.0]
NGT	25	105 [101;111]	6.3 [3.0;9.2]	0.2 [0;1]	0.6 [0.1;1.8]	5.3 [5.2;5.5]	5.8 [5.7;6.0]
IGT	12	114 [110;124]	16.8 [9.2;26.5]	1.3 [0.2;5.1]	0.8 [0.2;1.5]	5.6 [5.5;5.9]	6.1 [6.0;6.2]

	n()	SD (mg/dL)	CV	LBGI	HBGI	MAGE (mg/dL)	CONGA (mg/dL)
Overall	37	20.6 [17.9;23.5]	0.19 [0.17;0.22]	1.5 [1.1;1.8]	1.1 [0.8;1.5]	40.6 [36.1;49.0]	19.36 [15.46;22.04]
NGT	25	19.9 [17.7;22.0]	0.18 [0.17;0.20]	1.5 [1.1;1.8]	1.0 [0.7;1.3]	39.8 [33.4;44.9]	16.66 [15.08;21.33]
IGT	12	25.6 [19.5;30.1]	0.22 [0.19;0.24]	1.6 [1.0;1.8]	1.8 [1.1;2.7]	52.2 [41.1;59.8]	21.11 [19.36;24.27]

%TAR, percent of time above range; %TBR, percent of time below range; 2hPG, oral glucose tolerance test (oGTT)-derived 2-h plasma glucose; CONGA, continuous overall net glycemic action; CV, coefficient of variation; GMI, glucose management indicator; HBGI, high blood glucose index; IGT, impaired glucose tolerance; LBGI, low blood glucose index; MAGE, mean amplitude of glycemic excursions; NGT, normal glucose tolerance; SD, standard deviation.

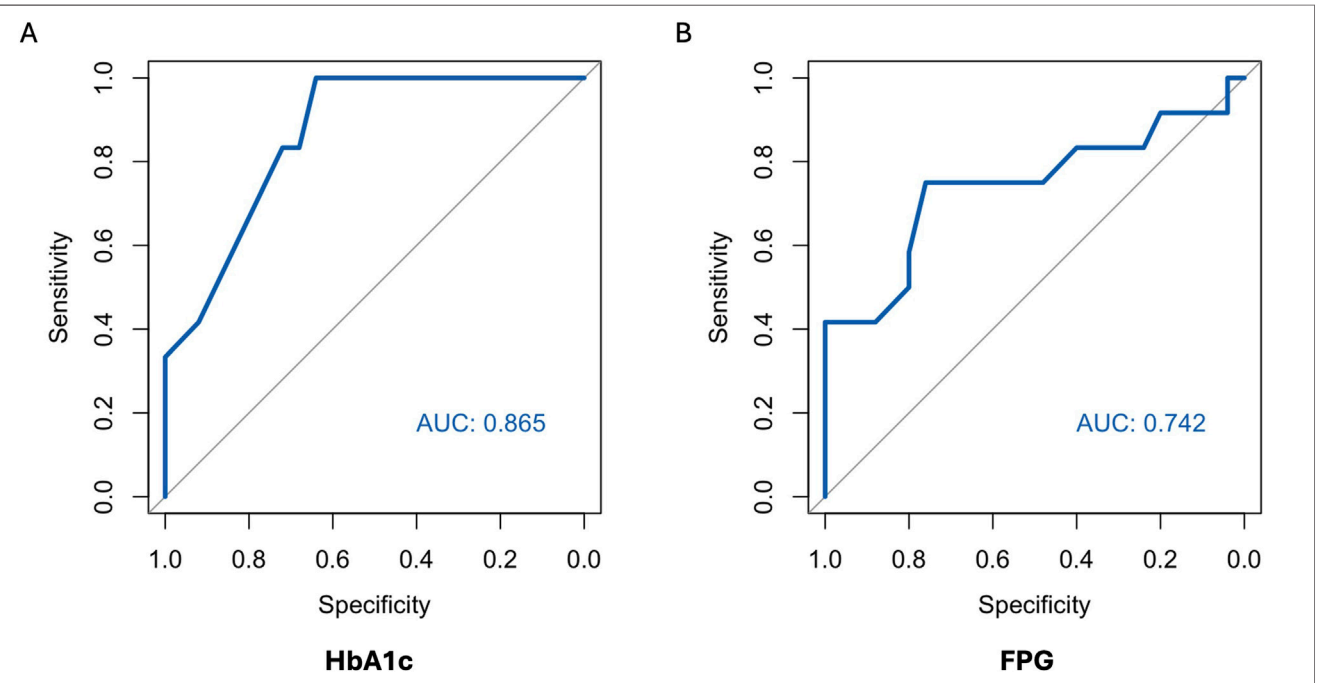


FIGURE 5 | AUC (area under the curve) derived by receiver operating characteristics curve analysis. Diagnosis of IGT vs. NGT with HbA1c **(A)** and FPG **(B)**. Reference test: IGT defined by 2hPG. 2hPG = oGTT-derived 2-h plasma glucose, FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; IGT, impaired glucose tolerance; NGT, normal glucose tolerance.

Detailed in-sample test characteristics of current ADA-defined HbA1c- and FPG-prediabetes thresholds as well as exploratory screening thresholds of CGM-readouts mean sensor readings and %TAR (140 mg/dL) regarding IGT vs. NGT are provided in **Tables 7**, **8**.

Feasibility and Tolerability of CGM

Overall, 41 sensors were returned. Three patients displayed recording durations <10 days thus leading to study exclusion. On a scale from 0 ("no discomfort at all") to 10 ("highest discomfort"), mean patient vote was 1.1, indicating low discomfort. No infectious complications associated to CGM-sensors were noted. 82% of patients (31/38) would have preferred CGM in an unblinded fashion.

DISCUSSION

In this prospective cross-sectional pilot study of 38 KTR without known preexisting diabetes mellitus (by means of HbA1c or glucose-lowering therapy) one to 5 years after transplantation, prevalence of PTDM and IGT, as defined by the gold standard 2hPG, amounted to 3% and 32% respectively. The major finding of this study is that HbA1c exhibits good diagnostic test

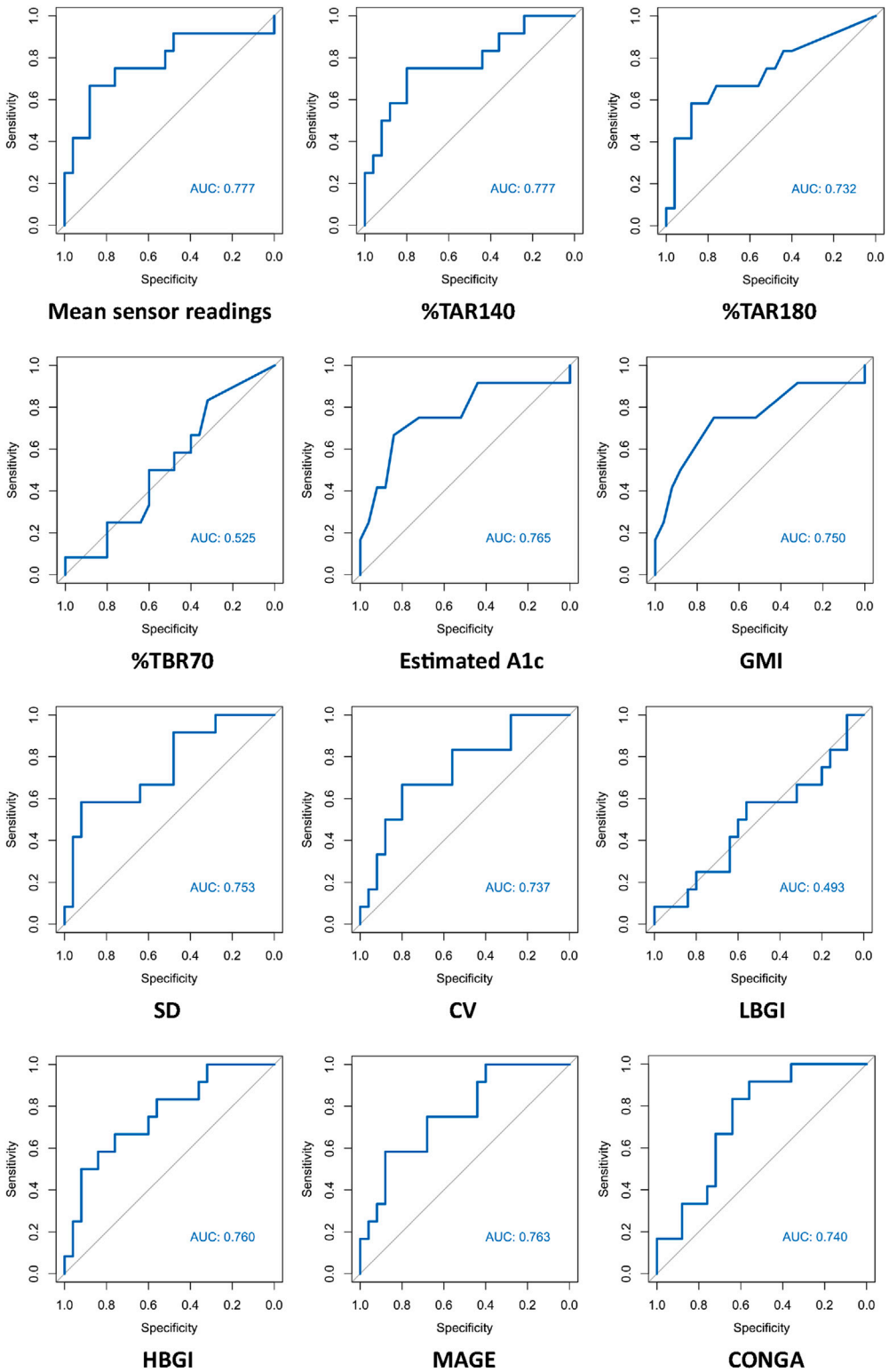


FIGURE 6 | AUC (area under the curve) derived by receiver operating characteristics curve analysis. Diagnosis of IGT vs. NGT with CGM-readouts. Reference test: IGT defined by 2hPG. %TAR, percent of time above range; %TBR, percent of time below range; 2hPG, oral glucose tolerance test (oGTT)-derived 2-h plasma glucose; CONGA, continuous overall net glycemic action; CV, coefficient of variation; GMI, glucose management indicator; HBGI, high blood glucose index; IGT, impaired glucose tolerance; LBGI, low blood glucose index; MAGE, mean amplitude of glycemic excursions; NGT, normal glucose tolerance; SD, standard deviation.

TABLE 6 | Diagnosis of IGT vs. NGT.

Laboratory parameters						
			HbA1c			**FPG**
ROC AUC (CI)			0.87 (0.75–0.98)			0.74 (0.54–0.94)
CGM-readouts						
	Mean Sensor	**%TAR (140 mg/dL)**	**%TAR (180 mg/dL)**	**%TBR (70 mg/dL)**	**Estimated A1c**	**GMI**
ROC AUC (CI)	0.78 (0.59–0.96)	0.78 (0.60–0.95)	0.73 (0.54–0.92)	0.53 (0.33–0.72)	0.77 (0.58–0.95)	0.75 (0.56–0.94)
	SD	**CV**	**LBGI**	**HBGI**	**MAGE**	**CONGA**
ROC AUC (CI)	0.75 (0.58–0.93)	0.74 (0.56–0.91)	0.49 (0.28–0.70)	0.76 (0.59–0.93)	0.76 (0.60–0.93)	0.74 (0.58–0.90)

%TAR, percent of time above range; %TBR, percent of time below rang; 2hPG, oral glucose tolerance test (oGTT)-derived 2-h plasma glucose; CONGA, continuous overall net glycemic action; CV, coefficient of variation; GMI, glucose management indicator; HBGI, high blood glucose index; IGT, impaired glucose tolerance; LBGI, low blood glucose index; MAGE, mean amplitude of glycemic excursions; NGT, normal glucose tolerance; SD, standard deviation.

TABLE 7 | Test characteristics of HbA1c- and FPG-prediabetes thresholds regarding IGT vs. NGT (based on the current criteria of the American Diabetes Association).

	Threshold	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**TP**	**FN**	**TN**	**FP**
HbA1c	5.7% (NGSP) 39 mmol/mol (IFCC)	1	0.64 (0.44–0.84)	0.57 (0.46–0.75)	1	12	0	16	9
FPG	100 mg/dL	0.42 (0.17–0.67)	0.96 (0.88–1)	0.83 (0.5–1)	0.77 (0.70–0.86)	5	7	24	1

HbA1c, hemoglobin A1c; FN, false negatives; FP, false positives; FPG, fasting plasma glucose; IFCC, international federation of clinical chemistry and laboratory medicine; NGSP, national glycohemoglobin standardization program; NPV, negative predictive value; PPV, positive predictive value; TN, true negatives; TP, true positives.

TABLE 8 | Test characteristics of exploratory CGM-screening thresholds regarding IGT vs. NGT. Screening thresholds were calculated for sensitivities directly above and directly below 90%.

Timepoint	**Threshold**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**TP**	**FN**	**TN**	**FP**
CGM – mean sensor readings	104.7 mg/dL	0.92 (0.75–1)	0.48 (0.28–0.68)	0.46 (0.37–0.58)	0.92 (0.77–1)	11	1	12	13
	105.6 mg/dL	0.83 (0.58–1)	0.52 (0.32–0.72)	0.45 (0.35–0.59)	0.87 (0.71–1)	10	2	13	12
CGM - %TAR (140 mg/dL)	4.4%	0.92 (0.75–1)	0.36 (0.16–0.56)	0.41 (0.32–0.50)	0.90 (0.67–1)	11	1	9	16
	5.3%	0.83 (0.58–1)	0.44 (0.24–0.64)	0.42 (0.32–0.53)	0.85 (0.67–1)	10	2	11	14

%TAR (140 mg/dL), percent of time >140 mg/dL; FN, false negatives; FP, false positives; NPV, negative predictive value; PPV, positive predictive value; TN, true negatives; TP, true positives.

characteristics for IGT vs. NGT from years one to five after kidney transplantation. This potentially re-established diagnostic capacity of HbA1c in the stable phase after kidney transplantation, leading to according diagnoses and treatment, could be one explanation for the low PTDM-prevalence in our study. In a large multi-centric prospective study, Porrini et al. had quantified oGTT-based PTDM- and prediabetes-rates from year one to five after kidney transplantation between 21%–34% and 17%–22%, respectively [23]. In our study, maximum Youden's index was noted for HbA1c 5.7% (NGSP) or 39 mmol/mol (IFCC); at this cut-off sensitivity and specificity regarding IGT were 1.0 and 0.64, respectively. The results of our study are in contrast to those of Kurnikowski et al. [10]. Though showing a progressive improvement over time, HbA1c cut-off of 5.7% (NGSP) or 39 mmol/mol (IFCC) at 2 years still displayed limited diagnostic test characteristics regarding 2hPG (sensitivity 0.55 and specificity 0.82 for IGT) [10]. The discrepancy between our findings might be attributed to the progressive harmonization between HbA1c and the oGTT with time from transplantation. In addition, both studies did not employ confirmatory oGTTs; though the current gold standard for diagnosis of PTDM and prediabetes, limited reproducibility of the OGTT remains a well-known weakness of the test [24]. Since prediabetes, when diagnosed by an oGTT 12 months after transplantation, is an established potentially reversible cardiovascular risk factor [1], our data imply that HbA1c can aid in timely diagnosis and treatment.

Our second major finding is that best-performing CGM-readouts mean sensor readings and %TAR (140 mg/dL) display acceptable test characteristics regarding IGT from years one to five after kidney transplantation (ROC-AUC 0.78 for both). Though not studied extensively, differences in CGM-readouts between non-transplanted oGTT-defined normoglycemic and prediabetic subjects have been described; in the study of Costa et al. mean %TAR (140 mg/dL) was 19% for prediabetic and 13.9% (diabetes high risk group)/3.9% (control

group) for normoglycemic patients [25], while in the study of Hanefeld et al. mean %TAR (140 mg/dL) was 13% for prediabetic and 5.7% for normoglycemic patients [26]. Inter-group differences in %TAR (140 mg/dL) were more pronounced in our study (mean: 19% for IGT-vs. 7.7% for NGT-patients). Though in need of prospective validation, this intriguing finding could be a result of immunosuppressive medications (especially steroids) amplifying patient-specific CGM-signatures, thus enhancing discrimination between 2hPG-subgroups over the duration of 14 days CGM.

To our knowledge, this is the first study to assess the diagnostic performance of CGM-readouts compared to traditional glycemic parameters in the stable phase after KTR. Strengths of this study are its prospective design and the use of the oGTT as gold standard (as recommended for clinical practice by the international consensus meeting on PTDM in 2013 [4] and 2022 [5]). The main limitation of the study is its restricted sample size with 38 patients. All patients were Caucasian and a combination of calcineurin inhibitor, mycophenolate and steroids was used for immunosuppression, limiting generalizability to other patient groups or immunosuppressive regimens. Nutritional uptake and physical activity were not assessed.

Our study adds to the existing knowledge around PTDM by highlighting the high prevalence of IGT from years one to five after kidney transplantation and reassessing the role of HbA1c as a reliable parameter for the diagnosis of IGT during this phase. Best-performing CGM-readouts mean sensor readings and % TAR (140 mg/dL) displayed acceptable diagnostic performance. Prospective studies to determine whether CGM-readouts can predict clinically relevant nonglycemic outcomes better than the oGTT in KTR remain of interest.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by the Ethics Committee of Charité – Universitätsmedizin Berlin. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Authors GE and KB conceived of the presented idea. Authors GE, MN, BO, LL, FH, MC, ES, BZ, AT, and KB recruited study participants. Author GE performed the data analysis. Authors GE and KB wrote the manuscript; all authors commented and reviewed the final manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

GE received travel support from Chiesi. MN received research funding from Berlin Institute of Health; travel support from Deutsche Gesellschaft für Nephrologie; lecture honoraria from Novartis, Thermo Fisher Scientific, Deutsche Gesellschaft für Nephrologie. BO received travel support from Oncocyte. FH received grants from MSD, Hansa Pharma, Chiesi; consulting fees from Orifarm, Sanofi; honoraria from Hansa Pharma, Thermo Fischer, Aey Congresse; travel support from Hansa Pharma and is part of the advisory board of TolerogenixX TOL-2 Study. MC received grants from DFG, BMBF, EKFS Exzellenzstipendium; honoraria from Alexion, AstraZeneca, Berliner Dialyseseminar, Freunde der Berliner Charité, GSK, Novartis. KB received grants/contracts from Alexion, Astellas, AstraZeneca, Chiesi, CSL Behring, MSD, Otsuka, Stada, Takeda; consulting fees from Alcuris, Alexion, Astellas, AstraZeneca, Bayer, Bristol-Myers Squibb, Carealytics, CareDx, Chiesi, CSL Behring, Fresenius, Hansa, HiBio, MSD, Natera, Neovii, Paladin, Pfizer, Pirche, Sanofi, Stada, Takeda, Veloxis, Vifor, Xenothera; honoraria from Astellas, AstraZeneca, Chiesi, Fresenius, MSD, Paladin, Sanofi, Takeda; travel support from AstraZeneca, Chiesi, HiBio, MSD, Neovii, Paladin, Stada, Takeda, Veloxis; is part of the advisory board of Alcuris, Alexion, Astellas, AstraZeneca, Bristol-Myers Squibb, Carealytics, CareDx, Chiesi, CSL Behring, HiBio, MSD, Natera, Neovii, Paladin, Pfizer, Stada, Takeda, Veloxis, Vifor and has a leader or fiduciary role for Deutsche Transplantationsgesellschaft, Eurotransplant

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.

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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.2024.13724/full#supplementary-material

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GLOSSARY

%TAR (140 mg/dL) percent of time >140 mg/dL

%TAR (180 mg/dL) percent of time >180 mg/dL

%TBR (70 mg/dL) percent of time <70 mg/dL

2hPG oral glucose tolerance test-derived 2-h plasma glucose

ADA American Diabetes Association

ADPKD autosomal dominant polycystic kidney disease

AUC area under the curve

CGM continuous glucose monitoring

CI 95% confidence intervals

CONGA continuous overall net glycemic action

CV coefficient of variation

ESKD end stage kidney disease

FPG fasting plasma glucose

GMI glucose management indicator

HbA1c hemoglobin A1c

HBGI high blood glucose index

HDL high density lipoprotein

IFCC International Federation of Clinical Chemistry and Laboratory Medicine

IGT impaired glucose tolerance

IQR interquartile range

KTR kidney transplant recipient

LBGI low blood glucose index

LDL low density lipoprotein

MAGE mean amplitude of glycemic excursions

NGSP National Glycohemoglobin Standardization Program

NGT normal glucose tolerance

oGTT oral glucose tolerance test

PTDM post-transplantation diabetes mellitus

ROC receiver operating characteristic

SD standard deviation

WHO World Health Organization

ESOT | Transplant International

ORIGINAL RESEARCH
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Normothermic Machine Perfusion Reconstitutes Porcine Kidney Tissue Metabolism But Induces an Inflammatory Response, Which Is Reduced by Complement C5 Inhibition

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Normothermic machine perfusion (NMP) is a clinical strategy to reduce renal ischemia-reperfusion injury (IRI). Optimal NMP should restore metabolism and minimize IRI induced inflammatory responses. Microdialysis was used to evaluate renal metabolism. This study aimed to assess the effect of complement inhibition on NMP induced inflammatory responses. Twenty-two pig kidneys underwent 18 h of static cold storage (SCS) followed by 4 h of NMP using a closed-circuit system. Kidneys were randomized to receive a C5-inhibitor or placebo during SCS and NMP. Perfusion resulted in rapidly stabilized renal flow, low renal resistance, and urine production. During SCS, tissue microdialysate levels of glucose and pyruvate decreased significantly, whereas glycerol increased ($p < 0.001$). In the first hour of NMP, glucose and pyruvate increased while glycerol decreased ($p < 0.001$). After 4 h, all metabolites had returned to baseline. Inflammatory markers C3a, soluble C5b-9, TNF, IL-6, IL-1β, IL-8, and IL-10 increased significantly during NMP in perfusate and kidney tissue. C5-inhibition significantly decreased perfusate and urine soluble C5b-9 ($p < 0.001$; $p = 0.002$, respectively), and tissue IL-1β ($p = 0.049$), but did not alter other inflammatory markers. Microdialysis can

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Abbreviations: DGF, delayed graft function; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assays; HMP, hypothermic machine perfusion; IL, interleukin; IQR, interquartile range; IRI, ischemia reperfusion injury; KPS, kidney preservation solution; MAP, mean arterial pressure; NGAL, neutrophil gelatinase-associated lipocaline; NMP, normothermic machine perfusion; PAS, periodic acid-schiff; SCS, static cold storage; sC5b-9, fluid-phase C5b-9; TNF, tumor necrosis factor.

accurately monitor the effect of NMP on renal metabolism. Closed-circuit NMP induces inflammation, which appeared partly complement-mediated. Targeting additional immune inhibitors should be the next step.

Keywords: normothermic machine perfusion, ischemia-reperfusion injury, renal metabolism, microdialysis, inflammation

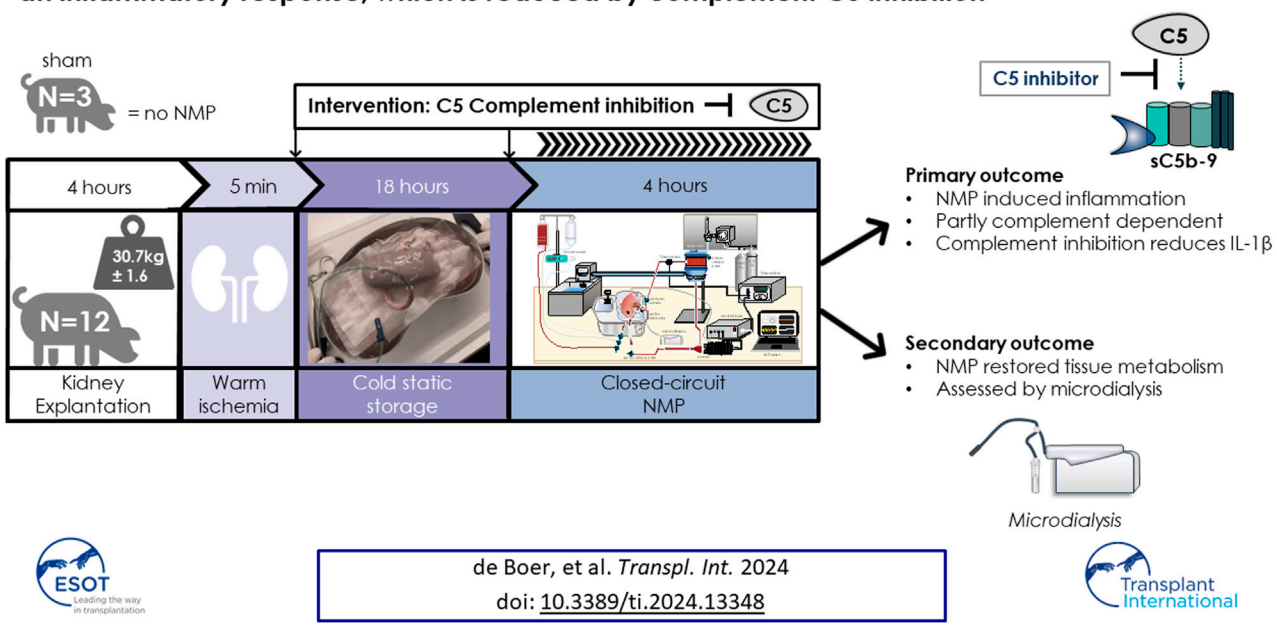
INTRODUCTION

The global shortage of suitable donor kidneys necessitates transplant centers to accept suboptimal allografts which are more susceptible to ischemia-reperfusion injury (IRI) [1–3]. As a consequence, a rise in incidence of clinical manifestations of IRI such as delayed graft function (DGF), primary nonfunction and rejection has been observed [3–5].

Normothermic machine perfusion (NMP) is a promising safe and feasible *ex situ* machine perfusion technique [6, 7]. NMP may alter ischemia and reperfusion induced IRI. During NMP, nutrients and oxygen are delivered to the graft, allowing the continuation of cellular metabolism under near-physiological conditions [8, 9]. Proof-of-concept studies using short-term NMP in human kidney transplantations demonstrated its potential to substantially mitigate IRI [10–12]. Additionally, NMP could be used as a research platform to evaluate non-systemic drug treatment. Yet, NMP itself might lead to inflammation and possible injury, and reliable monitoring tools to track *ex situ* renal metabolic tissue changes are absent.

The hallmark of ischemic injury includes a switch to anaerobic glycolysis leading to increased local accumulation of toxic metabolites [13]. Early detection of anaerobic metabolism during reperfusion is crucial for enabling interventions to optimize compromised grafts. Currently, there are no standard renal metabolic evaluation guidelines, most NMP protocols include estimations of the respiration status based on renal in- and effluent calculations including perfusion dynamics, oxygen- or glucose consumption, final glycolysis products, adenosine triphosphate depletion or focus on mitochondrial evaluation by measuring flavin mononucleotide [14, 15]. Real-time *in vivo* metabolic monitoring of renal metabolism is warranted as it offers the potential to improve nephron viability during NMP. Although invasive, microdialysis is safe, clinically approved, and importantly allows detection of reliable time-dependent metabolic changes in the renal interstitial fluid by using a small probe placed in the renal cortex [16, 17]. Studies on microdialysis in renal grafts, have revealed time-dependent increases in glycerol levels during static cold storage (SCS), and increases in pyruvate levels during hypothermic machine perfusion (HMP). None of the studies on microdialysis have evaluated kidney metabolism during NMP [18–20].

Despite the promising results of NMP in organ preservation, little is known about the inflammatory effect of NMP itself, which



GRAPHICAL ABSTRACT

might add to tissue damage [21]. The complement system is central in the innate inflammatory response and can be rapidly activated upon contact with foreign (bio)material, damaged cellular components, and blood-gas interfaces, all present during NMP [22–25]. Furthermore, various studies using animal models have demonstrated that complement activation plays an important role as a mediator of kidney IRI [26, 27]. Pharmaceutical targeting of the central complement component C5 seems promising, since C5aR1 and C6 blockade has been shown to ameliorate IRI in mice models [28, 29].

This study evaluated the feasibility of microdialysis to monitor renal cellular metabolism during NMP. The primary aim was to investigate the impact of complement C5 inhibition on renal inflammation during preservation.

MATERIALS AND METHODS

Animals

A total of 15 healthy Norwegian Landrace pigs (*Sus scrofa domesticus*), aged 6 months (30.7 ± 1.6 kg) of either sex were used. Exclusion criteria were: (i) haemoglobin <5 g/dL, (ii) SaO2 < 90% while receiving conventional (0.3) FiO2, (iii) mean arterial pressure (MAP) < 50 mmHg and/or heart rate >150 bpm before cross-clamping of the aorta, and (iv) death before kidney retrieval. The day before the experiment the pigs were housed in the animal facility and provided food and water *ad libitum*. All experiments were conducted by certified researchers in concordance with the European Ethical Guidelines for Use of Experimental Animals and the study was approved by the Norwegian Food Safety Authority (Ref. number: 20/78106).

Surgical Procedure

Anaesthesia was induced with intramuscular ketamine (60 mg/kg), atropine (1 mg), and droperidol (0.6 mg/kg). Pentobarbital sodium (25 mg) bolus injections were administered if needed for sedation and analgesia was provided using morphine (bolus and continuous infusion 1 mg/kg/hour) until no reaction to sharp hoof-pinching was elicited. After tracheostomy, controlled mechanical ventilation (flow 3 L/min, TV 10 mL/kg, RR 18/min, PEEP 5 cm H_2O, FiO_2 30%) was established and anaesthesia was maintained by 1% isoflurane. An indwelling urinary catheter, arterial pressure monitoring, and a central venous catheter were inserted. Once both kidneys and their vessels were isolated, two microdialysis catheters (CMA 71, 100-kDa pore size, length of 30mm, M Dialysis AB, Stockholm, Sweden) were inserted superficially into the lateral renal cortex using a splitable introducer. These catheters were perfused with Hydroxy-ethyl-starch 130/0.4 (Voluven®, Fresenius Kabi, India) through microinjection pumps (CMA 107, M Dialysis AB) at a velocity of 1 µL/min. After 1 h stabilization, sodium heparin (10.500 IE) was given, whole blood was collected and the aorta cross clamped prior to kidney retrieval. Both kidneys endured *in situ* warm ischemia time when systemic blood pressure dropped <50 mmHg of approximately 10–15 min. Once retrieved, the kidneys were immediately flushed with ice-cold Ringer's acetate at low pressure through a Lifeport cannula (Organ Recovery Systems, Itasca, IL) inserted in the renal artery until the effluent was clear from residual blood. Ureters were cannulated with a neonatal feeding catheter (8Fr). Kidneys from each animal were flushed with preservation solution (KPS)-1 (Organ Recovery Systems). Animals were sacrificed by an intravenous injection of 500 mg pentobarbital, 30 mg morphine, and 50 mmol potassium chloride. Ethylenediaminetetraacetic acid (EDTA; 0.5 M) blood samples were collected throughout surgery, centrifuged at 3000 *g* for 15 min at 4°C and stored at −80°C until further analysis.

Normothermic Machine Perfusion

All kidneys were preserved at 4°C for 18 h in University of Wisconsin based preservation solution (KPS-1, Organ recovery systems, Itasca, IL) prior to NMP; sham kidneys were not perfused (n = 6). Closed-circuit NMP was initiated using a pressure-controlled perfusion system (Software: SophistiKate, UMCG, Groningen, the Netherlands), a centrifugal pump providing pulsatile flow (Medos Deltastream DP2; Xenious AG, Heilbronn, Germany), a pediatric oxygenator with integrated heat exchanger (D100: Sorin Group, Arvada, CO) and an organ chamber (**Figure 1**) [30]. Components were connected by phosphorylcholine coated tubes, sampling ports were situated before and after the organ chamber. Perfusion pressure was obtained via pressure transducers (Edwards Lifesciences, Irvine, CA), and perfusion flow was measured via inline flow sensors (Transonic Systems Europe BV, Elsloo, the Netherlands). The NMP circuit was primed for 20 min with autologous plasma at 39°C (centrifuged at 3000 *g* for 15 min at 4°C). The renal vein was cannulated (12Fr catheter; Sorin Group). NMP was started with oxygenated (atmospheric air/oxygen 70%/30%) whole blood [hematocrit 20%, glucose 1 mg/mL, heparin 5 IU/mL, creatinine 1 mM, 0.1 mL sodium nitroprusside (25 mg/mL: Hospira Inc., Lake Forest, IL)] at 39°C with a mean arterial pressure of 60 mmHg and conducted for 4 h. Volume loss due to urine production was managed by 1:1 volume replacement with the recirculation of urine, administration of Ringer´s acetate, or autologous whole blood in 20 mL intervals based on the blood gas results. Throughout the perfusion, urine and perfusate-preparation samples were collected in EDTA tubes and stored at −80°C. Blood gas analyses were performed (ABL90 Flex/Plus: Bergman Diagnostika, Kjeller, Norway), and electrolyte imbalances were corrected to regulate the pH value. After 4 h of NMP, kidneys were flushed with 200 mL NaCl 0.9% at room temperature and thereafter tissue biopsies (cortex and calyx) were excised and fixed in formalin or snap-frozen at −80°C. Perfusion characteristics including renal blood flow, renal resistance, mean arterial pressure and urine production were constantly monitored.

C5 Inhibitor

Kidneys from each animal were randomized to receive either 20 µg/mL C5 inhibitor [Ra101295 peptic C5 inhibitor, comparable mode of action to Zilucoplan®, provided by Ra Pharma part of UCB Pharma (Brussels, Belgium)] or saline (NaCl 0.9%), thus every animal was its own control. C5 inhibitor or saline was given during SCS (20 µg/mL), as bolus at the start of NMP (20 µg/mL) and as a continuous

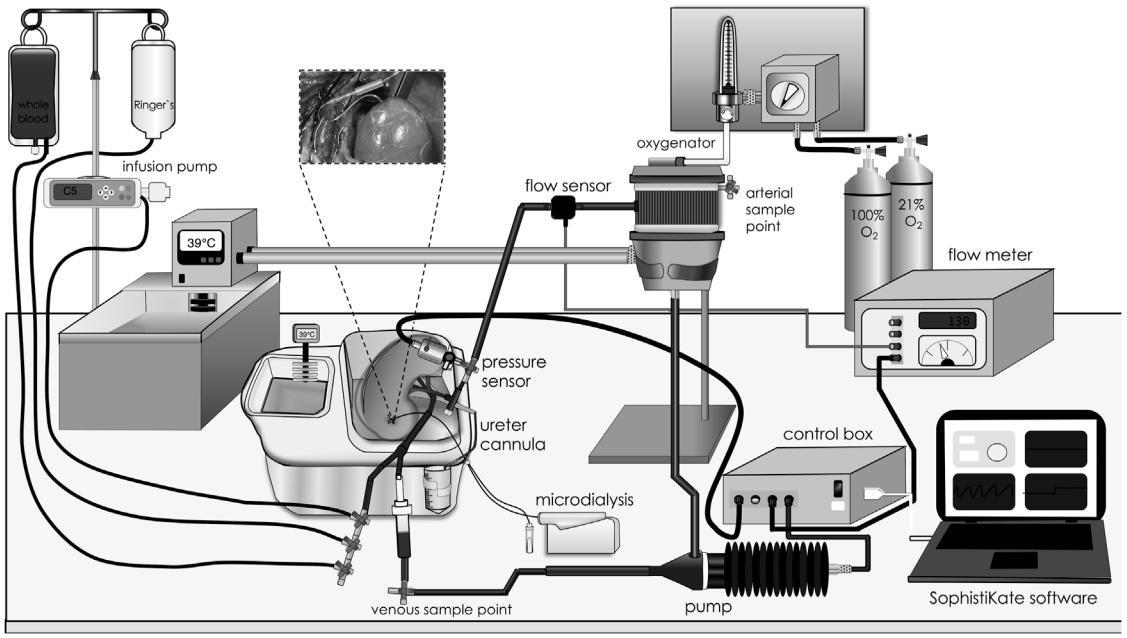


FIGURE 1 | Closed-circuit normothermic machine perfusion. Graphic illustration of the different components of the closed-circuit normothermic machine perfusion model. Blood was driven in a sinusoidal manner at a fixed rate of 60 oscillations per minute by a centrifugal pump. Pump speed was adjusted by setting a mean arterial pressure target in the software. Blood was warmed to 39°C, oxygenated, and cleared for CO2 in an oxygenator prior to entering the kidney through an arterial cannula. At the venous side, treatment, whole blood, Ringer's solution and urine were infused and pushed into a bubble trap to prevent perfusion of air bubbles from entering the circuit. Perfusate sampling ports were placed before and after the kidney chamber, and the microdialysis syringe pump was placed beside the organ chamber, allowing sampling throughout the perfusion period.

TABLE 1 | Perfusion solution characteristics during machine perfusion.

	C5 inhibitor				*Placebo*			
	T60	**T120**	**T180**	**T240**	**T60**	**T120**	**T180**	**T240**
Blood gas analysis								
pH	7.2 (7.1–7.3)	7.1 (7.1–7.3)	7.1 (6.9–7.2)	7.2 (7.0–7.2)	7.2 (7.2–7.2)	7.2 (7.1–7.3)	7.1 (7.0–7.2)	7.0 (6.9–7.1)
pO2 (kPa)	16.5 (11.8–17.1)	16.0 (14.8–17.0)	16.6 (13.7–17.8)	16.8 (15.0–18.5)	12.3 (9.2–15.0)	15.3 (13.3–16.3)	15.0 (13.6–17.8)	15.9 (14.0–18.7)
pCO2 (kPa)	3.5 (2.3–5.0)	2.9 (2.5–3.8)	3.2 (2.1–4.1)	2.8 (2.4–3.4)	3.8 (3.1–5.4)	4.1 (3.0–4.5)	4.3 (3.4–4.8)	2.8 (2.3–3.1)
Hb (g/dL)	6.6 (5.0–8.2)	6.3 (5.2–7.5)	6.0 (4.6–6.7)	8.2 (6.0–10.7)	7.7 (7.0–8.0)	6.7 (5.5–7.0)	6.4 (5.7–8.5)	6.9 (4.7–7.3)
Glucose (mmol/L)	4.1 (2.5–4.7)	6.1 (3.1–8.9)	3.7 (2.3–7.3)	7.1 (3.3–9.4)	4.5 (2.9–5.8)	5.7 (3.4–6.7)	5.7 (1.9–9.6)	6.0 (4.1–10.8)
Normothermic machine perfusion								
MAP	59.8 (53.3–63.2)	60.7 (56.3–62.3)	60.7 (55.0–65.0)	65.0 (62.3–67.6)	62.0 (58.7–64.0)	63.3 (58.0–69.5)	63.0 (60.6–64.3)	64.3 (60.6–69.0)

Abbreviations: MAP, mean arterial pressure.

infusion for the whole study period (1.75 μg/h, **Supplementary Figure S1**).

Microdialysis

The microdialysis samples were collected in microvials (M Dialysis AB) during surgery (before and after warm ischemia), SCS (1 h, 3 h, 16 h, and 18 h) and reperfusion (10 min, 30 min, 60 min, 120 min, 180 min, and 240 min). Concentrations of glucose, pyruvate and glycerol were immediately analyzed with the Iscus analyzer (M Dialysis AB).

Immunoassays

In-house enzyme-linked immunosorbent assays (ELISA) were used to measure C3a [31] and fluid-phase C5b-9 (sC5b-9) [32] concentrations in EDTA perfusate, urine samples and whole protein tissue extracts. Commercially available porcine ELISA assays were used to detect interleukin (IL)-10 (e-bioscience, Waltham, MA), tumor necrosis factor (TNF), IL-6 and IL-1β (R&D, Minneapolis, MN) in whole protein tissue extracts and EDTA perfusate. IL-8 quantification was performed using a Luminex assay (Merck, Darmstadt, Germany). All assays were

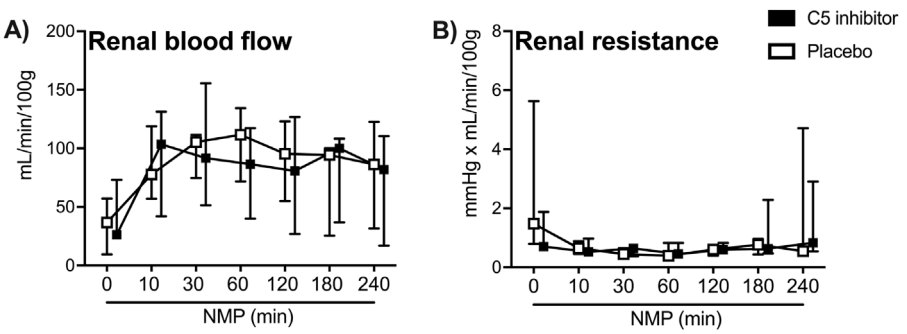


FIGURE 2 | Perfusion characteristics. Arterial renal blood flow **(A)** and renal resistance **(B)** over a 240 min period of normothermic machine perfusion. Data are presented as median ± IQR. General mixed model analyses.

used according to the manufacturer's instructions. Tissue extraction was performed as previously described [33], using CytoBuster protein extraction reagent (EMD Millipore Corp., Billerica, MA) and cOmplete protease inhibitor cocktail (Roche, Basel, Switzerland).

Kidney Damage Biomarkers and Function

Neutrophil gelatinase-associated lipocaline (NGAL) levels were detected by a commercially available porcine ELISA (Abcam, Cambridge, UK) according to the manufacturer´s instructions. Perfusate concentrations of creatinine were obtained through arterial blood gas, while the concentration in urine was measured using routine procedures at the clinical chemistry laboratory, Oslo University Hospital. Total protein concentrations in urine were measured by detergent compatible protein assay of Bio-Rad (Hercules, CA). Formulas used to estimate creatinine clearance and oxygen consumption are available in the **Supplementary Material**.

Histological Evaluation

Histopathological injury was examined using hematoxylin & eosin and periodic acid-Schiff (PAS) staining techniques on paraffin-embedded biopsies. Glomerular capillary microthrombi and fibrin deposition were examined through a Maurits, Scarlet, and Blue (MSB) stain, as described in detail elsewhere [34]. Loss of glomerular integrity was scored on a scale of 0–100; 0 (none), 0–1 (occasional), 1–10 (mild), 10–50 (moderate) and severe (>50), the abundance of tubular protein casts was scored on an ordinal scale. Signs of tubular ischemic injury including intratubular cellular detachment and tubular necrosis were observed, but not quantified due to concerns raised about the accuracy of such subjective measurements in our setup. All histological analyses were performed by an experienced pathologist blinded to group allocation.

Study Design and Statistical Analysis

In this prospective, blinded, controlled randomized study, kidneys were allocated randomly into two intervention groups using the random allocation tool in Microsoft Excel, the investigators handling the kidneys were blinded to the intervention. The sample size was calculated by power analyses, revealing that 10 kidneys in each treatment group would be sufficient to detect a 20% difference in the inflammatory markers (sC5b-9, TNF-α, IL-1β, IL-6, and IL-8) between the groups with a power of 0.8. In total, twenty-eight kidneys were included (sham, n = 6), two kidneys were excluded from analyses; one due to a technical perfusion defect and one due to morphologic abnormalities in the renal artery. NMP was terminated early when blood flow dropped below 10% of the maximum flow or severe perfusate leakage occurred, which was not possible to resuscitate within 5 minutes and/or kidney perfusion ceased. Six kidneys ceased functioning during NMP, in which five belonged to the C5-inhibitor treated group. One kidney ceased functioning after 74 min and one after 150 min and these were therefore excluded from analyses later than 60 min and 120 min of NMP, respectively. Four kidneys ceased functioning between 180 and 198 min of NMP and were excluded from analyses later than 180 min of NMP. Kidneys with perfusion times of ≥220 min were included in 240 min analyses. Values are presented as median ± interquartile range (IQR). Differences between C5 inhibitor-treated and control animals as well as differences over time throughout the study period were investigated using generalized linear mix model analyses (intervention as fixed effect and subject number as random effect). Non-parametric tests i.e., Mann-Whitney *U* test and Wilcoxon signed-rank test were used to compare differences between the groups. All statistical analyses were conducted using IBM SPSS Statistics for Macintosh 28 (IBM Cooperation, Armonk, NY) and GraphPad Prism 9 (GraphPad Software, San Diego, CA). P values less than 0.05 were considered statistically significant.

RESULTS

Perfusion Characteristics During NMP

Kidney weight did not differ between control and the C5 inhibitor group at baseline (109 g versus 108 g, p =

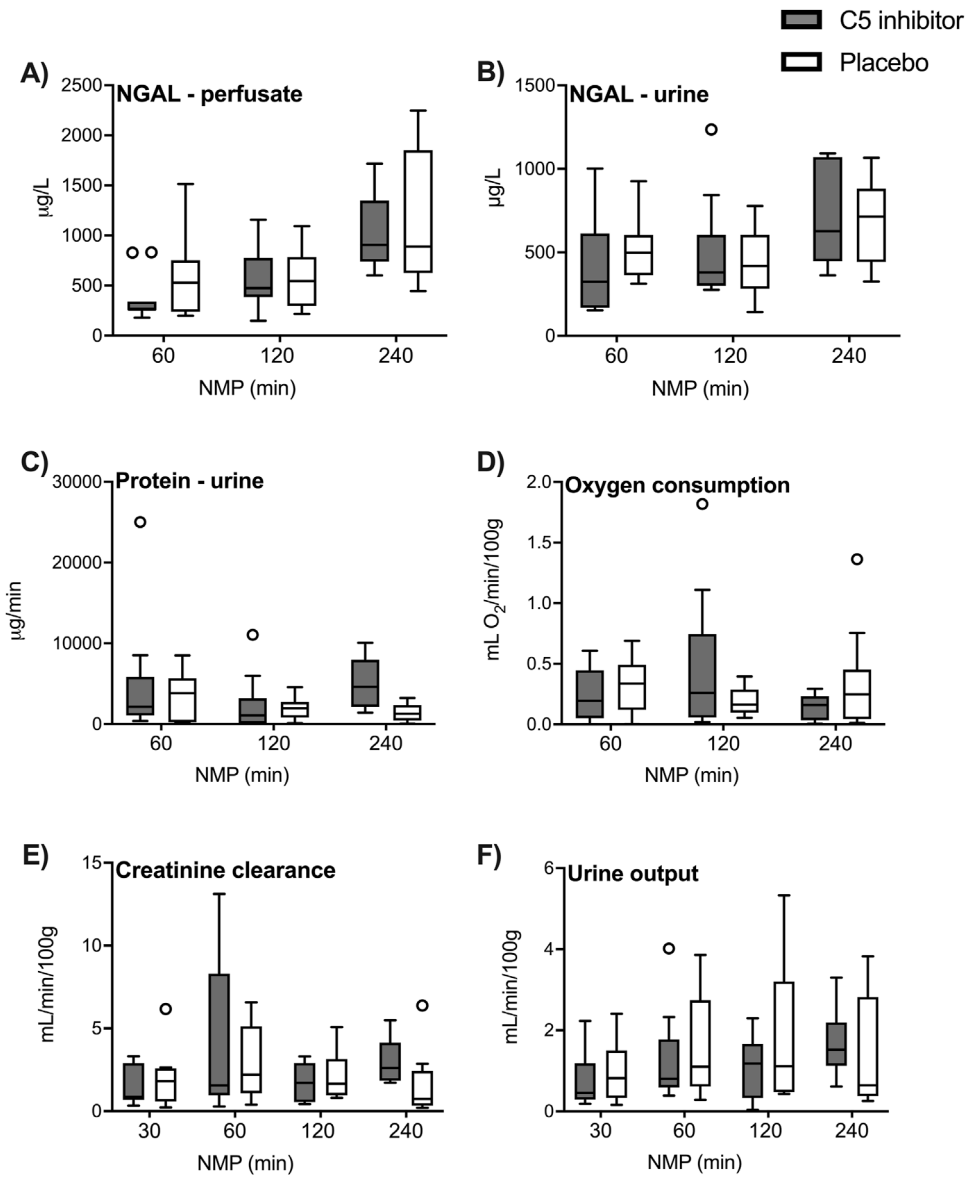


FIGURE 3 | Renal function and injury. The renal function and injury markers in the control and the C5 inhibited group were compared over a 240 min period of normothermic machine perfusion. NGAL levels in perfusate and NGAL levels in urine **(A, B)**, excretion rates of protein in urine **(C)** and oxygen consumption creatinine clearance and urine production **(D–F)**. Data are presented as median ± IQR. Generalized mixed model analyses. NGAL, neutrophil gelatinase-associated lipocalin; NMP, normothermic machine perfusion.

0.784) or after NMP (150 g versus 151 g, $p = 0.720$). Perfusate characteristics were comparable between groups throughout the NMP period (**Table 1**). Mean arterial pressure was kept stable during NMP. The renal blood flow showed a steep increase during the initial 30 min and was stable thereafter until the end of the 4 h of NMP, with no difference between the control and C5-inhibitor treated group ($p = 0.849$, **Figure 2A**). The renal resistance decreased within the first 10 min and remained continuously low throughout the perfusion with no difference between the control and C5-inhibitor treated group ($p = 0.282$, **Figure 2B**).

Renal Function and Injury

Perfusate and urinary NGAL excretion rates significantly increased after 60 min ($p = 0.001$; $p < 0.001$, respectively **Figures 3A, B**). Throughout reperfusion, significantly higher levels of proteinuria were observed during the first hour of reperfusion (p = 0.032, **Figure 3C**). Generally low oxygen consumption levels were observed during NMP and plateaued at 0.20 (0.12–0.51) mL O_2/min/100 g (**Figure 3D**). The creatinine clearance plateaued after 120 min NMP at 1.85 (0.94–2.98) mL/min/100 g (**Figure 3E**). Urine production rates slowly increased over the 4 h reperfusion period (Δ 0.72 mL/min/100g, $p < 0.001$,

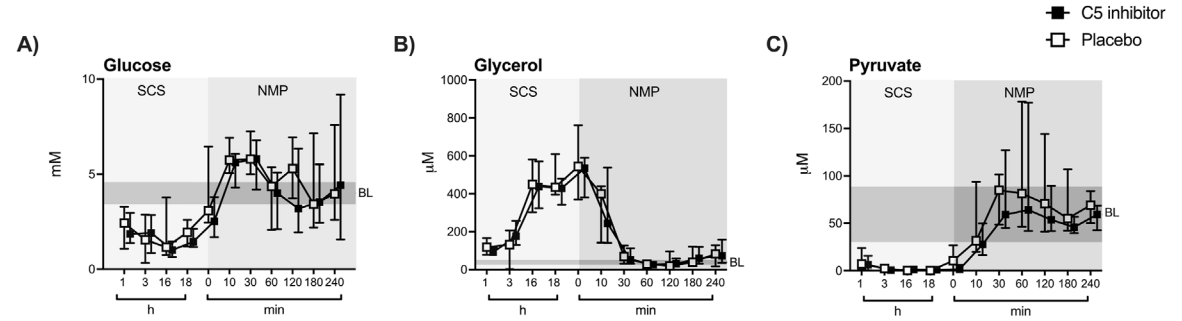


FIGURE 4 | Renal tissue metabolism. Glucose, pyruvate and glycerol **(A–C)** were measured in the renal microdialysate during SCS and NMP. Data are presented as median ± IQR. General mixed model analyses. SCS, static cold storage; NMP, normothermic machine perfusion; BL, *in vivo* baseline measurements (mean ± 2x sd).

Figure 3F). No significant differences were observed between the control and the C5 inhibitor groups for assessed kidney function and injury markers.

Renal Local Metabolism Assessed by Microdialysis

SCS led to a significant decline in tissue microdialysate levels of glucose (Δ −2.05 mM, $p < 0.001$) and pyruvate (Δ −56.82 µM, $p < 0.001$) **Figures 4A, C**), whereas glycerol levels increased (Δ +427.8 µM, $p < 0.001$) (**Figure 4B**) compared to *in vivo* baseline levels assessed prior to kidney procurement. A significant increase in glucose (Δ +3.81 mM, $p < 0.001$) and pyruvate (Δ +84.18 µM, $p < 0.001$) levels were observed during the initial 30 min of NMP, while glycerol levels decreased (Δ −378.8 µM, $p < 0.001$). Lactate was reduced upon SCS (Δ −1.91 mM, $p < 0.001$) and increased gradually during NMP (Δ +5.66 mM, $p < 0.001$) in comparison to baseline levels (**Supplementary Figure S2**). After 4 h of NMP, all metabolites settled at levels comparable to *in vivo* baseline levels except lactate, which showed a steady increase (**Supplementary Figure S2**). No statistical differences in microdialysis assessed metabolites were observed between the control and the C5 inhibitor group during SCS or NMP. Throughout NMP, correlations were observed between the level of tissue microdialysate levels of glycerol and urinary NGAL excretion rates ($p = 0.0324$, $r = 0.316$). None of the other kidney functional markers showed significant correlations (data not shown).

Complement System Activation

C3a and sC5b-9 levels in the perfusate and urine increased during the initial 30 min of NMP and remained elevated for up to 4 h (**Figures 5A, B, E, F**). Over the whole NMP period, C5 inhibition led to significantly reduced levels of sC5b-9 in perfusate and urine ($p < 0.001$; $p = 0.002$, respectively), except for a modest but significant increase at 240 min NMP in perfusate compared to the start of NMP ($p < 0.001$). Lower urine sC5b-9-to-proteinuria ratios were observed in C5 inhibitor treated kidneys compared to non-treated kidneys ($p = 0.033$, **Figure 5G**). In contrast, C3a perfusate and urine levels did not differ between the control and the C5-inhibitor treated group according to inhibition at the C5 level (**Figures 5B, F**). No significant differences were observed in sC5b-9 from tissue extracts between the control and the C5 inhibitor group (**Figure 5C**). C3a tissue levels were significantly elevated after 4 h of NMP compared with sham-treated kidneys ($p < 0.001$) and were significantly higher in medulla tissue compared to cortex tissue (**Figure 5D**). In the medulla tissue, C5 inhibitor treated kidneys had significantly higher C3a tissue levels ($p = 0.03$) compared to placebo.

Cytokine Production and Release

All tissue cytokine concentrations, except IL-10, were significantly elevated after NMP compared to sham kidneys ($p < 0.001$ for all, **Figures 6A–D**). Cytokine concentrations did not significantly differ between medulla and cortex region, except for IL-10, which showed lower levels in medulla compared to cortex ($p = 0.021$, **Figure 6E**). C5 inhibitor treatment led to a 46% reduction of IL-1β levels in medulla tissue ($p = 0.049$), while only non-significant trends were observed for the other cytokines. Perfusate levels of IL-1β, IL-6, IL-8, TNF and IL-10 significantly increased after 120 min of NMP and remained elevated up to 4 h; no differences were observed between the control and the C5 inhibitor group (**Supplementary Figure S3**).

Histopathology

Glomerular basement membrane integrity loss together with a reduction in cell density of the mesangium was observed in several of the kidneys exposed to NMP; no differences were observed between the groups (**Figure 7**). In separate analyses, MSB staining showed no signs of intracapillary fibrin deposition. Protein casts were observed in the lumen of the tubules; most prominent in the calyces, without differences observed between the control and the C5 inhibitor group. No evident lesions were present among the sham-treated kidneys.

DISCUSSION

In this study, we demonstrated that microdialysis detected changes in the renal metabolites glucose, pyruvate and glycerol, comparable between both intervention groups in

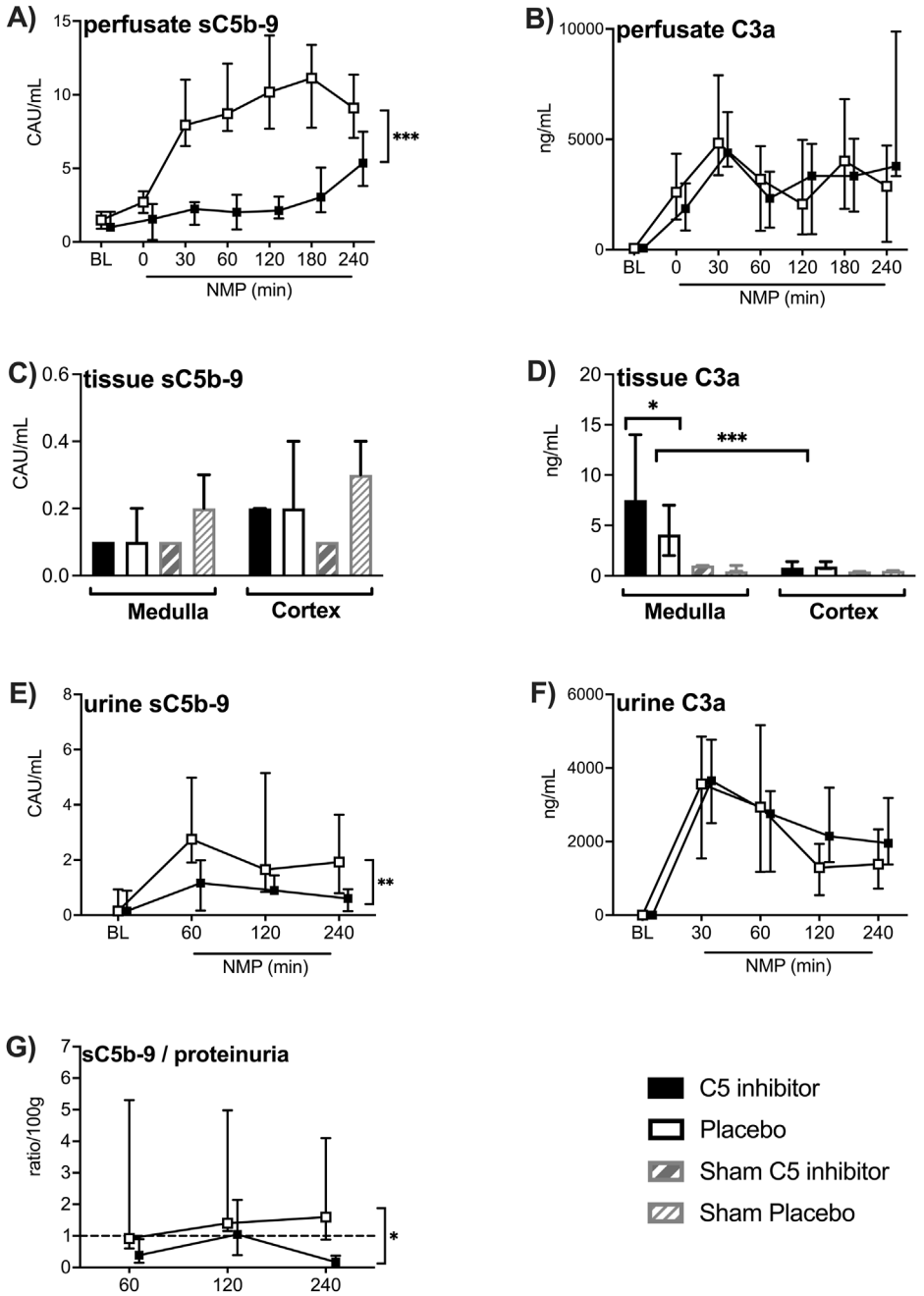


FIGURE 5 | Effect of C5 complement inhibition on complement activation. The complement markers in the control and the C5 inhibited group were compared during and after a 240 min period of normothermic machine perfusion. sC5b-9 levels and C3a levels in the perfusate **(A, B)**, sC5b-9 and C3a levels in medulla and cortex tissue **(C, D)**, sC5b-9 and C3a levels in the urine **(E, F)** and urine sC5b-9-to-proteinuria ratio **(G)**. Data are presented as median ± IQR. General mixed model analyses, Wilcoxon signed rank test and Mann-Whitney-U test. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. BL, *in vivo* baseline measurements; CAU, complement arbitrary units; NMP, normothermic machine perfusion.

response to SCS and NMP. The primary aim of this study was to assess the effect of complement inhibition on NMP induced inflammatory responses. We observed that NMP induced inflammation with increase in complement and cytokine levels in perfusate, urine, and kidney tissue. C5 inhibition completely blocked sC5b-9 formation and substantially and significantly reduced IL1-β, a central component of the NLRP3 inflammasome.

During organ transplantation, the metabolic state of kidney grafts is affected and NMP is used to reconstitute metabolism with the aim to reduce organ damage upon reperfusion [14]. Here, SCS caused a decrease in glucose and pyruvate levels while

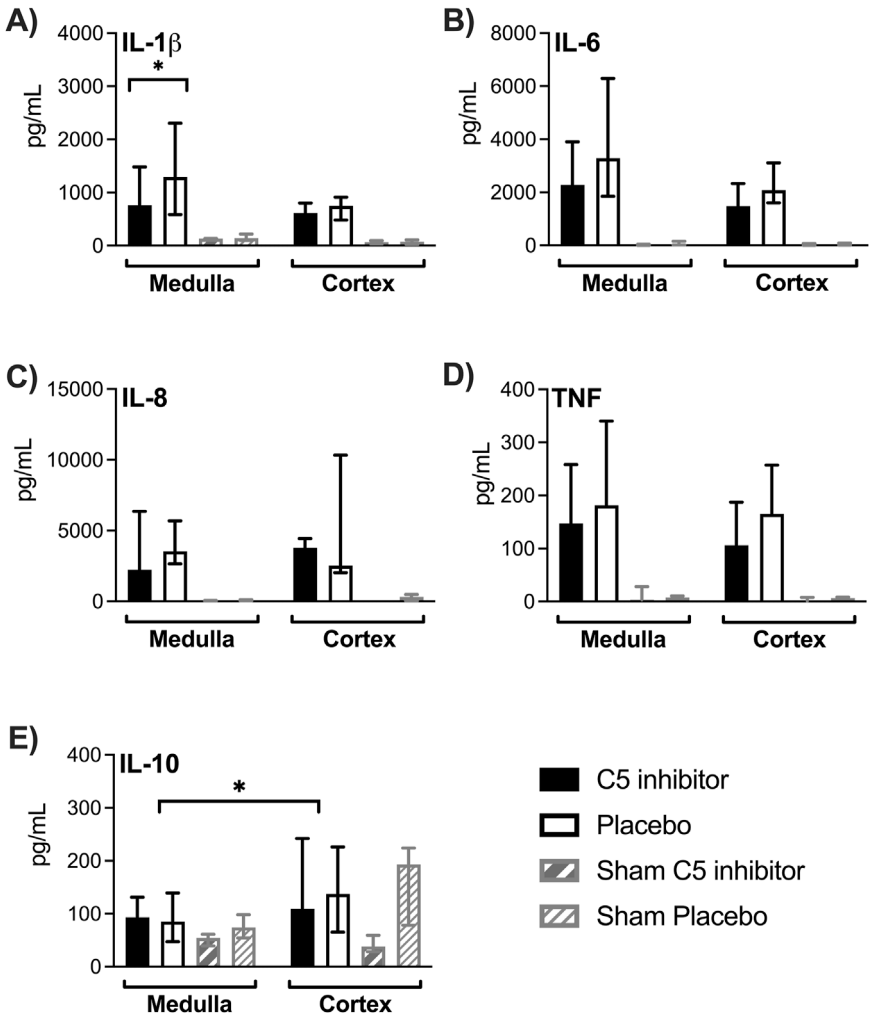


FIGURE 6 | Effect of C5 complement inhibition on cytokine levels in renal tissue. The complement markers in the control and the C5 inhibited group were compared after a 240 min period of normothermic machine perfusion. IL-1β, IL-6, IL-8, TNF and IL-10 cytokine concentrations measured in medulla and cortex tissue **(A–E)**. Data are presented as median ± IQR. Wilcoxon signed rank test and Mann-Whitney-U test. * = $p < 0.05$. IL, interleukin; TNF, tumor necrosis factor.

glycerol increased. These findings are consistent with previous studies and confirm the lack of metabolic function during SCS and cellular membrane break-down reflected by glycerol increase [18, 35, 36]. Upon the initiation of NMP, microdialysis detected an immediate glucose increase, followed by pyruvate, whereas the level of glycerol dropped significantly. Thus, NMP leads to return of renal cellular metabolism and decreases fatty-acid breakdown. Lactate increased progressively during NMP. Similar metabolic trends have also been found in the perfusate of previous NMP studies [12, 37–39] and are due to the limited ability of the kidney to metabolize lactate. The accumulation of lactate might be explained by the release through activated erythrocytes and leucocytes present in the perfusate [40, 41]. Renal lactate production caused by reduced oxidative phosphorylation [42] is less likely since pyruvate stabilized at prior *in vivo* levels. Renal oxygen consumption was stable. Thus, lactate was not produced by renal but leucocyte hypermetabolism.

NMP led to a significant activation of the complement system as revealed by increase in the activation products C3a and sC5b-9 in perfusate, urine, and renal tissue. These findings are consistent with studies assessing complement activation in other extracorporeal blood circulations such as cardiopulmonary bypass, hemodialysis and plasmapheresis [25] and in-line with previous findings in pig and human kidney NMP from our group [43]. The introduction of foreign material or a gas-blood interface into the circulation could initiate complement activation [44]. Artificial and air surfaces have been shown to induce IgG and C3 conformational changes resulting in the activation of the classical and alternative pathways [44–46]. Here, we have minimized gas-plasma interfaces, by using a closed-NMP system. Local synthesis of complement proteins by the kidney itself could be an important contributor [47]. Human kidney NMP uses plasma-free perfusates, but still shows complement activation, which can be explained by small amounts of plasma

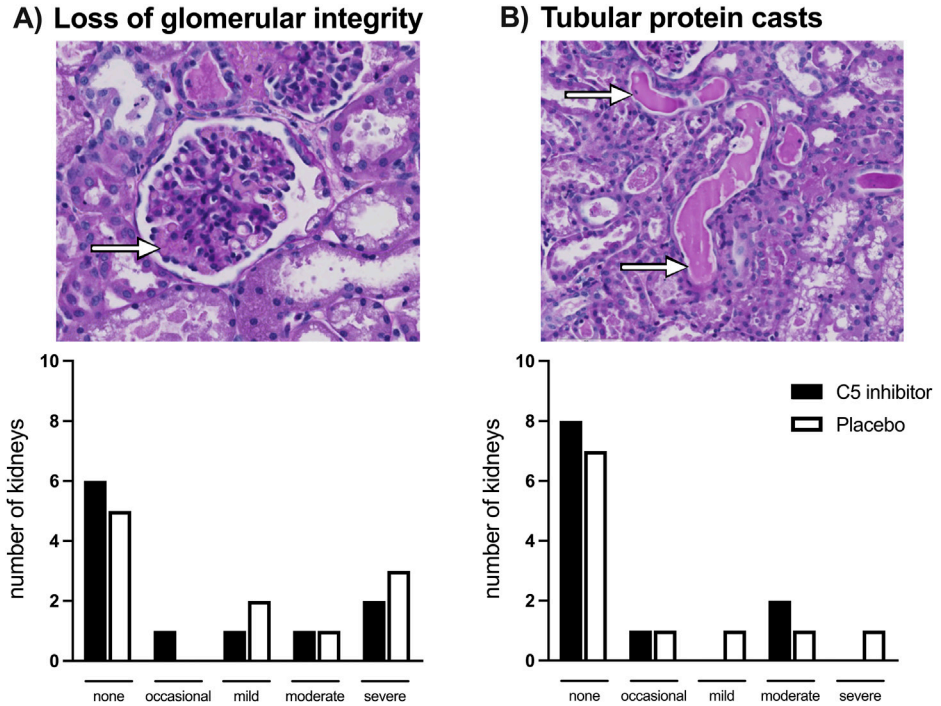


FIGURE 7 | Histology. Loss of glomerular basement membrane integrity depicted by the arrow **(A)** and protein casts in the lumen of tubules depicted by the arrows **(B)** assessed after a 240 min period of normothermic machine perfusion in PAS-stained biopsies. No differences were observed between the control and the C5 inhibited group.

left in the kidney as well as *de novo* synthesis of complement in the kidney [43]. Thus, although NMP reconstituted metabolism, a strong innate immune reaction was induced, which might hamper organ function and could at least in part explain high delayed graft function rates in clinical trials of kidney NMP after SCS preservation [6].

C5-inhibition blocked perfusate and urine sC5b-9 formation throughout NMP. sC5b-9 concentrations extracted from tissue were low and comparable between groups. Thus, we did not assess the deposition of C5b-9 in tissue sections. Furthermore, it is known that complement activation can induce endothelial cell and immune cell activation without detectable tissue complement activation [48]. Clinical trials evaluated the efficacy of the C5 inhibitor eculizumab when given minutes prior to reperfusion of kidney grafts and reported no benefit on delayed graft function [49]. In our study, kidney reperfusion was mimicked by using whole blood during NMP and our results are consistent with findings from these clinical trials. Here, C5 inhibition was extended and started immediately after organ procurement. However, C5 inhibition did not affect metabolic or physiological markers of kidney function, implying that transplant-induced IRI is only partly C5 dependent. Studies in mice imply that the lectin and alternative complement pathways contribute to renal IRI; mice deficient in MBL, factor B, or C3 showed reduced renal injury [50, 51]. Activation of these pathways results in the cleavage of C3 into C3a and C3b fragments. Since C3a-receptors are expressed on renal tubular epithelial cells and granulocytes, C3a is thought to play a role in the pathogenesis of renal IRI [28, 52]. In this study, C5 was inhibited and thus C3 cleavage led to similar C3a generation in both groups. Thus, targeting C3-cleavage might provide better outcomes. Unfortunately, there is no effective porcine C3 inhibitor currently available.

NMP caused a significant increase in the level of cytokines in the perfusate and tissue after 60–120 min from the start of NMP. Concordant with our findings, Stone *et al.* observed an inflammatory storm after kidney NMP, demonstrated by the increase of a range of pro-inflammatory cytokines at high concentrations [53], which has been confirmed in discarded human kidneys [43]. Interestingly, C5 inhibition resulted in a decrease of 46% in IL-1β levels in kidney tissue. Increased IL-1β levels have been linked with decreased graft function following IRI and co-occur in many diseases caused by complement dysregulation [50, 54]. We speculate that cytokines were induced by DAMPS originating from the initial oxidative allograft injury as the use of autologous blood only allows for stimulation by "self" molecules [13, 55]. In line with Jager *et al.*, TNF perfusate levels rapidly increased upon NMP whereas the other cytokines increased first after 1 hour [43]. This strengthens the notion that a TNF-dependent pathway might be involved in generation of cytokines [43]. The levels of cytokines decreased at the end of perfusion. A dilution effect is less likely as we observed steady hemoglobin concentrations, implying that the observed decrease reflects a biological mechanism. Taken together, all studied cytokines increased in our study after NMP. As cytokines are induced by several innate immune sensor

systems and renal IRI has been shown to enhance both TLR2 and TLR4 expression, the combined inhibition of complement with TLR co-factor CD14 may be more effective [56–58].

A limitation of this study is that we used whole blood as perfusate. Initial experiments had shown vast complement activation during leukocyte filtration of pig whole blood, which could have influenced the results [59]. However, also NMP performed with leucoyte- and even plasma-free perfusate has been reported to activate complement and cytokine production during NMP [43]. Thus, the results of this study might be useful also in clinical settings of kidney NMP. However, C5 inhibition in a clinical study [49] and this study did not lead to improvement in immediate kidney function and tissue damage. Thus, future research might have higher chances of success if optimization of NMP includes metabolic and inflammatory interventions in combination. A small number of kidneys were investigated in this study, but the paired approach using both kidneys from each individual created an ideal platform for assessing the C5 intervention. All kidneys returned function. Nonetheless, the observed histological injury along with enhanced NGAL levels and proteinuria confirmed renal IRI in our model. Some kidneys cease functioning due to high resistance before the 4-h endpoint. While up to 8 h kidney NMP is described, most NMPs are carried out in an open system [60]. Our closed-circuit setup required continuous monitoring to correct for volume loss and was highly susceptible to obstruction caused by collapse of the renal vein. Future research comparing the degree of complement activation in both systems should provide an answer to whether there is a rationale for using the more labor-intensive closed-circuit system.

In conclusion, metabolism can be assessed by microdialysis in kidney NMP and reveals metabolic demands during NMP. NMP induced complement activation and production and release of cytokines. Renal inflammation upon IRI appeared to be partially mediated by the complement system as C5 inhibition mainly led to non-significant changes except for a marked and significant decrease of IL-1β. However, C5 inhibition did not lead to improvement of kidney function and tissue damage. Further metabolic optimization of the NMP model and the assessment of additional immune inhibitors, should be the next step to reduce NMP-induced renal IRI.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal studies were approved by the Norwegian Food Safety Authority. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

EB participated in research design, performed experiments, performed data analyses and wrote the manuscript. MS participated in research design, performed experiments, performed data analyses and revised the manuscript. NJ participated in research design and revised the manuscript. CS participated in research design, performed experiments, performed data analyses and revised the manuscript. MW participated in research design and revised the manuscript. OL performed experiments and revised the manuscript. HM participated in the histological analysis and revised the manuscript. HG performed histological analysis and revised the manuscript. ET performed experiments and revised the manuscript. KP participated in the data analysis and revised the manuscript. DC participated in the data analysis and revised the manuscript. JL participated in the data analysis and revised the manuscript. BJ participated in research design and revised the manuscript. TM participated in research design and revised the manuscript. HL participated in research design and revised the manuscript. SP participated in research design, supervision, performed experiments and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

TM is a consultant for UCB Pharma.

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.2024.13348/full#supplementary-material

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BRIEF RESEARCH REPORT
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Overcoming Lung Challenges in TA-NRP Assisted Heart Recovery in Donation After the Circulatory Determination of Death

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Thoraco-abdominal normothermic regional perfusion (TA-NRP), utilizing Extra Corporeal Membrane Oxygenation (ECMO) devices, has emerged as an effective strategy for heart recovery in donors declared dead by circulatory criteria (DCDD). After death declaration, TA-NRP restores heart activity by reperfusing the arrested heart with oxygenated blood at normothermia. Mechanical ventilation resumption in the donor enables weaning from ECMO and restores systemic circulation and oxygenation using the donor's heart and lungs. However, if pre-existing conditions prevent the donor's lungs from oxygenating blood post-cardiac activity restoration, weaning from veno-arterial ECMO may lead to systemic hypoxia, jeopardizing the restored cardiac function. Anticipating this scenario may guide planning a split ECMO circuit to facilitate earlier and more effective recovery of donor heart function post-ECMO weaning. This manuscript describes three cases of DCDD donors with hypoxic respiratory failure undergoing TA-NRP for heart recovery. By establishing a bridge in the arterial portion of the circuit, clamped out after weaning from veno-arterial ECMO, donor heart function was assessed exclusively with veno-venous ECMO support, leading to successful heart transplantation.

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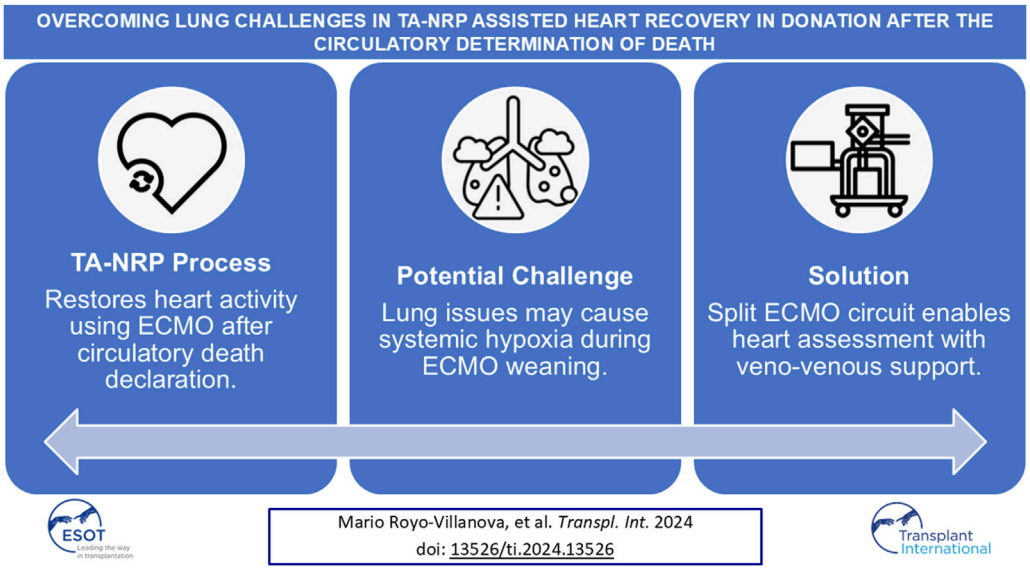
Keywords: NRP, thoracoabdominal normothermic regional perfusion, heart donors, lung, ECMO

INTRODUCTION

Heart recovery in donation after circulatory determination of death (DCDD) represents a recent advancement that expands the pool of hearts available for transplantation. Outcomes for heart transplants from DCDD donors appear comparable to those from donors declared dead by neurological criteria [1] (DNDD). As the demand for heart transplants continues to grow, innovative strategies like DCDD are critical to increasing the availability of viable donor hearts.

The utilization of thoraco-abdominal normothermic regional perfusion (TA-NRP), initially introduced by the Papworth group [2, 3], has emerged as an advantageous method for *in situ* reperfusion and restoration of activity in the asystolic heart. This technique, based on the use of Extra Corporeal Membrane Oxygenation (ECMO) devices, enables the functional evaluation of the heart after the declaration of death and prior to organ recovery. TA-NRP allows restoring heart activity by reperfusing the arrested heart with oxygenated blood at normothermia. Once heart activity is



GRAPHICAL ABSTRACT

restored, the reinstatement of mechanical ventilation in the donor facilitates the weaning of ECMO, allowing systemic circulation and oxygenation via the donor's own cardiac and pulmonary functions.

However, challenges arise when the donor has succumbed to severe and advanced lung disease or severe hypoxemic respiratory failure. In such cases, the donor's lung function may be insufficient to provide proper systemic oxygenation, thus hindering adequate cardiac contractility. Anticipating and managing these scenarios is crucial for the successful recovery and transplantation of donor hearts.

This manuscript reports a series of three DCDD donors with hypoxic respiratory failure prior to death where heart recovery was successfully performed through TA-NRP using a specific technical approach. We describe the methodologies used, the clinical outcomes, and discuss the implications of these findings for future DCDD heart transplantation protocols.

METHODS

TA-NRP Protocol

In Spain, the national DCDD heart transplant protocol [4] is based on the use of TA-NRP for the recovery and *in situ* assessment of heart viability. This assessment is performed using transesophageal echocardiography and/or Swan-Ganz catheterization. Once recovered, the DCDD heart is subject to static cold storage before transplantation.

In the standard procedure, after the provision of life support measures, waiting to circulatory arrest, a 5-min "no-touch" period is observed, and the patient is declared deceased. Subsequently, a rapid sternotomy is performed with clamping and sectioning of the supra-aortic trunks to avoid restarting brain flow [5], followed by the initiation of TA-NRP.

Upon restoration of the donor heart's activity, it is crucial to wait for the restoration of optimal cardiac function capable of producing adequate systemic output to perfuse both the coronary circulation and the donor's other organs. Typically, with the commencement of TA-NRP, heart function experiences prompt recovery, enabling the gradual weaning of TA-NRP. Ongoing assessment of donor heart function is performed using a transesophageal echocardiogram, which is particularly valuable in evaluating cardiac performance in the now heart-beating donor.

To facilitate a gradual transition from normothermic regional perfusion, reintubation of the recipient and gradual oxygenation of their blood using the donor's native lungs allow for the gradual weaning of ECMO support.

TA-NRP in Donors With Hypoxic Respiratory Failure

In scenarios where donors have severe hypoxic respiratory failure, a Y-shaped bypass integrated into the ECMO circuit can be used to allow quick diversion of the loop and immediate re-conversion from veno-arterial TA-NRP to veno-venous TA-NRP (**Figure 1**). This configuration is achieved using a simple diverting clamp, which permits *in situ* oxygenation of the donor's organs when the donor's lungs are insufficient to perform this function independently.

We report a series of three DCDD donors with hypoxic respiratory failure prior to death and cardiac procurement. Written informed consent for their recruitment and publication of data was authorized by close family members and the study was approved by the Ethics Committee for Medical Research at Virgen de la Arrixaca University Hospital (CEIM).

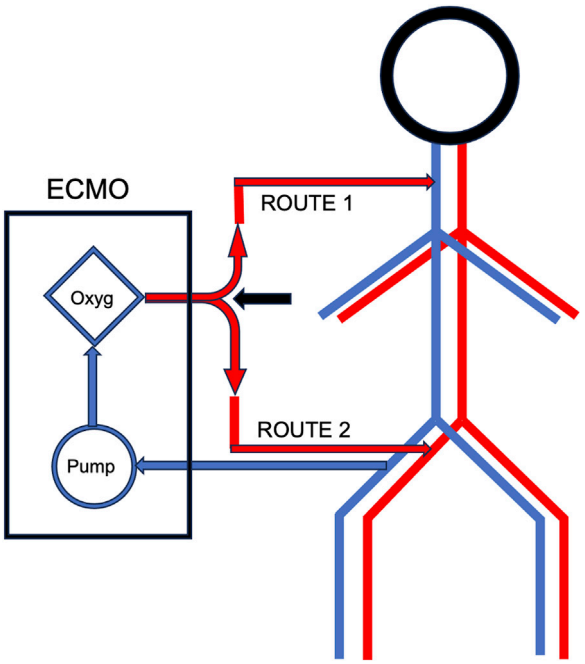


FIGURE 1 | ECMO Y-shape bypass.

RESULTS

Two of the donors were already on VV ECMO support and had a femoral arterial cannula placed for NRP prior to the withdrawal of life-sustaining therapies (WLST). The third donor experienced rapid respiratory deterioration immediately before WLST and was placed on VA ECMO as a bridge to convert to VV ECMO.

The first two cases had femoral and jugular veno-venous ECMO, requiring only the insertion of an additional femoral arterial cannula. In the third case, separate arterial and venous cannulas were inserted into the femoral vessels, along with an additional cannula in the right jugular vein. Thus, all donors had three cannulas before WLST (Spanish legislation allows pre-mortem preservation maneuvers with appropriate family consent). The ECMO circuit was initially connected to the femoral vessels (veno-arterial) to restart systemic circulation once the patients were declared dead.

In all cases, cardiac activity resumed within 2 minutes after initiating TA-NRP. Following adequate initial cardiac contractility, attempts to withdraw VA TA-NRP resulted in a significant and immediate decrease in contractility and cardiac output, accompanied by severe hypoxia. Through the Y-bypass of the ECMO, the NRP circuit flow was redirected to veno-venous by extracting blood via the femoral venous route and re-infusing oxygenated blood via the jugular vein. Under these conditions, cardiac contractility and output were maintained optimally until organ validation and subsequent perfusion with cold cardioplegia solution.

All three hearts were deemed suitable for transplantation, and all three were successfully implanted in the recipients (**Table 1**).

DISCUSSION

The successful recovery of hearts from DCDD donors with severe hypoxic respiratory failure demonstrates the viability of using TA-NRP in combination with a modified ECMO circuit. If, due to any pre-existing condition, the donor's lungs are unable to oxygenate the blood after restoring cardiac activity, weaning from VA-ECMO may be impossible due to systemic hypoxia, compromising the newly restored cardiac activity.

When there is suspicion that mechanical ventilation of the lungs may be insufficient to oxygenate the blood of the DCDD donor, such as in cases previously supported with VV-ECMO, it is critical to preserve the VV-ECMO circuit. This ensures adequate oxygenation during the weaning of VA-ECMO by quickly reconfiguring it to VV-ECMO with a double bypass. Anticipating the insertion of a second jugular venous cannula in potential donors not already on VV-ECMO, but exhibiting severe hypoxia prior to the withdrawal of life support measures, can facilitate the conversion of the femoro-femoral VA circuit to a femoro-jugular VV circuit if necessary. This approach can be highly beneficial for heart resuscitation and successful transplantation.

The findings from these cases suggest that preemptive strategies and modifications to the ECMO circuit can significantly enhance the outcomes of DCDD heart recovery, even in donors with compromised pulmonary function. Future protocols for DCDD heart transplantation should incorporate these techniques to maximize the pool of eligible donors and improve transplantation success rates.

TABLE 1 | Outlines the clinical characteristics of the cases.

Donor								Recipient						
	Age	ICU days	Diagnosis	ECMO (days)	Weight	Height	CIT	VA ECMO time	VV ECMO time	LVEF at retrieval	PIcuD	PIS	PDS	DAFH
Patient 1	51	12	ILS	11	100	180	96	16	21	65	13	Yes	No	Yes
Patient 2	33	39	Polytrauma (thoracic trauma)	36	92	182	108	23	33	65	9	Yes	No	Yes
Patient 3	42	12	Polytrauma (VAP)	0	75	170	73	12	18	60	16	Yes	No	Yes

ILS: interstitial lung disease, VAP: Ventilator-Associated Pneumonia, Age (years), Weight (Kilograms), Height (centimeters), CIT: Cold ischemic time (minutes), VA ECMO, time (minutes), VV ECMO, time (minutes), LVEF: left ventricular ejection fraction, PIcuD: postoperative ICU, days, PIS: postoperative inotropic support, PDS: postoperative device support, DAFH: discharge alive from the hospital.

The use of TA-NRP combined with strategic modifications to the ECMO circuit can effectively overcome the challenges posed by donors with severe hypoxic respiratory failure. By ensuring adequate oxygenation and maintaining cardiac function during the weaning process, it is possible to achieve successful heart transplantation outcomes. These findings underscore the importance of tailored approaches in DCDD heart recovery and highlight the potential for expanding the donor pool through innovative techniques.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) OR legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article and none of the individuals were under the age of 18.

AUTHOR CONTRIBUTIONS

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

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CONFLICT OF INTEREST

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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ChatGPT was used to review the text for possible English language or syntax errors.

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