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Predicting the outcomes of a bridge to lung transplantation

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TRANSPLANT TRIAL WATCH
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Transplant Trial Watch

Simon R. Knight[1,2]*, *John Fallon*[1,2] *and Keno Mentor*[3]

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Keywords: delayed graft function, deceased donor kidney transplantation, randomised controlled trial, liver transplant, normothermic 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.

RANDOMISED CONTROLLED TRIAL 1

Balanced Crystalloid Solution Versus Saline in Deceased Donor Kidney Transplantation (BEST-Fluids): A Pragmatic, Double-Blind, Randomised, Controlled Trial.

by Collins, M. G., et al. Lancet 2023; 402(10396): 105–117.

Aims
To assess if use of balanced crystalloid vs. saline reduces rates of delayed graft function.

Interventions
The intervention group received a balanced crystalloid in the form of Plasma-Lyte 148 intra- and post-operatively for intravenous volume replacement vs. standard care who received 0.9% sodium chloride.

Participants
808 participants, adults and children receiving kidney only transplant.

Outcomes
The primary outcome was DGF, which they defined as need for dialysis within the first 7 days. Secondary outcomes included: number of dialysis treatments, duration of dialysis in days, ranked composite of DGF and day 2 creatinine reduction ratio, post-op hyperkalaemia, peak potassium, fluid overload, urine output, use of inotropes, acute rejection, number of biopsies, mortality, graft survival, graft function and hospital stay.

Follow-Up
52 weeks.

CET Conclusion
This large, multi-centre, double-blinded, randomised control trial found a reduction in DGF rate with the use of balanced crystalloid (30%) compared with normal saline (40%), a RR of 0.74 ($p < 0.0001$).

Transplant Trial Watch

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The trial was well designed, with exceptional blinding, the Plasma-Lyte and saline were packaged in custom identical bags only identifiable by serial numbers, so all parties were blinded for the duration of the trial. The trial was conducted across 16 sites, with a representative trial cohort of deceased donor kidneys (DBD:DCD of 3:1), containing only 16 (2%) of pre-emptive recipients and 20 (2%) kidneys that received HMP as preservation, which is crucial given their primary outcome. This reduction in DGF equated to 190 fewer dialysis sessions in the balanced crystalloid group and a number needed to treat of 10 to prevent 1 case of DGF. Their hypothesised effect of the fluid on post-transplant biochemistry, with reduced chloride burden, increase bicarbonate and pH with minimal effect on potassium was demonstrated and thus reduced tubular acidosis and improve blood flow leading to lower rates of DGF is sound. The trial has few limitations, laboratory data was not collected beyond post-operative day 2 and other minor data points such as blood pressure and surgical anastomosis time, however given the trial size and randomisation strategy limiting centre effect, this is likely of no consequence. It is important to note that the effect is not necessarily generalisable to other balanced crystalloids such as Hartman's, given that contains more chloride as well as lactate and further work would be needed to assess their benefit. This trial provides robust evidence sufficient to warrant consideration of changing practice, Plasma-Lyte is readily available, relatively inexpensive and in the context of renal transplant providing likely reduction in DGF.

Jadad Score
5.

Data Analysis
Modified intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
ACTRN12617000358347; ClinicalTrials.gov—NCT03829488.

Funding Source
Non-industry funded.

RANDOMISED CONTROLLED TRIAL 2

Normothermic Machine Perfusion of Donor Livers for Transplantation in the United States—A Randomized Controlled Trial.

by Chapman, W. C., et al. Annals of Surgery 2023 [record in progress].

Aims
The aim of this study was to investigate the effectiveness of normothermic machine preservation (NMP) versus static cold storage (SCS) in the prevention of preservation-related graft injury.

Interventions
Donor livers were randomised to undergo either NMP or SCS.

Participants
383 donor livers were randomised out of which 266 donor livers were transplanted.

Outcomes
The primary endpoint was early allograft dysfunction (EAD). Secondary endpoints included graft survival, patient survival, incidence of postreperfusion syndrome, biochemical liver function, biliary complications, histological evidence of ischemia-reperfusion injury, feasibility and safety, health economics and organ utilization.

Follow-Up
12 months.

CET Conclusion
This unblinded randomised trial compared the outcomes of liver transplantation following either normothermic machine perfusion (NMP) or static cold storage (SCS). The study employed a "device-to-donor" methodology where the Organox metra device is transported to the site of organ retrieval, which the authors highlight is logistically more challenging. 266 livers were included in the analysis. The primary endpoint was early allograft dysfunction (EAD), defined as abnormal liver parameters 7 days after transplantation. There was no significant difference in EAD between the two groups. Although the difference in EAD was numerically greater when using an as treated or sub-group analysis of higher risk groups (high DRI, DCD donor), this to failed to reach statistical significance. The authors reached conclusions similar to that of previous European trials—NMP is a safe modality and shows potential to improve outcomes in marginal organs.

Jadad Score
3.

Data Analysis
Strict intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
ClinicalTrials.gov—NCT02478151.

Funding Source
Industry funded.

CLINICAL IMPACT SUMMARY

The use of machine preservation technologies in liver transplantation has been gaining pace over recent years, with

centres using a mixture of normothermic machine perfusion (NMP), hypothermic oxygenated machine perfusion (HOPE) and normothermic regional perfusion (NRP). Machine preservation has the potential to resuscitate the liver, reverse retrieval-related injury, allow longer safe preservation times and enable viability assessment prior to implant. In particular, NMP allows functional assessment of the liver with well-defined parameters predicting early allograft function [1].

The first multicentre randomised controlled trial (RCT) of normothermic machine perfusion in Europe was published in 2018, and demonstrated a significant (50%) reduction in the incidence of early allograft dysfunction (EAD) in machine perfused livers, despite longer preservation times [2]. These results were replicated in a US study (using a different NMP device), which also demonstrated a significant reduction in the incidence of EAD with NMP [3]. Whilst not specifically designed to demonstrate differences in organ utilisation, both studies also showed a reduction in organ discard rates, particularly for donation after cardiac death (DCD) livers.

In a recent publication in the Annals of Surgery, Chapman et al. report the results of the large multicentre US experience of NMP [4]. They used a protocol very similar to that followed in the European RCT. Livers were randomised to either conventional static cold storage (SCS) or NMP, with perfusion initiated at the donor hospital and the liver transported on the device to the implanting centre. In contrast to the European study, the trial did not meet its primary endpoint of demonstrating an overall reduction in EAD. Per-protocol analysis showed similar trends to the prior European and US studies, with greater reduction in EAD rates seen with NMP in DCD and high donor-risk index (DRI) subgroups. Interestingly, there was evidence of a learning curve, with a reduction in EAD rates in the NMP arm following enhanced training during the study. Unlike the previous two RCTs, there was no difference in transplant rate between the arms.

One important point to note is that all three RCTs used NMP in a "device-to-donor" configuration, with initiation of NMP at the donor hospital and transport on the device. This has significant logistical challenges, particularly in countries like the US where travel distances are longer and travel by plane is more common. In reality, most centres using NMP routinely in the UK and Europe are using NMP in a "back-to-base" configuration, with transport of the liver under SCS and initiation of perfusion in the recipient centre. Whilst small studies suggest that this does not compromise outcomes for the majority of livers [5], there is no large-scale RCT evidence to support the back-to-base NMP perfusion strategy that many centres are employing.

Overall, whilst this study demonstrates a smaller effect size than previous RCTs, it does confirm that the technology is safe and that the main benefit of this technology appears to be for more marginal (high DRI and DCD) livers.

Clinical Impact

3/5.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST

SK has received past consultancy income from OrganOx Ltd. for assistance in the design of clinical trials.

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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POINT OF VIEW
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Embracing the Wisdom of Ancient Greece in the Era of Personalized Medicine—Uncertainty, Probabilistic Reasoning, and Democratic Consensus

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Further improvements of outcome after solid organ transplantation will depend on our ability to integrate personalized medicine in clinical routine. Not only better risk stratification or improved diagnostics, also targeted therapies and predictive markers of treatment success are needed, as there is a virtual standstill in the development and implementation of novel therapies for prevention and treatment of allograft rejection. The integration of clinical decision support algorithms and novel biomarkers in clinical practice will require a different reasoning, embracing concepts of uncertainty and probabilistic thinking as the ground truth is often unknown and the tools imperfect. This is important for communication between healthcare professionals, but patients and their caregivers also need to be informed and educated about the levels of uncertainty inherent to personalized medicine. In the translation of research findings and personalized medicine to routine clinical care, it remains crucial to maintain global consensus on major aspects of clinical routine, to avoid further divergence between centres and countries in the standard of care. Such consensus can only be reached when experts with divergent opinions are willing to transcend their own convictions, understand that there is not one single truth, and thus are able to embrace a level of uncertainty.

Keywords: transplantation, biomarkers, prediction, precision medicine, artificial intelligence

Point of View

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INTRODUCTION

In the closing plenary session of the 2023 Congress of the European Society for Organ Transplantation (ESOT) in Athens (17th September to 20th), I gave my impression of the past congress, building a bridge between the past, the present and the future of transplantation, drawing inspiration from the wisdom of ancient Greece and the earliest years of the democratic city-state of Athens 2,500 years ago.

I embarked on a journey into the world of transplantation 20 years ago. Those were the days of bustling international conferences. The field was vibrant, driven by collaborations between academia, clinical centres, and the pharmaceutical industry. New drugs emerged from decades of research, and clinical trials like the Symphony trial in kidney transplantation [1] shaped post-transplant patient management with effective immunosuppressive regimens that minimized the risk of acute rejection. The energy, innovation, and enthusiasm continue to inspire many of us working in transplantation today.

Fast forward to today, and the protocols established in the early 2000s remain largely unchanged. For instance, in the US, a country that usually embraces and implements innovation quickly, tacrolimus-mycophenolic acid is still the baseline immunosuppressive regimen for the vast majority of patients [2]. In another example, data from an ESOT survey presented at the ESOT Congress 2023 illustrates that the first-line treatment of T-cell-mediated rejection in kidney transplantation consists mainly of high-dose steroids, toxic therapy with numerous side effects. Second-line treatment, often needed as first-line therapy fails, consists of lymphocyte depleting antibodies, strong immunosuppressants that also have considerable additional risks; also these antibodies are in routine clinical use for 25 years. No new therapies are approved for the treatment of T cell-mediated rejection since several decades.

The clinical protocols developed several decades ago are largely unbeaten up to today, leaving us with the idea that we are playing extensions, no longer the real game. However, the field cannot relax given the very negative balance between moderate efficacy and long-lasting side effects, and excess mortality associated with the old-fashioned and limited therapeutic armamentarium we have available to prevent and treat rejection.

HIPPOCRATES AND PERSONALIZED TRANSPLANT MEDICINE

In contrast to the lack of novel therapies entering our field and the protocolized care using standard regimens from 20–30 years ago, a remarkable transformation has taken place within the academic and research sphere—the advent of personalized medicine. The idea that "one size does not fit all" has never been more relevant. We hear this often, but the true meaning of it is underestimated. We must tailor our approach to individual patients, using tools to assess risk, monitor their health or disease, predict potential complications [3]. Notably, post-transplant care is not the only aspect that needs personalization; organ donor characteristics and organ quality vary significantly, impacting post-transplant outcomes and recipient wellbeing [4]. This need for personalization of our approaches was emphasized in many sessions at the ESOT Congress 2023.

Although one might think that this focus on personalized medicine is new, that is clearly not true. Already 2,500 years ago, Hippocrates, the father of medicine, noted that medicine is not absolute, thus its directions cannot be generalized to everybody [5]; that each human body/organism is different and responds differently to therapy, and therefore, the same treatment cannot be suitable for everybody; finally, that the physician should choose the appropriate treatment, depending on the patients' individual characteristics, such as different health status and lifestyle (activities, diet, etc.). These words sounded 2,500 years ago in Ancient Greece and still resonate as the definition of personalized medicine as we know it today.

CHALLENGES IN THE IMPLEMENTATION OF PERSONALIZED MEDICINE AND THE PATH FORWARD

In this evolving landscape, diagnostic companies have taken center stage. Boosted by easy access to molecular analysis as well as data scientists and fast computing, many thousands of studies have produced a wealth of biomarkers, algorithms, prediction models, and other clinical decision support tools to help us navigate clinical care, also in the field of transplantation.

However, the challenge lies in the implementation of these innovations in clinical practice, which is very often not

FIGURE 1 | Certainty vs. uncertainty in medicine. People who are overly certain of themselves or their ideas risk to be fixed in them, and command others to follow these ideas, even if they are absurd. In medicine, certainty is also an absurdity, where diagnosis, prognosis, predicted treatment effects, etc. are usually oversimplified. It leads to paternalism towards patients and colleagues, excessive testing to find the ground truth, which can lead to rising costs. Protocolized medicine suggests such certainty to healthcare professionals, but in medicine, one size does not fit all. In contrast, uncertainty is essentially uncomfortable and vulnerable, but it this leads to a listening and consensus-driven attitude, and open-mindedness. In medicine, it is very often not possible to measure the ground truth, diseases are spectrums and overlap with other diseases, which leads to differential diagnoses. In personalized medicine, where biomarkers and clinical decision support systems inherently produce results with levels of uncertainty, probabilistic reasoning is key, both for healthcare providers and patients.

happening. This relates to a lack of coordination and focus, leading to dispersion. Research in our field is often driven by local funding, with very few international, unified European, or even global programs. Furthermore, there is no consensus on the best approaches, and small sample sizes, single-centre retrospective data, and conflicts of interest can bias the development and implementation of algorithms. Many biomarkers lack independent validation and net benefit, or risk-benefit analyses are not performed. Even if validated extensively, clinical access to these tools and algorithms is hindered by regulatory requirements like the *In Vitro* Diagnostics Regulations (IVDR) in the European Union (EU) [6]. This leads to great heterogeneity in clinical practice between, for example, the EU and the United States, but also between countries and even individual centres within one country.

I am convinced that each of these hurdles to implement personalized medicine can be overcome by strong collaboration like we have observed at the ESOT Congress 2023. For instance, over the past two decades, we have clearly advanced personalized medicine for kidney transplantation [3], with much more detailed risk stratification tools with advanced immunogenetics analyses of donors and recipients and anti-HLA donor-specific antibody evaluations, which is routinely implemented in clinical practice [7, 8]; with novel non-invasive diagnostics entering clinical use [9–11]; with improved classification of rejection and biopsy-based molecular diagnostics analysis integrated in the Banff classification [12, 13], with classification of disease stage (activity vs. chronicity [12]); and with validated prognostication tools even acceptable as endpoints in drug registration trials [14, 15].

INNOVATIVE THERAPIES AND BIOMARKERS PREDICTIVE OF THERAPY RESPONSE

One major shortcoming is that we do not yet have predictive biomarkers that are able to predict therapy response [3]. To move our field forward and improve outcomes for our patients, we need to focus on the discovery and validation of novel therapeutic targets, test therapies that halt disease pathobiology, and find predictive biomarkers that indicate which therapies will work best in which particular patients. We can personalize care as much as we want, but if the therapeutic armamentarium sticks with toxic high-dose steroids as alpha and omega of, e.g., rejection treatment protocols, we will not improve outcomes much.

To move forward and improve patient outcomes, we need to couple the promise of personalized medicine with the extensive pipeline–outside of transplantation–of innovator drugs. This merging of personalized medicine with drug development should be our primary focus. The global immunology market is booming, and if we can attract even a fraction of it to transplantation, it can make a substantial difference for our patients.

SOCRATES AND THE CONCEPTS OF UNCERTAINTY

The ESOT Congress 2023 focused on realistic care, digital transformation, innovation, technology, and shared decision making. So, in essence, about how we can implement personalized medicine in our daily clinical practice. But without new drugs, this will only have marginal effects on the outcome of our patients. To move our field forward, we will need the brightest people among us to work together and move things forward.

2,500 years ago, the brightest man on Earth, according to the Oracle of Delphi, was Socrates, whose statue was used as the symbol for the ESOT Congress 2023. Socrates, a philosopher in Ancient Greece who worked and lived here on the very same ground as the congress, taught his students lessons, which are still of great value today. Socrates indicated that progress will be made through open dialogue, education, critical thinking, and most importantly, self-criticism. We indeed have to remain critical to our results and achievements. In the era of social media and self-promotion, we must embrace these principles and remain humble and critical.

Next to these principles, Socrates initiated discussions about uncertainty. He would have said: "To be uncertain is to be uncomfortable, but to be certain is to be ridiculous." Also today, we need to embrace uncertainty. People who are very certain about themselves or their ideas or getting front stage in all aspects of society, but we observe that sometimes this is not just absurd, but also counterproductive and even dangerous.

UNCERTAINTY AND PERSONALIZED MEDICINE

This concept of uncertainty is crucial in the implementation of personalized medicine in our clinical practice (**Figure 1**) [16]. A level of uncertainty is inherent to every aspect of personalized medicine, e.g., when we use risk biomarkers like donor-recipient genetic mismatch analysis or antibody evaluation, when non-invasive tests indicate a probability for ongoing disease. For disease diagnosis and disease severity, it is clear that we cannot assess the final ground truth, that we rely on consensus-based classifications like Banff, which are inherently imperfect. Prognostic algorithms for outcome prediction are available, but this is not a magic crystal ball that accurately predicts the future; there remains a lot of uncertainty in our prognostications, at the individual patient level. Treatment outcomes are often unpredictable, especially when we lack predictive biomarkers that provide information on the probability of response to a particular therapy.

Recently, it was outlined how important it becomes to embrace uncertainty in the era of clinical algorithms, but also how difficult it is to implement the thinking about uncertainty in our clinical reasoning [17]. Paraphrasing Hippocrates, no patient is just like the average patient. Many clinical decision support systems use algorithms to make predictions, in uncertain medical conditions. It is important to realize that positive and negative predictive

values are very dependent on disease prevalence, which can greatly differ between populations and centres. These predictions are typically expressed as probabilities; a diagnosis becomes more or less likely, with some explicit degree of uncertainty.

These probabilistic results do not align with how most doctors typically think about whether a disease is present or absent, in a black-and-white simplistic world where certainty is readily achievable [16]. The quest for diagnostic certainty quickly leads to excessive testing, not only increasing healthcare costs but also risking false positive results and iatrogenic injury. Moreover, the principles of probabilistic clinical decision support systems perhaps clash somewhat with pathophysiology-based reasoning, which is still very relevant in the development and clinical implementation of targeted therapies, e.g., also for rejection [18].

Clinical decision making requires integration of probabilistic reasoning with acceptance of uncertainty around disease causality, because true causality usually cannot be proven in the clinical setting. I believe that Socrates would agree with this modern translation of his ideas on uncertainty. Embracing the concept that certainty is not always the end goal will be key for the future of medicine.

THE DEMOCRATIC LEGACY OF THE CITY-STATE OF ATHENS: CONSENSUS NEEDS UNCERTAINTY

The wisdom of Ancient Greece is not only related to the thoughts of Socrates. Athens is also the city-state where 2,500 years ago, democracy, the power to the people, was invented. In the earliest years, the direct democracy in Athens was not only accessible but, in fact, obligatory for every male citizen aged 20 and above. In contrast to what was depicted by Raphael in his fresco "School of Athens" in the Vatican 2000 years later (1,509–1,511), democracy in Athens did not take place in a splendid palace. Democracy was merely an open space where the people of Athens were expected to come to listen to each other. The assembly meeting place and speaker's platform were located near the site of ESOT Congress 2023; its ruins can still be visited today.

As is the case for personalized medicine, uncertainty is also vital for democracy. It is only when we are critical and uncertain about our own ideas and conclusions, and accept that there is not one single truth, that other people can have other ideas, that we can form consensus. We have to listen to others' ideas, and find common ground. Otherwise, we risk to end up in toxic leadership and tyranny. Around us, we see many examples of what can happen when we give too much power to people who are too self-confident and complacent and stop listening to other opinions.

Recent examples of critical self-reflection and successful democratic processes in our field are the ESOT Transplant Learning Journey [19], the ongoing ENGAGE consensus for sensitization in transplantation [7, 8], the Banff consensus for allograft pathology [20], and the SONG-Tx initiative for defining standardized outcomes in transplant nephrology [21, 22]. Especially the latter is a good example of how important the democratic processes are for the field. Using Delphi methodology, not only health professionals, but also patients and their caregivers were able to contribute to the definition and validation of outcome measures, that will become relevant for clinical trial design.

Perhaps most importantly, the SONG-Tx initiative [21, 22] illustrates that we can access robust methods that enable to integrate patients' perspectives in further development of the field. This allows to put the focus of research to what matters most to the patients. Explicit democratic processes enable us to integrate all opinions, also those from our main stakeholders, the patients. Such processes enable full patient centrality.

INTEGRATING THE CONCEPTS OF UNCERTAINTY IN PATIENT INTERACTIONS

With such focus on what matters to patients, it is also very important to interact with the patients and their caregivers on what are the implications of personalized medicine. As described above, uncertainty is central to personalized medicine, and it will be crucial to be honest with patients about this uncertainty as well [23]. In crisis management, it is sometimes said that in times of uncertainty, honesty is the best policy. The same counts for medicine. We need to discuss together with the patients what is the impact of the new discoveries and advancements of personalized medicine, and we should not be afraid to talk openly about the uncertainties inherent to it. Not only we need to train the healthcare professionals in probabilistic thinking [17], also patients and society in general should be informed and educated about the key concepts of probabilistic reasoning in clinical decision making [23].

Only in open and honest discussions with patients as equals, away from medical paternalism, we will be able to truly individualize care. Not only must we adapt clinical approaches to individual patients' medical conditions and the output of the biomarkers and clinical decision support algorithms, but we must also take into account less quantifiable aspects of risk appetite or aversion, expectations, quality of life, social support, and even economic considerations.

CONCLUSION

In conclusion, we must learn from the wisdom of ancient Greece and the city-state of Athens, where democracy thrived, and where Socrates championed critical thinking and preaching uncertainty. The coming years, we really will need to focus on what matters most to the organ transplant recipients. Patient centricity will be key. We need much more structured concertation and collaboration, especially making the bridge between personalized medicine and innovative drug development, an important gap that is halting progress in clinical care. EU research frameworks and international funding for the transplant field are urgently necessary. Last but not least, we need different ways of communication with each other and with patients. We should embrace the democratic processes we are

increasingly implementing in our community. We should allow levels of uncertainty in our discussions and train ourselves in probabilistic thinking, admitting that there is more we do not know than we know. And this very much echoes what Socrates said 2,500 years ago.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

CONFLICT OF INTEREST

The author declares that the text was written in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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MEETING REPORT
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Banff Digital Pathology Working Group: Image Bank, Artificial Intelligence Algorithm, and Challenge Trial Developments

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The Banff Digital Pathology Working Group (DPWG) was established with the goal to establish a digital pathology repository; develop, validate, and share models for image analysis; and foster collaborations using regular videoconferencing. During the calls, a variety of artificial intelligence (AI)-based support systems for transplantation pathology were presented. Potential collaborations in a competition/trial on AI applied to kidney transplant specimens, including the DIAGGRAFT challenge (staining of biopsies at multiple institutions, pathologists' visual assessment, and development and validation of new and pre-existing Banff scoring algorithms), were also discussed. To determine the next steps, a survey was conducted, primarily focusing on the feasibility of establishing a digital pathology repository and identifying potential hosts. Sixteen of the 35 respondents (46%) had access to a server hosting a digital pathology repository, with

Abbreviations: 2D, 3D, 2-dimensional, 3-dimensional; AI, Artificial intelligence; DL, deep learning; DPWG, digital pathology working group; EU GDPR, European Union General Data Protection Regulation; HPC, High-performance computing; IFTA, interstitial fibrosis and tubular atrophy; IT, information technology; ML, machine learning; NLP, natural language processing; WSI, whole slide image/imaging.

2 respondents that could serve as a potential host at no cost to the DPWG. The 16 digital pathology repositories collected specimens from various organs, with the largest constituent being kidney (n = 12,870 specimens). A DPWG pilot digital pathology repository was established, and there are plans for a competition/trial with the DIAGGRAFT project. Utilizing existing resources and previously established models, the Banff DPWG is establishing new resources for the Banff community.

Keywords: Banff, digital pathology, artificial intelligence, machine learning, image analysis

INTRODUCTION

The Banff Digital Pathology Working Group (DPWG) was formed in 2019, followed by a publication describing the DPWG's main goals and the current state of transplant digital pathology [1]. Since then, the DPWG meets regularly in video conferences (nearly every 2 weeks) to discuss new digital pathology initiatives, innovative investigations, and digital pathology's current status and future (2), particularly computer vision applied to transplantation, considering the fact that digital pathology has enabled the development of "computational pathology" as a new science [2–4]. "Computational Pathology" is a novel approach to precision medicine incorporating multiple data sources using artificial intelligence (AI) to generate actionable knowledge to improve disease diagnosis, prognostication, and prediction [5].

The development of new digital pathology-based tools, computer vision algorithms, and machine learning (ML) models for the study of kidney diseases has stimulated the pathology and nephrology community to build large digital pathology repositories to allow for the integration of data from clinical, molecular, pathology, and other domains. While this effort has been in place for over a decade for native kidney diseases [5], the use of digital pathology repositories, computer vision, and computational pathology in transplant pathology remains largely unexplored.

As also detailed in the last Banff Meeting Report [6] and the DPWGs' first paper [1], the DPWG's goals are detailed in **Tables 1**, **2**; **Figure 1**. Notably, future plans can be summarized in three aims:

- Aim 1: Image banks and/or digital pathology repositories for benchmarking algorithms so that research groups can test their AI and other algorithms similar to what is being done in the computer science community overall, with ImageNet supervised natural image classification being a main example.[1]
- Aim 2: Algorithms will developed for the transplant community. One future goal potentially includes the release of "official" Banff algorithms that could be used by the Banff community and beyond. As mentioned in the previous Banff DPWG working group paper, these could include targeted, handcrafted algorithms (e.g., for parameters such as fibrosis, inflammation, steatosis, etc.) [1]; or these could include thoroughly validated AI/ML algorithms. Furthermore, data pipelines for the integration of "–omic" data could be provided so that centers could have mechanisms for mining data within their center as well as sharing with other centers.
- Aim 3: Competitions or trials will be conducted so that groups can compare their algorithms on standard transplant pathology image sets.

This current DPWG paper serves as an update on the DPWG's progress with selected examples and is not a comprehensive review, and we apologize for related studies that are not cited. The DPWG's survey research on the current state of digital transplant pathology will be covered, and additional details regarding each of the three aims above will be discussed.

IMAGE BANK SURVEY

A survey was conducted from 27 April, 2020, to 23 July, 2020, primarily to determine image bank possibilities for the DPWG. Questions were sent via SurveyMonkey (Palo Alto, California, United States) to both the NEPHROL and NEPHNPPT Discussion Groups (with 701 members and 456 members, respectively) moderated by Kim Solez aimed primarily toward renal pathologists and clinicians interested in renal pathology. The NEPHROL group includes mostly nephrologists and pathologists, and the NEPHNPPT group is a subset of the Renal Pathology Society (RPS) membership.

The Banff DPWG Image Bank Survey had 35 respondents from 13 countries, 19 from the US, 4 Canada, 2 Netherlands, and one each from 10 countries (**Supplementary Material**). Most (24 or 69% of respondents) specified pathology as their specialty. Of these, 16 (46%) specified that they had a server to manage whole slide images (WSIs) from multiple institutions, and these used various server software and image formats and had a range of storage and bandwidths. In this regard, it is recognized that setting up servers and workflows is quite a complex endeavor; and our survey reflected these complexities [7–10].

Of 13 answering a question regarding the ability of their server to de-identify slide information (including the slide label) automatically, 9 (69%) responded yes; 2 (15%) no; and 2 (15%) not sure. Of 12 answering a question regarding their server's ability to allow customized and commercial algorithms installation, 8 (67%) answered yes; 2 (17%) no; 1 (8%) only customized algorithms; and 1 (8%) not sure. Of 10 answering a question regarding their server's ability to allow the correction/standardization of staining variability and other variables in images from multiple laboratories, 9 (90%) answered yes.

[1]https://www.image-net.org/

TABLE 1 | Banff digital pathology working group (DPWG) issues and plans. The Banff DPWG issues and future plans are depicted as laid forth in the original DPWG paper (1).

Topic	Items
Issues to address	• Digital automation of pathology practice o Computing, Artificial intelligence (AI), Nanotechnology, Machine learning, Slide numeration
Future plans	• Standardization of practices • Classification for studies using integrative approaches • Interstitial fibrosis and tubular atrophy (IFTA) scoring • Inflammation scoring • Algorithms to fit to the classification and decrease interobserver variability (e.g., "official" Banff algorithms) • Validation of algorithms using slides prepared at different institutions with different laboratory protocols (processing, staining, etc.) • Archetypes to be validated across multiple institutions • Delivery of precision diagnostic, molecular pathways, and therapeutics (e.g., through established data pipelines and natural language processing) • Image bank for groups to test AI and other algorithms

TABLE 2 | Banff digital pathology working group (DPWG) issues and plans. The Banff DPWG issues and future plans are depicted as further refined through DPWG discussions (1).

Primary goals
Aim 1: Image bank for AI/ML & other algorithms
Aim 2: Algorithms
Algorithm validation using different institutions and laboratory protocols
Algorithms for classification (e.g., "official" Banff)
Banff Parameter algorithms (e.g., IFTA & Inflammation)
Aim 3: Competition/trial
Competition/trial to test algorithms
Secondary Goals
Computing, AI/ML, nanotechnology, slide numerationetc.
Standardization of practices
Decrease interobserver variability
Classification using integrative approaches
Precision diagnostics, molecular, & therapeutics, NLP, etc.
Archetypes validated across multiple institutions

Abbreviations: AI/ML, artificial intelligence; machine learning; IFTA, interstitial fibrosis and tubular atrophy; NLP, natural language processing.

Survey questions regarding the possibility of image bank hosting were asked; and of nine responding, 7 (78%) had an associated cost; and only 2 (22%) had no associated cost. The two responding there would be no cost were contacted; and it became clear that one of these would not be able to host the image bank due to logistical issues. Thus, based on the survey, only one respondent at Georgia State University could host an initial image bank pilot. Later discussions in the community revealed another image bank could possibly be hosted at RWTH Aachen University in the future, particularly regarding specimens in Europe subject to European Union General Data Protection Regulation (EU GDPR).

Survey questions also covered existing image bank material available among respondents. Of 28 respondents responding to the question of whether they had an existing transplant WSI repository, 16 (57%) said they had such a repository. When asked for the number of their specimens, the combined specimens included 12,870 kidney, 670 heart, 55 pancreas/islet, 50 lung, 30 liver, 20 intestine, and 2 vascularized composite allograft. Thus, the survey showed that the community already has a substantial specimen number; however, the number of specimens obtained is likely an underestimate.

It is likely that this survey could be repeated in the future with an increased response rate, since interests in AI, ML, and deep learning (DL) are likely increasing [11]. Furthermore, the survey was conducted during the COVID-19 pandemic, which could have hindered response rates. In the future, such a survey could likely find additional servers and material for collaboration.

AIM 1: IMAGE BANK AND DIGITAL PATHOLOGY REPOSITORY PILOT

Our Banff DPWG conducts discussions, planning, testing, and implementations of appropriate vehicles for pathology AI method dissemination, deployment, and comparison readily accessible by end users. An image bank or digital pathology repository is a goal that the Banff DPWG would like to achieve, similar to the "Big Picture" European digital pathology project,[2] the Nephrotic Syndrome Study Network (NEPTUNE),[3] and Kidney Precision Medicine Project (KPMP[4]). In contrast to desktop applications, web-based platforms are preferred by many since they do not require any user-involved installation process [12]. Although some web-based tools have been developed, they are either commercial software with license purchase requirement [12] or limited for new algorithm integration (e.g., Omero [13]).

The one respondent available to host a pilot for the DPWG is the Digital Pathology Laboratory (DPLab[5]), a publicly available web platform allowing researchers to visualize, annotate, analyze, and share 2D and 3D pathology images via web-enabled devices. This platform allows users to upload their own WSIs, annotate regions of interest, invoke AI analysis methods, visualize analysis outputs, and download outputs for follow-up statistical comparisons. Due to its web-based framework, DPLab enables WSI annotation and analysis data sharing. Since AI method training and execution relies on a reliable and

[2]https://bigpicture.eu/
[3]https://www.neptune-study.org/
[4]https://www.kpmp.org
[5]https://dplab.gsu.edu/

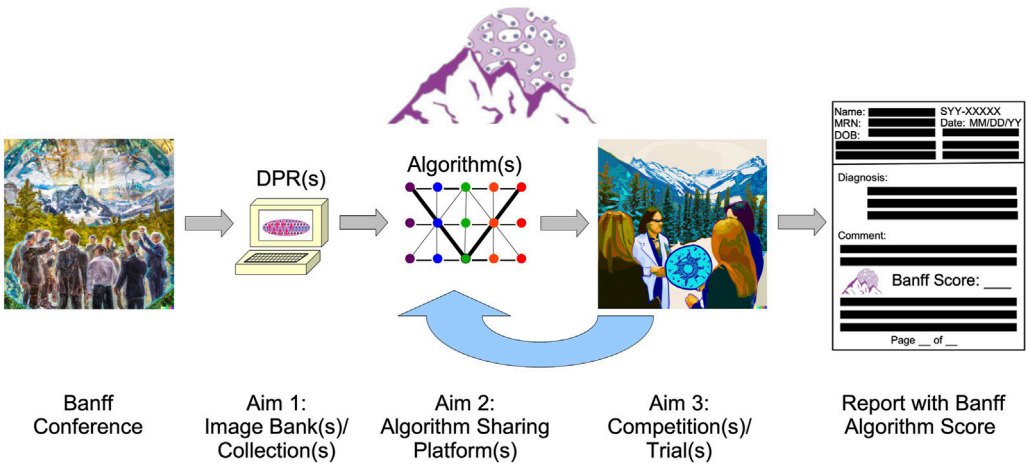

FIGURE 1 | The Banff Digital Pathology Working Group (DPWG) main aims are shown. The primary aims of the Banff DPWG include 1) image bank/collection establishment, to possibly include other data in digital pathology repositories (digital pathology repositories); 2) algorithm sharing platform initialization; and 3) competition/trial organization. Multiple solutions for each of these aims may be possible. After competition/trial conduction among the Banff community and other collaborators, the algorithm performance will be characterized in a process that will affect the future performance and sharing of algorithms; and thus, the competition(s)/trial(s) will provide "feedback" to algorithm sharing. Ultimately, effective, precision patient care could be provided with Banff algorithm scores. (The "Banff Conference" and "Aim 3" image were produced by Kim Solez using DALL-E 2.).

powerful computational infrastructure, DPLab allows running these AI methods without local computational resources. All requested analysis jobs from the front end are executed through a backend computational environment, addressing a frequent WSI analysis computational obstacle. Currently, DPLab is equipped with numerous WSI analysis algorithms, ranging from color deconvolution, cell detection, nuclei segmentation, histology component quantification, to serial WSI image registration (with some demonstrated in **Figure 2**). Because DPLab is designed as an open environment, AI methods from the research community can be contributed for method enrichment, validation, and comparison. In the future, additional components are planned for DPLab, such as a quality control component (e.g., similar to those seen in the open-source tool HistoQC [14]). As this software becomes more mature, we envision it and others like it can become useful tools for digital pathology community [12].

Complete digital pathology implementation will require digitization of all workflow steps. For example, in renal pathology, this will require light, immunofluorescence, and electron microscopy digitization. Regarding this, immunofluorescence staining is an integral part of kidney transplant biopsy evaluation, both for C4d staining for detection of antibody-mediated rejection and for immunoglobulins and other complement components for recurrent and *de novo* glomerulonephritis detection. Factors to consider include the ability to support automated scanning with minimal operator input, available immunofluorescence filters, scanning speed, automated tissue detection, image quality, tissue focusing ability, scanning magnification, degree of image bleaching (fading), and price. Major challenges with currently available immunofluorescence slide scanners include inability of scanners to focus on tissue, inability to reflect negative/dim staining, and excessive human technologist time for scanning setup (Dr. Lynn Cornell in DPWG communications).

Digital pathology repositories can include a variety of "omic" data types in the future. Digital pathology "pathomic" data can be included with other "omic" data including genomic, transcriptomic, proteomic, and metabolomic data. "Pathomics" refers to the morphological examination of tissue on the macroscopic, microscopic, and ultrastructural level. "Pathomics" was used at least as far back as a 2007 editorial by Robert Colvin (11, 12) commenting on a study investigating microarray analysis of rejection that later become available in the molecular microscope diagnostic (MMDx) system (13). Using this terminology, the "pathome" can refer to the entirety of morphological histology features, particularly when examined using enhanced ancillary techniques; and enhanced techniques to examine the "pathome" can be termed "Next-Generation histoMorphometry (NGM)." Of note, standard "omic" technologies are increasingly being applied in a "spatial" manner (e.g., spatial transcriptomics and spatial proteomics) [15]. Digital pathology repositories will likely be crucial for the integration of "pathomic" with other "omic" data.

AIM 2: AI/DEEP LEARNING ALGORITHMS

To effectively develop deep learning (DL)-based support systems for diagnosis and research, including in transplant pathology, three main prerequisites are needed (e.g., when thinking of setting up transplant digital pathology central resources), including: 1) hardware and software infrastructure, 2) interdisciplinary expert teams, and 3) diverse and clinically annotated datasets [16].

(1) The hardware and software infrastructure are becoming more available and affordable, and many pathology labs now have at least partial digital infrastructure. Based on a particular study's extent and the computational demands of newer DL architectures, however, the introduction of robust digital pathology resources within a single institution can be challenging. Digital pathology and WSIs produce the largest

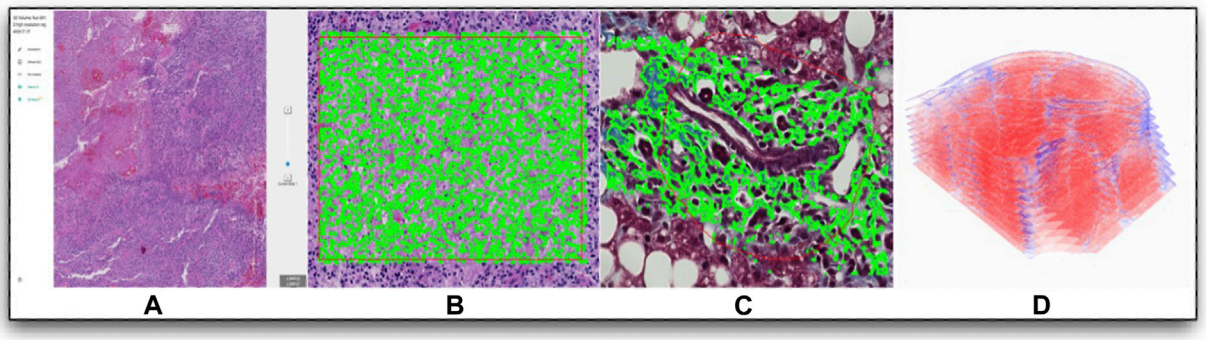

FIGURE 2 | Digital Pathology Laboratory: a publicly available web platform for multi-dimensional pathology image analytics example image manipulations are shown, including the following: **(A)** A representative WSI visualized from DPLab at multiple image resolutions; **(B)** Cell detection result in a user-annotated rectangle region; **(C)** Liver fibrosis quantification with a region annotated by a free-hand annotation tool; **(D)** Detailed 3D liver tissue sub-volume visualization after serial WSI registration and collagen quantifications.

imaging data in clinical medicine. When setting up large digital pathology repositories, sufficient storage capacity is required, which can easily be in the petabyte (PB) range. Such storage must be secure, both in terms of security of access and sufficient backup. Modern DL systems are increasingly computationally expensive to train due to the model size, with many trainable parameters and large datasets. Thus, central high-performance computing (HPC) centers or cloud providers might be needed for model development. Frequently, such HPC centers (or cloud providers) are not used to handling sensitive medical data and privacy concerns (e.g., HIPPA and GDPR); and the legal aspects can be complicated. Also, such centralized algorithmic training requires secure data transfer between institutions. This may also be challenging for security and compliance. Cloud providers and download possibilities can tackle some of these issues. Another potential solution for this could be the use of federated learning approaches, which are becoming more popular not only in computational pathology. These approaches train (parts of the) models on locally stored data (i.e., without the need to move the data from the hospitals) [17–19]. Such federated approaches require scaling up local computing power, which, in our recent experience, is not available everywhere, and sometimes not even considered in some larger repositories. This is not completely surprising, since digital pathology possibilities are still new and emerging. Digital pathology infrastructure maintenance costs (e.g., security updates and other services) need to be kept in mind and can present a challenge when aiming for a long-term digital pathology repository. Thereby, solutions for long-term infrastructure financing are required, and might be a challenge.

(2) AI/DL development and infrastructure maintenance requires experts from information technology (IT), computer science, medicine, research, and other areas [20]. Such an interdisciplinary team is required 1) to ensure a relevant use case and the datasets are defined for meaningful application scenarios in a realistic workflow, 2) a suitable model architecture can be modified to fit the use case, 3) software best practices are followed during training, and 4) to ensure model safety. Ultimately, models should be thoroughly audited before clinical testing, uncovering potential risks and developing mitigation strategies [21]. User studies should test whether systems will be useful in later day-to-day work. The workforce needs of industry vs. academia may be in competition. Generating an environment that motivates IT and AI experts to join academia will be imperative to building up domain-specific expert teams. Also, such teams should have a minimal "critical" size of the particular specialty (e.g., Having only a single AI or IT expert makes the team heavily dependent on a single person, while it does not provide a suitable environment for discussion and exchange for the expert.). It is our experience that large and strongly interdisciplinary teams, directly embedded within the specific application domain might be most efficient in new approach development testing. This direction also helps educate "hybrid" experts (e.g., pathologists with expertise in AI development and AI developers knowledgeable of real-world pathology workflows). Such "hybrid" experts can be augmented by automated systems such as those that help codify the complexity of the Banff classification system [22].

(3) Finally, and currently one of the major challenges in this field, is the availability of relevant, sufficiently large datasets. Sample size is determined by the ML system's efficiency and the problem complexity. Datasets should be multicentric and reflect the population(s) in which the system will ultimately be used. In addition, it is important to invest time uncovering existing dataset biases before fitting a model to the data and reducing biases as much as possible [23, 24]. To uncover such biases, datasets must be deeply phenotyped, and in the case of pathology, enriched at least with clinical and pathological data. It is essential to validate any DL models using independent cohort(s), which were not used for DL training. While tremendous thought has previously been given to the collection of training datasets [2], only recently have recommendations for the collection of test datasets been issued for the case of computational pathology [20]. Test datasets must be independent from the development datasets. The ML community has long recognized the need for diverse multi-center datasets to reliably assess the generalizability of DL systems. This is now also well established in computational pathology and should be a common standard [2].

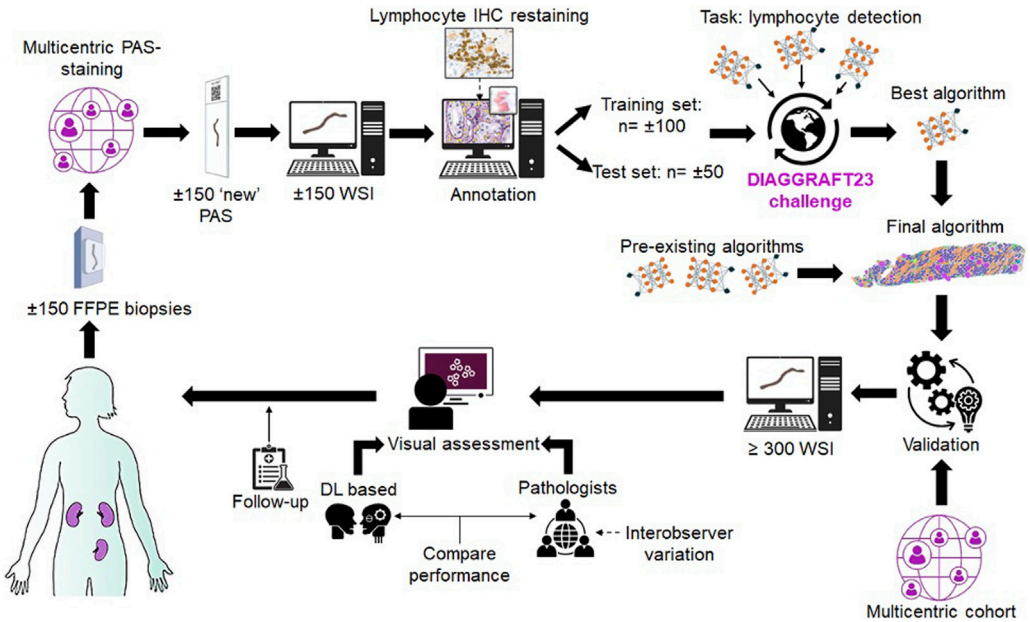

FIGURE 3 | The DIAGGRAFT Challenge Work Plan is shown. Abbreviations: FFPE, formalin-fixed paraffin-embedded; PAS, periodic acid Schiff; WSI, Whole Slide Images; IHC, immunohistochemistry.

One example of how the integration of all prerequisites and joined international cooperation can lead to promising DL algorithm development was previously shown in the DEEPGRAFT study, which involves transplant biopsy weakly-supervised slide-level diagnosis classification using DL [25]. This is currently the largest multicentric dataset of renal transplant biopsies assembled and analyzed centrally, with more than 5,000 WSIs, including consecutive biopsies from a center not included in training, representing a "real-world" scenario and enabling validation and assessment of the model's generalizability.

Other novel algorithms for efficiently analyzing very large renal tissue biopsy digital WSIs have been integrated into ML pipelines for nephropathology. The developed tools employ a human-AI-loop (HAIL) approach [26] via integrating human with AI for efficiently detecting and segmenting multi-compartmental structures (e.g., glomeruli, tubules, interstitium, and arteries and arterioles). The tool's performance is shown in computational histologic classification of diabetic nephropathy [27], as well as computational detection and segmentation of interstitial fibrosis and tubular atrophy [28]. The tool has been extended to computationally detect and count podocytes from WSIs, and also subsequent feature extraction for various inference studies [29]. HAIL's utility has been further shown via integrating the tool with the VIPR (Validated Identification of Pre-Qualified Regions) algorithm [30]. HAIL operates at segmenting large renal structural levels, and VIPR operates at deriving renal micro-compartments using pixel level vector features. In tandem, these tools are being used to conduct unsupervised classification of tubules in the KPMP. Features quantified from HAIL-derived image structures are currently being used for fusing with tissue molecular signatures, such as those derived by CODEX and spatial transcriptomics, to discover new molecularly distinct structural motifs with implications in chronic kidney disease and acute kidney injury. It is anticipated that the tools developed herein will contribute to renal transplant biopsy assessment to automate Banff scoring for chronicity assessment as well as automatically predict graft outcome from pixel level image features.

While retrospective studies have inherent value in showing system applicability or useability, prospective evidence of the clinical benefit of DL systems must be generated through well-designed clinical trials. Promising studies include those examining the classification of rejection versus other diseases [25] and antibody-mediated rejection under Banff criteria [31] in the kidney; and in cardiac endomyocardial biopsies, allograft rejection has been distinguished from benign mimics (Quilty B lesions) using AI [32]. However, clinical trials implementing DL systems are currently largely missing in the field of computational pathology, but in some scenarios might also be hard to provide.

AIM 3: COMPETITION/TRIAL AND CURRENT IMAGE ANALYSIS TRIAL WORK

As mentioned previously, our last aim deals with competitions or trials will be conducted so that groups can compare their algorithms on standard transplant pathology image sets. In this regard, the Banff DPWG has an ongoing collaboration that has been discussed in DPWG meetings entitled "DIAGGRAFT: leveraging artificial intelligence technology for accurate quantitative histological diagnostic assessment of transplant renal biopsies." The Dutch Kidney Foundation recently awarded a Success Accelerator Grant for the DIAGGRAFT project. DIAGGRAFT was started in January 2022 by Dominique van Midden, Meyke Hermsen, Jeroen van der Laak, et al, and will be executed in close collaboration with the

DPWG. This project builds upon former research by Hermsen et al. [33, 34] that developed AI (more specifically: DL) for automated assessment of histopathologic features in digitized kidney tissue sections. DIAGGRAFT aims to take developed AI a step further, extending these techniques and preparing them for large-scale research- and even diagnostic use. The DIAGGRAFT consortium will organize a so-called "grand challenge": an international competition, similar to challenges previously organized (e.g., PANDA [35] for prostate cancer,[6] CAMELYON [36–38] for breast cancer and lymph node metastasis, and other Kaggle efforts[7, 8]). In the DIAGGRAFT challenge, a large, annotated, multi-center data set will be established and provided to participants with the goal to collectively build AI for inflammatory cell detection in periodic-acid Schiff-stained slides. The best inflammatory cell detection model(s) from the DIAGGRAFT challenge will be combined with existing structure segmentation AI to quantify Banff classes. In the last part of DIAGGRAFT, AI will be validated on a large patient cohort, originating from multiple international medical centers and scored by an expert renal pathologist panel. DIAGGRAFT aims to develop powerful DL tools for objective and reproducible Banff scoring, validated in a multicenter setting against graft function and survival. The resulting DL models will be made available to the Banff community for subsequent validation studies. DIAGGRAFT is visually displayed in **Figure 3**.

CONCLUSION

The Banff DPWG plans to continue the efforts of fostering the establishment of image banks and digital pathology repositories, of stimulating algorithm development, and supporting the validation of these algorithms. The DPWG's efforts will be disseminated through a variety of venues (e.g., during the annual meeting of the American Society of Transplantation), to stimulate engagement of the entire transplant community. Funding sources are being explored to financially support efforts of the DPWG. Digital pathology techniques allow computational pathology, which provides automated histopathology analyses with more throughput scalability, reproducibility, and precision [5, 15, 39–42]. Indeed, these new technologies will essentially allow numerous novel manipulations, such as the translation/augmentation of one stain to another [43, 44]. It is possible that AI/ML will serve as a "gold standard" in some sense, although we foresee AI/ML augmenting pathologists rather than replacing them as the "gold standard." Algorithms and other advances for the Banff community may eventually arise from these efforts, with the ultimate goal of providing more effective, precision patient care.

[6]https://www.kaggle.com/c/prostate-cancer-grade-assessment/overview

[7]https://www.kaggle.com/c/hubmap-kidney-segmentation, https://cns.iu.edu/docs/publications/2021-Godwin-FTUs.pdf

[8]https://hubmapconsortium.github.io/ccf/pages/kaggle2.html

AUTHOR CONTRIBUTIONS

AF and KS devised the survey and manuscript structure. AF drafted the manuscript along with contributions from LB, PB, RB, LC, MH, JeK, JuK, MN, PS, JvL, and DvM. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

JvL has been a member of the advisory boards of Philips, Netherlands and ContextVision, Sweden, and received research funding from Philips, Netherlands, ContextVision, Sweden, and Sectra, Sweden in the last 5 years. He is chief scientific officer (CSO) and shareholder of Aiosyn BV, Netherlands. JeK is a consultant for Aiosyn BV and Novartis AG Switzerland and received speaker fees from Chiesi Pharmaceuticals, Netherlands.

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.2023.11783/full#supplementary-material

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MINI REVIEW
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The Role of the Duffy Blood Group Antigens in Renal Transplantation and Rejection. A Mini Review

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Finding a compatible donor for kidney transplant candidates requires overcoming immunological barriers such as human leukocyte antigens (HLA) compatibility and ABO compatibility. Emerging data suggest a role for red blood cell antigens (RCA) in renal transplant outcomes. The incidence of RCA alloimmunization is high in chronically transfused individuals, such as end stage renal disease patients, but whether antibodies to RCA can mediate renal graft rejection remains debatable. The Duffy blood group antigens (Fy) has been shown to be expressed in the kidney, among other tissues. There are some data to suggest that donor-recipient Fy mismatches may increase the risk for chronic allograft damage and that anti-Fy antibodies may be involved in renal graft rejection, however, while it is routine to screen renal transplant candidates for ABO antigens, detailed RCA phenotyping of the donor kidney is not routinely tested. In this paper, we review the current data on the role of Fy in renal transplantation and discuss the potential mechanisms of its biological function.

Keywords: Duffy, red blood cells (RBC), renal transplant, rejection, alloantibody

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INTRODUCTION

Finding a compatible donor for kidney transplant candidates requires overcoming immunological barriers such as human leukocyte antigens (HLA) compatibility and ABO compatibility, which may prolong time to transplant. Emerging data suggest a role for red blood cell antigens (RCA) in renal transplant outcomes [1–3]. As of July 2023, there are 45 recognized blood group systems, many of which are expressed on tissue other than the surface of red blood cells (RBC) [4]. The functions of these antigens are not all fully understood but some function as chemokine receptors and others as membrane transporters [5]. Of the 45 recognized blood groups the kidney is known to express Duffy, Lewis, Kidd and MNS blood groups [1, 5]. Antibodies to RCA (other than ABO) are usually not naturally occurring and require exposure for development. Transfusions are the main source of exposure for most RCA with each transfusion event increasing the likelihood of alloimmunization by 0.2%. The probability of developing RCA allosensitization is dependent on multiple factors including underlying disease, immunogenicity of the antigen, dose and frequency of transfusion, age, and

Abbreviations: AA, African American; ABMR, antibody-mediated rejection; DARC, Duffy antigen receptor for chemokines; DGF, delayed graft function; Fy, Duffy blood group antigen; HLA, human leukocyte antigens; IRI, ischemia reperfusion injury; RCA, red blood cell antigen; RBC, red blood cells.

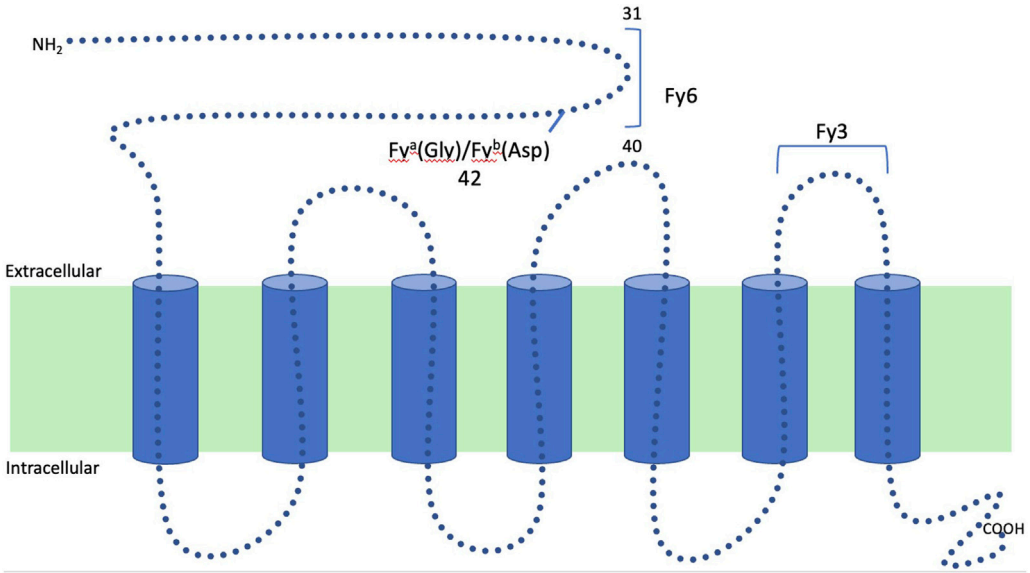

FIGURE 1 | Structure of the Duffy glycoprotein: Duffy is a seven transmembrane glycoprotein that has multiple epitopes for which antibodies can be formed. It is encoded on chromosome 1 by two codominant alleles (*FY*A* and *FY*B*). These two alleles differ by a single nucleotide polymorphism at position 125 (G/A) resulting in the presence either of glycine or aspartic acid in position 42 of polypeptide chain that gives rise to the two antigens, Fy^a and Fy^b.

gender among other factors [6]. The incidence of RCA alloimmunization in multiply transfused individuals, such as end stage renal disease patients requiring frequent transfusions, can be as high as 60% [2, 6].

Whether antibodies to RCA can mediate renal graft rejections remains debatable. Various studies produced mixed results when looking at retrospective data and trying to correlate presence of antibodies with kidney graft survival [1, 3]. There are some data to suggest that donor-recipient Duffy blood group (Fy) mismatches may increase the risk for chronic allograft damage [3]. However, while it is routine to screen renal transplant candidates for ABO antigens, detailed RCA phenotyping of the donor kidney is not routinely tested. In this paper, we review the current data on the role of Fy in renal transplantation and discuss the potential mechanisms of its biological function.

THE DUFFY BLOOD GROUP ANTIGENS

The Duffy blood group system was first described in 1950 in a patient named Duffy who presented with hemolytic transfusion reactions after having received multiple transfusions [7, 8]. Fy is a seven transmembrane domain glycoprotein that has multiple epitopes for which antibodies can be formed. It is encoded on chromosome 1 by two codominant alleles (*FY*A* and *FY*B*). These two alleles differ by a single nucleotide polymorphism at position 125 (G/A) resulting in the presence either of glycine or aspartic acid in position 42 of the polypeptide chain that gives rise to the two antigens, Fy^a and Fy^b (**Figure 1**). Depending on the alleles present, four main phenotypes may be found in the population: Fy(a+ b–), Fy(a– b+), Fy(a+ b+) and Fy(a– b–), although the phenotypic expression in tissue versus blood can vary depending on the specific genotype (**Table 1**) [7, 9]. The most common antigens are Fy^a and Fy^b, but Fy3, Fy5, and Fy6 have also been described [5]. Antibodies for Duffy are almost never naturally occurring and are a result of exposure to the antigen. Antibodies to Fy^a are more frequently seen than antibodies to Fy^b by about a 20-fold increase. These antibodies are predominately IgG1 with a small percentage of presentations consisting of IgM (25%) and IgG2 (18%) [5, 7]. Antibodies against both Fy^a and Fy^b cause immediate and delayed hemolytic transfusion reactions and have been associated with hemolytic disease of the fetus and newborn [5].

TABLE 1 | Examples of Duffy genotype and phenotypic expression in blood vs. tissue.

Genotype	Phenotype (blood)	Phenotype (tissue)
*FY*A/FY*A*	Fy(a+, b–)	Fy(a+, b–)
*FY*B/FY*B*	Fy(a–, b+)	Fy(a–, b+)
*FY*A/FY*B*	Fy(a+, b+)	Fy(a+, b+)
*FY*02N.01/FY*02N.01*[a]	Fy(a–, b–)	Fy(a–, b+)
*FY*02N.02/FY*02N.02*[b]	Fy(a–, b–)	Fy(a–, b–)

[a]*This genotype represents the GATA box mutation (erythrocyte silent phenotype).*
[b]*This genotype is one on many that cause the true null phenotype which prevents expression of Duffy on both blood and tissue. For more information, please refer to Höher et al. [9].*

Duffy Expression in Different Ethnic Populations

Duffy expression varies greatly in different ethnicities. Caucasians and Asians have near 100% expression on their RBC, whereas

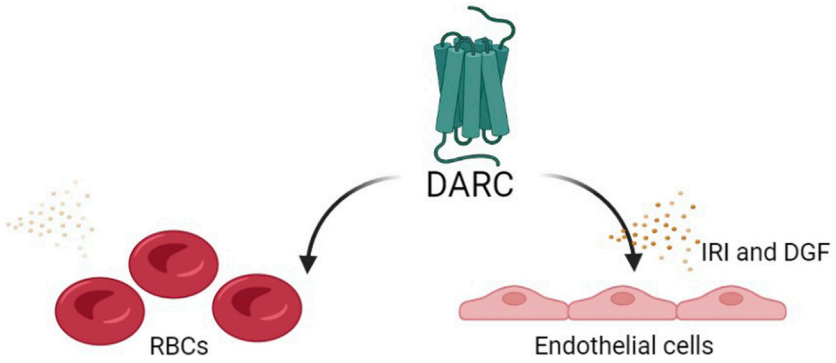

FIGURE 2 | The potential functional role of DARC in recruitment of inflammatory cells: The functional role of DARC in recruitment of inflammatory cells is still not fully understood, and several mechanisms have been suggested: 1) DARC helps presenting chemokines on the endothelial cells to leukocytes expressing corresponding receptors; 2) The binding of chemokines to DARC helps creating a gradient flow to keep an influx of chemokines coming to the site; 3) Neutralization of bound chemokines [19, 20]. The question remains whether this upregulation is an attempt to bind and neutralize chemokines and control the inflammation or is this an attempt to recruit more inflammatory cells? It is also possible that DARC is able to pursue different functions, proinflammatory in some scenarios and anti-inflammatory in others, according to the spatial and the temporal context.

only 33% of African Americans (AA) express Fy on their RBC [5]. Variants of the Duffy blood group system, their phenotype and genotype frequencies are reviewed elsewhere [9]. An explanation to this difference in expression can be provided by Duffy's functional role as a receptor for the malaria parasite *Plasmodium vivax*. RBC lacking Fy are more resistant to invasion from this parasite. The lack of expression of Fy in AA is caused by a point mutation in the GATA box promoter of the Fy^b allele (*FY*02N.01*; 1–67 T>C), which prevents expression of Duffy protein only on RBC [5, 7]. This mutation is seen with a high incidence of up to 95% in western and southwestern sub-Saharan Africa and correlates with a low prevalence of Plasmodium vivax [10]. However, individuals carrying this mutation rarely develop Fy3 alloantibodies since Fy expression is preserved on other tissues. The true Duffy null phenotype, resulting in complete loss of Fy expression on all tissues, is seen in patients homozygous for either a point mutation or deletion in exon 2 (e.g., *FY*02N.02*) which introduces a stop codon for the Duffy gene in all tissues (see example in **Table 1**). This phenotype allows for production of a Fy3 alloantibodies. Fy3 antibodies where first described in 1971 and have since been recognized with more case reports being presented [11–13]. The prevalence of Duffy null phenotype is unknown due to its rarity [5].

Duffy Antigen Receptor for Chemokines (DARC)

The Duffy antigen was identified as a transmembrane glycoprotein coupled receptor and was renamed the Duffy antigen receptor for chemokines (DARC) [14, 15]. DARC is found on the surface of RBC as well as the endothelium of postcapillary venules of lymph nodes, spleen, the kidney, and other organs [14]. DARC is shown to bind many different chemokines including Interleukin 8 (IL-8), monocyte chemoattractant protein 1 (MCP-1), CXC chemokines and regulated on activation normal T-cell expressed and secreted (RANTES) [16, 17]. Since no downstream effects have been identified after chemokine binding to DARC, it was hypothesized that it acts as a chemokine sink by attracting and binding chemokines, therefore reducing their bioavailability [17]. When challenged with lipopolysaccharide induced endotoxemia, DARC knockout mice showed an increase in chemokine production within multiple organs compared to control mice, supporting DARC's role in regulating the inflammatory response [18]. DARC has been shown to play a role in modulating immune responses in multiple diseases such as HIV, COVID-19 and atherosclerosis; given that, it is reasonable to think that DARC plays a role in renal transplantation [7].

DARC's Role in Renal Transplantation

One way in which DARC can affect renal transplant outcomes is by acting as a minor histocompatibility antigen. A Lerut et al. did a retrospective study of 370 renal transplant recipients comparing Fy matched (n = 239) versus mismatched (n = 131) donor-recipient pairs and correlated Fy mismatch status with histologic findings of kidney biopsies and overall survival [3]. Although graft survival and acute histologic lesions were similar between the groups, the study found increased incidence of chronic histologic lesions (e.g., interstitial fibrosis, tubular atrophy and intimal fibrosis) in Fy mismatched recipients despite the fact that the matched recipients had longer cold ischemia times and higher sensitization status (as indicated by PRA). Both *FY*A* mismatches and *FY*B* mismatches were associated with chronic lesions. However, the *FY*B* mismatched group showed only increased intimal fibrosis which may have been attributed to older age in this group compared with the Fy matched group. The *FY*A* mismatched group had no age or other demographic differences compared with the Duffy matched group, yet this group showed significantly more chronic histologic changes. The difference between *FY*A* and *FY*B* mismatches was attributed to the

higher immunogenicity of *FY*A* compared to *FY*B* [3]. Altogether, these results support Duffy's role as a minor histocompatibility antigen.

Another way in which DARC can affect renal transplant outcomes is through its expression levels. DARC expression is upregulated in the kidney in response to multiple environments of cell injury including HIV, nephropathy, hemolytic uremic syndrome, delayed graft function (DGF), ischemia reperfusion injury (IRI), and acute renal allograft rejection [16]. A study by Segerer et al. found upregulation of DARC positive venules in the setting of acute rejection post-transplant compared to pre-transplant biopsies [14]. This increase was even more prominent in cases with signs of both cellular and humoral rejection [14]. Akalin et al. studied a retrospective cohort of 117 kidney transplant recipients who were categorized based on their RBC Fy phenotype. The study showed a strong association between Fy(a−, b−) patients with DGF and graft failure, indicating that DARC may decrease the inflammatory response during DGF, causing DARC-negative patients to be more susceptible to DGF [16].

DISCUSSION

The functional role of DARC in recruitment of inflammatory cells is still not fully understood, and several mechanisms have been suggested: 1) DARC helps presenting chemokines on the endothelial cells to leukocytes expressing corresponding receptors; 2) The binding of chemokines to DARC helps to create a gradient flow to keep an influx of chemokines coming to the site; 3) Neutralization of bound chemokines [19, 20]. Although the mechanism of DARC is not clear, there is evidence of upregulation of DARC on peritubular capillaries during both humoral and acute cellular rejection episodes [19]. The question remains whether this upregulation is an attempt to bind and neutralize chemokines and control the inflammation or is this an attempt to recruit more inflammatory cells? It is important to note that most of the studies investigating Duffy's role in renal transplantation rely on RBC Fy phenotyping, which may be different from the Fy phenotype in the renal tissue. It is possible that DARC is able to pursue different functions according to the spatial and the temporal context (**Figure 2**).

The idea that anti-Fy antibodies may participate in renal allograft rejection is supported by a case report by Watorek et al. The authors reported a 41 years-old Caucasian woman, Fy(a− b+) phenotype, who had anti-Fy^a antibodies detected 2 years prior to her transplant, although at time of transplant her anti-Fy^a antibodies were undetectable. Her post-transplant hospital course was complicated by DGF and a biopsy at 26 days post-transplant revealed acute rejection, both of which are associated with upregulation of DARC expression in the kidney [14, 19]. Two months post-transplant her serum tested positive for anti-Fy^a antibodies and a repeat biopsy at 3 months showed acute and C4d+ antibody-mediated rejection in the absence of HLA donor-specific antibodies (DSA). The authors concluded that the unfavorable outcome of her transplant is a result of the presence of antibodies to Fy^a [21]. At the time of writing this paper, this is the only case report found linking the cause of kidney rejection to anti-Fy antibodies. However, case reports have suggested the involvement of antibodies to other RBC groups in renal allograft rejection [22–25].

At our center, we recently evaluated a 30 years-old African female with a history of multiple RBC transfusions due to anemia for a kidney transplant. Her HLA panel reactive antibody (PRA) was 0%. An ABO type and screen identified antibodies to Fy3, JkB, E, C, and K. The finding of the anti-Fy3 suggested that this patient carries the very rare true Fy null phenotype. Despite her ability (albeit limited) to receive transfusions due to the absence of Fy on RBC in most AA, her ability to find a compatible kidney donor is virtually impossible due to the presence of Fy on nearly every donor kidney. Even with a perfectly HLA and ABO matched donor, there is a potential for rejection due to anti-Fy3 reactivity and therefore this patient was deemed not suitable for transplantation at our center. Currently, there are no guidelines for donor-recipient RCA matching, mostly since more data is needed and since this will narrow down the donor pool for many transplant candidates. However, a more detailed screening process for RBC antibodies and RCA phenotyping of the donor may be warranted in patients experiencing antibody-mediated rejection (ABMR) with no HLA-DSA, especially if a recent post-transfusion reaction was observed prior to the diagnosis of ABMR. RCA antibody characterization might also be helpful prior to re-transplant following an ABMR involving RCA antibodies, and in cases where the recipient has a rare null phenotype. Differentiating between the true null and the erythrocyte silent mutations can be achieved by utilizing DNA-based typing instead of serologic phenotyping.

In conclusion, most of the emphasis in overcoming immunological barriers in solid organ transplantation is rightfully put on matching donors and recipients for HLA and ABO, however, the Duffy blood group system can present a unique and rare barrier to transplantation and potentially impact transplant outcomes.

AUTHOR CONTRIBUTIONS

RH-D, DH, JB, ME, BG, and ZS reviewed the literature and drafted the manuscript. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

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ORIGINAL RESEARCH
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Validated Prognostic Scores to Predict Outcomes in ECLS-Bridged Patients to Lung Transplantation

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Selection of patients who may benefit from extracorporeal life support (ECLS) as a bridge to lung transplant (LTx) is crucial. The aim was to assess if validated prognostic scores could help in selecting patients who may benefit from ECLS-bridging predicting their outcomes. Clinical data of patients successfully ECLS-bridged to LTx from 2009 to 2021 were collected from two European centers. For each patient, we calculated Sequential Organ Failure Assessment (SOFA), Simplified Acute Physiology Score III (SAPS III), Acute Physiology and Chronic Health Evaluation II (APACHE II), before placing ECLS support, and then correlated with outcome. Median values of SOFA, SAPS III, and APACHE II were 5 (IQR 3–9), 57 (IQR 47.5–65), and 21 (IQR 15–26). In-hospital, 30 and 90 days mortality were 21%, 14%, and 22%. SOFA, SAPS III, and APACHE II were analyzed as predictors of in-hospital, 30 and 90 days mortality (SOFA C-Index: 0.67, 0.78, 0.72; SAPS III C-index: 0.48, 0.45, 0.51; APACHE II C-Index: 0.49, 0.45, 0.52). For SOFA, the score with the best performance, a value ≥9 was identified to be the optimal cut-off for the prediction of the outcomes of interest. SOFA may be considered an adequate predictor in these patients, helping clinical decision-making. More specific and simplified scores for this population are necessary.

Keywords: outcomes, lung transplantation, bridge to transplant, SOFA, extracorporeal life support

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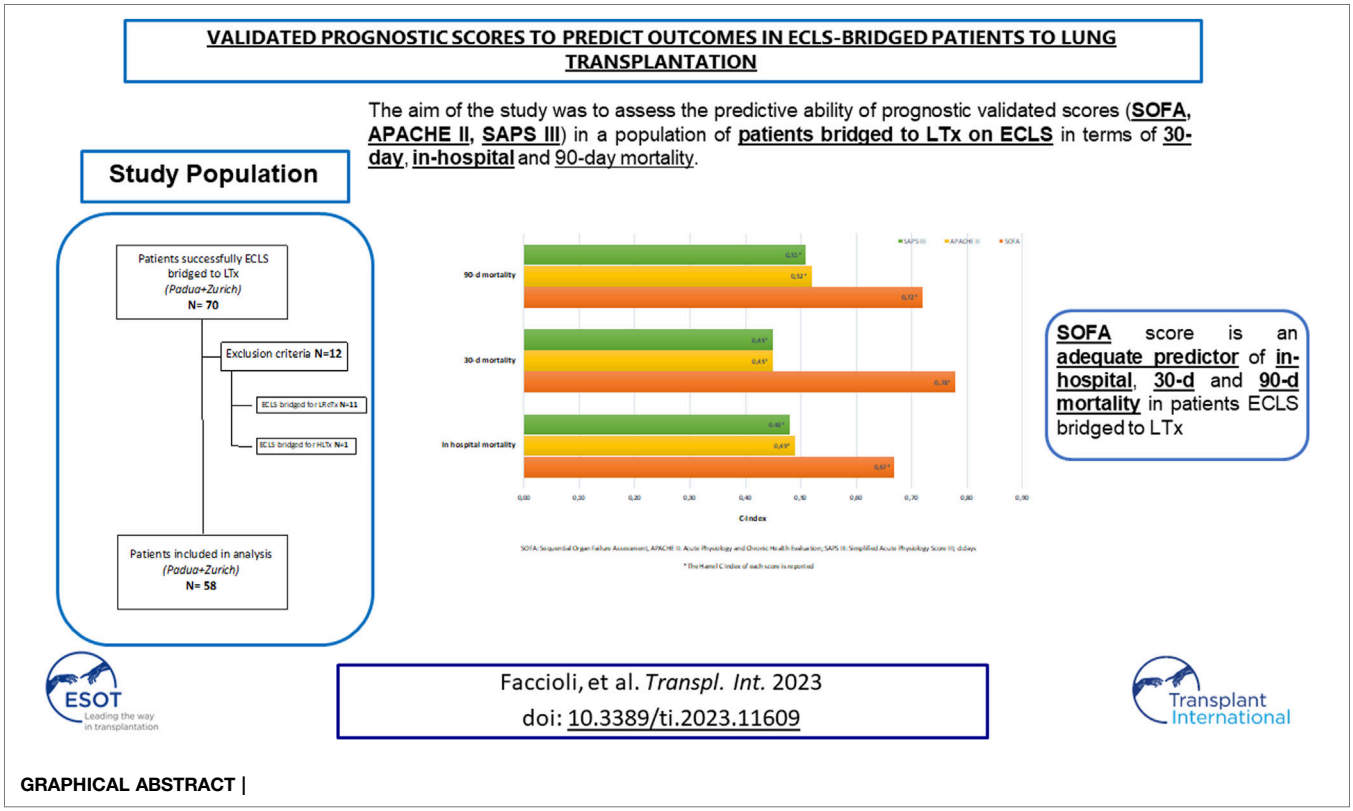
Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; CIFs, cumulative incidence functions; CLAD, chronic lung allograft dysfunction; ECCO2-R, extracorporeal carbon dioxide removal; ECLS, extracorporeal life support; ECMO, extracorporeal membrane oxygenation; ENCOURAGE, prEdictioN of Cardiogenic shock OUtcome foR AMI patients salvaGed by VA ECMO; EVLP, *ex-vivo* lung perfusion; HLTx, heart-lung transplantation; ICU, intensive care unit; IQR, interquartile range; MV, mechanical ventilation; LTx, lung transplantation; LreTx, lung retransplantation; pSOFA, pediatric Sequential Organ Failure Assessment; REMEMBER, pRedicting mortality in patients undergoing veno-arterial Extracorporeal MEMBrane oxygenation after coronary artEry bypass gRafting; RESP, Respiratory Extracorporeal Membrane Oxygenation Survival Prediction; SAPS III, Simplified Acute Physiology Score III; SAVE, Survival After Veno-arterial ECMO; SOFA, Sequential Organ Failure Assessment; STABLE, Recipient STratification Risk Analysis in Bridging Patients to Lung Transplant on ECMO; UNOS, United Network for Organ Sharing; VV, veno-venous; VA, veno-arterial.



GRAPHICAL ABSTRACT |

INTRODUCTION

The utilization of extracorporeal life support (ECLS) as a bridge to lung transplantation (LTx) has allowed critically ill patients to remain eligible for transplant.

The selection of patients who may benefit from ECLS as a bridge to LTx is a crucial aspect: highly urgent patients, with a high predicted pre-transplant mortality, are often the ones who would benefit the most from ECLS but at the same time they could be too compromised to be suitable candidates for this support [1].

The patients who can derive the greatest benefit from ECLS-bridge are generally those with cardiopulmonary dysfunction severe enough to limit their ability to maintain the necessary physical condition to tolerate a transplant (such as oxygen saturation <90% with high-flow levels and with non-invasive oxygenation devices, hemodynamic instability, and use of positive pressure ventilation that could lead to further lung injury and secondary organ dysfunctions) and it is mostly recommended in patients who have already been evaluated for LTx [1–4].

The effect of ECLS as a bridge to LTx and the consequences on recipients' clinical outcomes remain undetermined, indeed the results reported in current literature are divergent [5, 6].

Some authors [7, 8] reported negative experiences with ECLS as a bridge to LTx, showing a worse overall survival in bridged patients compared to unsupported ones. On the other hand, in more recent times, different authors have reported good outcomes for successfully bridged patients on ECLS with satisfying survival rates [2, 3, 9–11].

It is widely established that a careful patient selection, high volume transplant centers, and multidisciplinary teams are the key factors to obtain improvements in ECLS bridging strategies [1], even though a homogeneous consensus on which factors might help the clinicians in predicting outcomes of patients bridged to LTx with ECLS supports is still lacking. This is also demonstrated by the fact that currently no clinically validated tools, except the Recipient STratification Risk Analysis in Bridging Patients to Lung Transplant on ECMO (STABLE) score [12], exist to predict outcomes in this population. However, given the increasing use of different bridging devices (not only extracorporeal membrane oxygenation, ECMO) and strategies in the modern era, it is mandatory to define if validated prognostic scores might predict mortality in this population, helping to better select patients who may benefit from ECLS bridging in relation to post-operative outcomes.

Scoring systems, such as Sequential Organ Failure Assessment (SOFA), Simplified Acute Physiology Score III (SAPS III), and Acute Physiology and Chronic Health Evaluation II (APACHE II), are commonly used for risk assessment in critically ill patients, especially to predict in-hospital mortality [13–15].

The aim of this study was to assess the predictive ability of these scores in a population of patients bridged to LTx on ECLS in terms of in-hospital, 30 and 90 days mortality.

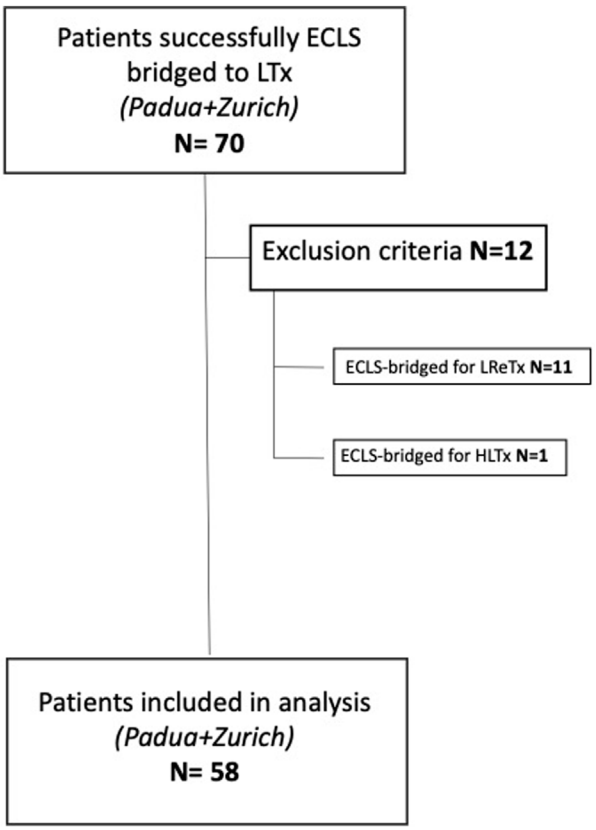


FIGURE 1 | Flowchart of patients' inclusion and exclusion criteria (ECLS, extracorporeal life support; LTx, lung transplant; LreTx, lung re-transplant; HLTx, heart-lung transplant).

These findings might play an important role in guiding physician decision making to better select patients who might benefit from a bridge from ECLS to LTx, also facilitating evidence-based rationing of limited healthcare resources in the future.

MATERIALS AND METHODS

All clinical data of 70 patients successfully ECLS-bridged to LTx from 2009 to 2021 were retrospectively collected from two European centers (Thoracic Surgery Unit of University Hospital of Padua, Italy and Department of Thoracic Surgery of University Hospital of Zurich, Switzerland) as anonymized records. The study was approved by the Ethical Committee of the University Hospital of Padua (4539/AO/18). Informed consent was waived due to the retrospective nature of this work.

Patients bridged to lung retransplantation (LReTx) or heart-lung transplantation (HLTx) with ECLS were excluded. Fifty-eight patients were finally enrolled in the study (**Figure 1**).

Demographic and clinical data, intra-operative characteristics, peri and post-operative outcomes were collected for each patient from both centers. Follow-up was achieved in each center by indirect contact via the treating physician. For each patient, organ dysfunction (SOFA) and illness severity (APACHE II, SAPS III) scores were collected when already available otherwise calculated retrospectively at the ICU arrival, before positioning the ECLS device and then correlated with outcomes. A comparison between the variables utilized in the abovementioned scoring systems is shown in **Table 1**.

Statistical Analyses

Descriptive statistics were reported as I quartile/median/III quartile for continuous variables and as percentages (absolute numbers) for categorical variables.

Survival distribution was evaluated using the Kaplan-Meier method. To assess the predictive ability of the scores (SOFA, APACHE II, SAPS III) on the outcomes of interest (in-hospital, 30 and 90 days mortality) logistic regression models were estimated. After models' validation using bootstrap resampling, the Harrel's C index, also known as "concordance index" [16] was computed.

Furthermore, the optimal cut-off for SOFA in predicting outcomes of interest was identified as the value that maximizes the sum of sensitivity and specificity.

Analyses were performed using R software version 4.1.3 [17] within the packages rms [18] and cutpointr [19].

RESULTS

Study Population

The main clinical and demographic characteristics of 58 patients bridged to LTx on ECLS support are presented in **Table 2**.

Thirty-four patients (59%) were females, 24 were males (41%) with a median age at time of LTx of 42 years-old (IQR 24–49). Seven patients (12%) were in pediatric age (age <18 years). The median BMI was 19.5 (IQR 17–24). The most common indication for LTx was cystic fibrosis (CF) (57%) followed by interstitial lung disease (ILD) (27%); almost all patients (98%) underwent bilateral lung transplantation while only a 65 year-old patient affected by ILD was submitted to single LTx.

The median waiting list time was 69 days (IQR 14–240). The most common ECLS bridge configuration was veno-venous (VV) (37 patients, 64%) although in 30% of cases an upgrading to another ECLS configuration was necessary during bridging. During ECLS bridging, 48 patients (83%) were mechanically ventilated while 10 patients (17%) were awake. The median time from ECLS bridge to LTx was 10 days (IQR 3–18). Median SOFA, APACHE II, and SAPS III values at the ICU arrival were respectively 5 (IQR 3–10), 21 (IQR 15–26), 57 (IQR 47.5–65). Median pediatric (p) SOFA, a special score tailored on pediatric patients [19], was also calculated but the median value did not differ from the one obtained in adults (5, IQR 3–10). The most common intra-operative ECLS configuration was the VV (18 patients, 31%) followed by the central veno-arterial (VA) ECLS (15 patients, 26%). 30 patients (51%) needed prolonged post-operative ECLS with a median duration of 3 days (IQR 2–8).

In 4 cases (7%) *ex-vivo* lung perfusion (EVLP) methods were used to recondition the organs before the implantation because of extended criteria donors and in 17 patients (29%) a size reduction with lobar LTx was performed due to the donor and the recipient size mismatch.

TABLE 1 | Variables employed in SOFA, APACHE II, SAPS III scoring systems.

	SOFA (range 0–20)	APACHE II (range 0–71)	SAPS III (range 16–217)
Variables	- PaO2/FiO2 (and if MV/CPAP) - Platelets - GCS - Bilirubin - MAP (and if vasoactive agents required) - Creatinine	- Temperature - Age - MAP - HR - RR - pH - Sodium - Potassium - Creatinine - Hematocrit - WBC - Chronic organ failure (heart, lung, liver, kidney) - GCS - FiO2	- Age - LOS before ICUA - In- hospital location (OR, ER, other ICU) - Cancer therapy (yes/no) - Chronic heart failure (yes/no) - Hematological cancer (yes/no) - Cirrhosis (yes/no) - AIDS (yes/no) - Cancer (yes/no) - Vasoactive drugs before ICUA (yes/no) - ICUA (planned/unplanned) - Reason for admission (cardiovascular, hepatic, digestive, neurologic) - Surgical status at ICUA (scheduled, emergency, no surgery) - site of surgery (transplant, trauma, cardiac, neurosurgery) - acute infection at ICUA (nosocomial, respiratory) - GCS - Bilirubine - Temperature - Creatinine - HR - WBC - pH - Platelets - SBP - pO2/FiO2 - MV (yes/no/CPAP)

APACHE II, acute physiology and chronic health evaluation II; CPAP, continuous positive pressure ventilation; ER, emergency room; FiO2, fraction of inspired oxygen; GCS, Glasgow coma scale; HR, heart rate; ICU, intensive care unit; ICUA, intensive care unit admission; LOS, length of stay; MAP, mean arterial pressure; MV, mechanical ventilation; OR, operative room; paO2, partial pressure of oxygen; RR, respiratory rate; WBC, white blood cells; SBP, systolic blood pressure; SAPS III, simplified acute physiology score III; SOFA, sequential organ failure assessment.

Short-Term Outcomes

Table 3 summarizes the main post-operative outcomes. The median duration of mechanical ventilation (MV) was 96 h (IQR 48–480) and in 28 patients (48%) a tracheostomy was performed for respiratory weaning. The median duration of ICU and hospital stay were respectively 11 days (IQR 6–28) and 44 days (IQR 31–71). Twenty-four patients (41%) required post-operative continuous veno-venous hemofiltration (CVVH) or dialysis for renal failure. In-hospital, 30, and 90 days mortality were respectively 21%, 14%, and 22%.

Long-Term Outcomes

One, 3, and 5 years survival rates were 72 % (95% CI 0.61–0.84), 55% (95% CI 0.43–0.70), and 51% (95% CI 0.38–0.66), respectively (**Figure 2**).

Predictive Ability of SOFA, APACHE II, and SAPS III

The ability of SOFA, APACHE II, and SAPS III in predicting post-LTx outcomes in ECLS-bridged patients is presented as C Index in **Table 4**.

SOFA, SAPS III, and APACHE II were analyzed as predictors of in-hospital, 30, and 90 days mortality respectively (SOFA C Index: 0.67, 0.78, 0.72; SAPS III C Index: 0.48, 0.45, 0.51; APACHE II C Index: 0.49, 0.45, 0.52). For SOFA, the score with the best performance in this population, a value ≥9, was identified to be the optimal cut-off for the prediction of all the outcomes of interest (**Table 5**).

DISCUSSION

Patients bridged to LTx with ECLS are often critically ill with a severe deterioration of clinical conditions. The investigation of predictors of outcomes in this population is mandatory, especially in a context of donors' paucity as well as to facilitate evidence-based rationing of limited healthcare resources in the future.

We decided to analyze the predictive ability of three scores (SOFA, APACHE II, SAPS III), which are widespread known and easily accessible for every patient at the ICU arrival, to predict post-operative outcomes in ECLS bridged patients. These scores have already been extensively validated as predictors of mortality in several clinical settings [20–22] including transplantation field [23] and in patients on ECMO for cardiac or acute respiratory failure [24, 25]. However, they were not validated in ECLS-bridged patients to LTx. In addition, a number of specific scores in VA ECMO settings like prEdictioN of Cardiogenic shock OUtcome foR AMI patients salvaGed by VA ECMO (ENCOURAGE), Survival After Veno-arterial ECMO (SAVE), and pRedicting mortality in patients undergoing veno-arterial

TABLE 2 | Patients characteristics.

Variable	Total (N = 58)
Female sex	34 (59%)
Age at LTx (y)	42 (24–49)
Diagnosis	
CF	33 (57%)
ILD	16 (27%)
COPD	4 (7%)
LAM	1 (2%)
Other	4 (7%)
BMI	19.5 (17–24)
Waiting list time (d)	69 (14–240)
Type of LTx	
BLTX	57 (98%)
SLTX	1 (2%)
Pre LTx MV	
No	10 (17%)
Yes	48 (83%)
Awake ECLS	10 (17%)
Time from ECLS bridge to LTx (d)	10 (3–18)
Initial ECLS bridge configuration	
VV-ECMO	37 (64%)
VA-ECMO	8 (14%)
ECCO2-R	13 (22%)
ECLS bridge configuration change	18 (30%)
SOFA	5 (3–9)
pSOFA (age <18 y)	5 (3–10)
APACHE II	21 (15–26)
SAPS III	57 (47.5–65)
Intraoperative ECLS configuration	
VV-ECMO	18 (31%)
pVA-ECMO	12 (21%)
cVA-ECMO	15 (26%)
CEC	4 (7%)
VAV	9 (15%)
Prolonged post-operative ECLS	
NO	28 (47%)
YES	30 (51%)
De novo	1 (2%)
Post-operative ECLS duration (d)	3 (2–8)
EVLP	4 (7%)
Lobar transplantation	17 (29%)

APACHE II, Acute Physiology and Chronic Health Evaluation II; BLTX, bilateral lung transplant; c, central; d, days; ECCO2-R, extracorporeal carbon dioxide removal; ECLS, extracorporeal life support; ECMO, extracorporeal membrane oxygenation; EVLP, ex-vivo *lung perfusion; f, female; LTx, lung transplant; MV, mechanical ventilation; p, peripheral; SOFA, Sequential Organ Failure Assessment; SAPS III, Simplified Acute Physiology Score III; y, years; VA, venoarterial; VAV, venoarterial venous; VV, venovenous.*

Data are reported as median (I-III interquartile range) for continuous variables and as absolute number (relative frequencies %) for categorical variables.

TABLE 3 | Clinical course and outcomes.

Variable	Total (N = 58)
ICU stay (post LTx, d)	11 (6–28)
MV duration (post LTx, h)	96 (48–480)
Post-operative tracheostomy	28 (48%)
Post-operative CVVH/dialysis	24 (41%)
Hospital stay (d)	44 (31–71)
CLAD	8 (14%)
In hospital mortality	12 (21%)
30 d mortality	8 (14%)
90 d mortality	13 (22%)

CLAD, chronic lung allograft dysfunction; CVVH, continous venovenous hemofiltration; d, days; h, hours; ICU, intensive care unit; LTx, lung transplant; MV, mechanical ventilation. Data are reported as median (I-III interquartile range) for continuous variables and as absolute number (relative frequencies %) for categorical variables.

Extracorporeal MEMBrane oxygenation after coronary artEry bypass gRafting (REMEMBER) have been proposed to predict mortality in selective cardiogenic shock subsets [26–28], limiting their application in our study population.

On the other hand, The Respiratory Extracorporeal Membrane Oxygenation Survival Prediction (RESP) Score predicts survival for patients receiving ECMO for severe acute respiratory failure [29] but again it is not tailored to chronic end-stage lung disease and it does not take into account those patients with an associated hemodynamic instability (like in idiopathic pulmonary hypertension).

To the best of our knowledge, the only available predictive tool for risk stratification in ECMO bridge patients to LTx is the STABLE score [12] but some of its limitations made it not applicable to our entire population: firstly, it is validated only in adults but our population was composed for 12% of pediatric patients. In our study, in accordance with what has been reported by some of the most consistent studies on ECLS bridge [2, 8], more than a half (57%) of our population had cystic fibrosis which is the most common indication in pediatric population, therefore a score also applicable in a pediatric population (<18 years old) is mandatory. In our pediatric patients, we have also calculated for each of them the pSOFA, an adapted and validated pediatric version of the SOFA score [20], finding the same median value of the adult population. Concerning the other two scores, APACHE II has already been utilized for pediatrics in other clinical setting [30] and in the SAPS III calculator, ages of <18 years old can be inserted, so we felt authorized to use these scores also in pediatrics.

Secondly, among extracorporeal supports, the STABLE score only considers ECMO and not other devices such as extracorporeal carbon dioxide removal (ECCO2-R), which was utilized in 22% of our population as a bridge to LTx. Finally, this score was created on a big number of patients extracted from United Network for Organ Sharing (UNOS) database but it was externally validated only on 31 American patients and so it could not be representative of the European reality.

Among the three scores utilized in our analysis, the predictor of in-hospital, 30- and 90-days mortality with the best performance was the SOFA with a cut-off value of 9. SOFA score is the easiest to calculate and based on easy repeatable variables available in all institutions. Originally, it was designed to describe morbidity expressing different degrees of organ failure, but then it has been extensively externally validated as a good predictor of hospital mortality [14, 25].

In contrast to our finding [24], in a previous study, compared the prognostic ability of different scores in ECMO patients, showing that APACHE had a superior ability to SOFA in predicting hospital mortality. Their study did not focus on patients bridged to LTx and furthermore the scores were calculated only on the first day of ECMO support and not at

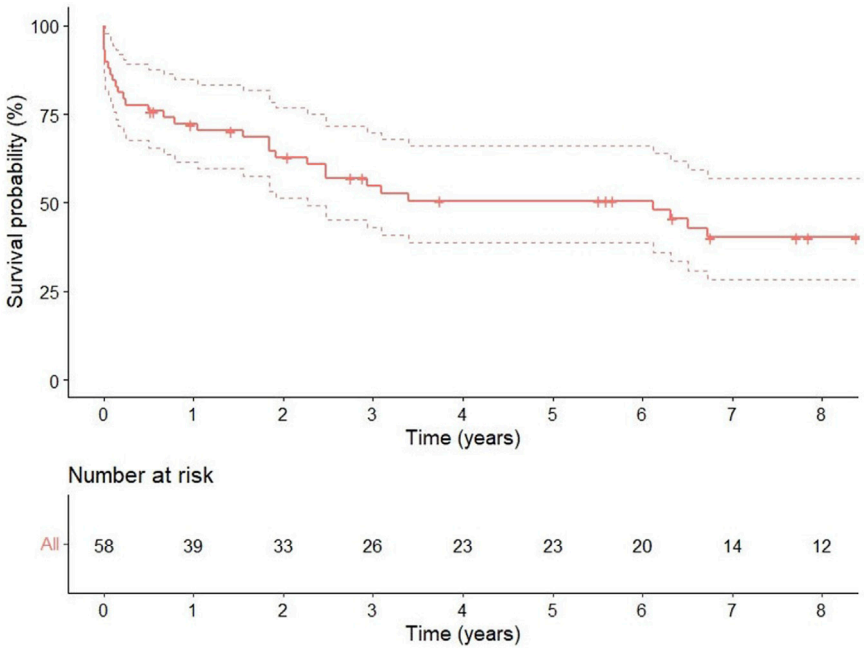


FIGURE 2 | Kaplan-Meyer curve of the overall survival in ECLS-bridged patients.

TABLE 4 | Predictive ability of SOFA, APACHE II, SAPS III scores in predicting outcomes.

Score	Outcomes			
	In hospital mortality	30 d mortality	60 d mortality	90 d mortality
SOFA	0.67	0.78	0.60	0.72
APACHE II	0.49	0.45	0.42	0.52
SAPS III	0.48	0.45	0.42	0.51

APACHE II, Acute Physiology and Chronic Health Evaluation II; d, days; SAPS III, Simplified Acute Physiology Score III; SOFA, Sequential Organ Failure Assessment. The Harrel's C Index of each score for in-hospital, 30, 60, and 90 days mortality is reported.

TABLE 5 | Performance of SOFA value ≥9.

Outcome	Sensitivity	Specificity	AUC
In-hospital mortality	0.714	0.780	0.775
30 d mortality	0.6	0.789	0.680
90 d mortality	0.636	0.810	0.717

AUC, area under curve; d, days.

the ICU admission as these models were originally developed and this may have affected the results. The low accuracy of APACHE II and SAPS III in predicting in-hospital mortality in transplant patients has already been established [23]. We also reported the same finding in ECLS bridged patients to LTx; this may be due to the multitude of physiologic aspects (such as for examples sodium, potassium, hematocrit, white blood cells, and platelets count) accounted by these two scores compared to the SOFA. These parameters are usually out of normality range in this population and tend to have a large and rapid variability during the pre- and post-transplant periods, making these scores unreliable in our patients.

Although in our study, a SOFA score of higher than or equal to 9 was associated with a poor short-term prognosis, this value should not be intended to arbitrarily exclude patients from life-sustaining therapies or from the possibility of a lung transplantation but just as a useful tool to better select the most appropriate LTx candidate or to help clinicians to identify which patients would need a stricter follow-up in the early post-operative period.

In conclusion, the results of this study serve as a first external validation of these scores in ECLS-bridged patients to LTX but it has some limitations. Even though it reflects the reality of two European lung transplant centers, the main limitation is the small number of patients and the absence of a control group. Then, in some situations, difficulties exist in performing this analysis as clinical/laboratory data to calculate the scores are not always collected at the same moment for all the subjects with a tendency towards high variability during pre-operative course during ECLS bridging. Again, it would be necessary to evaluate the evolution of these scores in different moments by sequential measurements (daily, weekly) and not only at the ICU admission, providing a more robust prediction of mortality.

Further studies are necessary to validate our results and to find a promising and accurate score in this peculiar subgroup of patients.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by Comitato Etico Azienda Ospedale-Università di Padova. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin due to the retrospective nature of the study.

AUTHOR CONTRIBUTIONS

EF: conceptualization, investigation, data curation, writing-original draft. GL: data curation software, methodology. DS: writing-review and editing. AD'A: writing-review and editing. SH: writing-review and editing. MS: writing-review and editing. CC: writing-review and editing. DG: data curation software, methodology. FR: writing-review and editing. IO: writing-review and editing. II: conceptualization, data curation, investigation, supervision, writing-original draft.

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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ORIGINAL RESEARCH
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Three Decades Single Center Experience of Airway Complications After Lung Transplantation

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Post lung transplantation airway complications like necrosis, stenosis, malacia and dehiscence cause significant morbidity, and are most likely caused by post-operative hypo perfusion of the anastomosis. Treatment can be challenging, and airway stent placement can be necessary in severe cases. Risk factors for development of airway complications vary between studies. In this single center retrospective cohort study, all lung transplant recipients between November 1990 and September 2020 were analyzed and clinically relevant airway complications of the anastomosis or distal airways were identified and scored according to the ISHLT grading system. We studied potential risk factors for development of airway complications and evaluated the impact on survival. The treatment modalities were described. In 651 patients with 1,191 airway anastomoses, 63 patients developed 76 clinically relevant airway complications of the airway anastomoses or distal airways leading to an incidence of 6.4% of all anastomoses, mainly consisting of airway stenosis (67%). Development of airway complications significantly affects median survival in post lung transplant patients compared to patients without airway complication (101 months versus 136 months, $p = 0.044$). No significant risk factors for development of airway complication could be identified. Previously described risk factors could not be confirmed. Airway stents were required in 55% of the affected patients. Median survival is impaired by airway complications after lung transplantation. In our cohort, no significant risk factors for the development of airway complications could be identified.

Keywords: lung transplant, anastomosis, bronchoscopy, airway complications, airway stent

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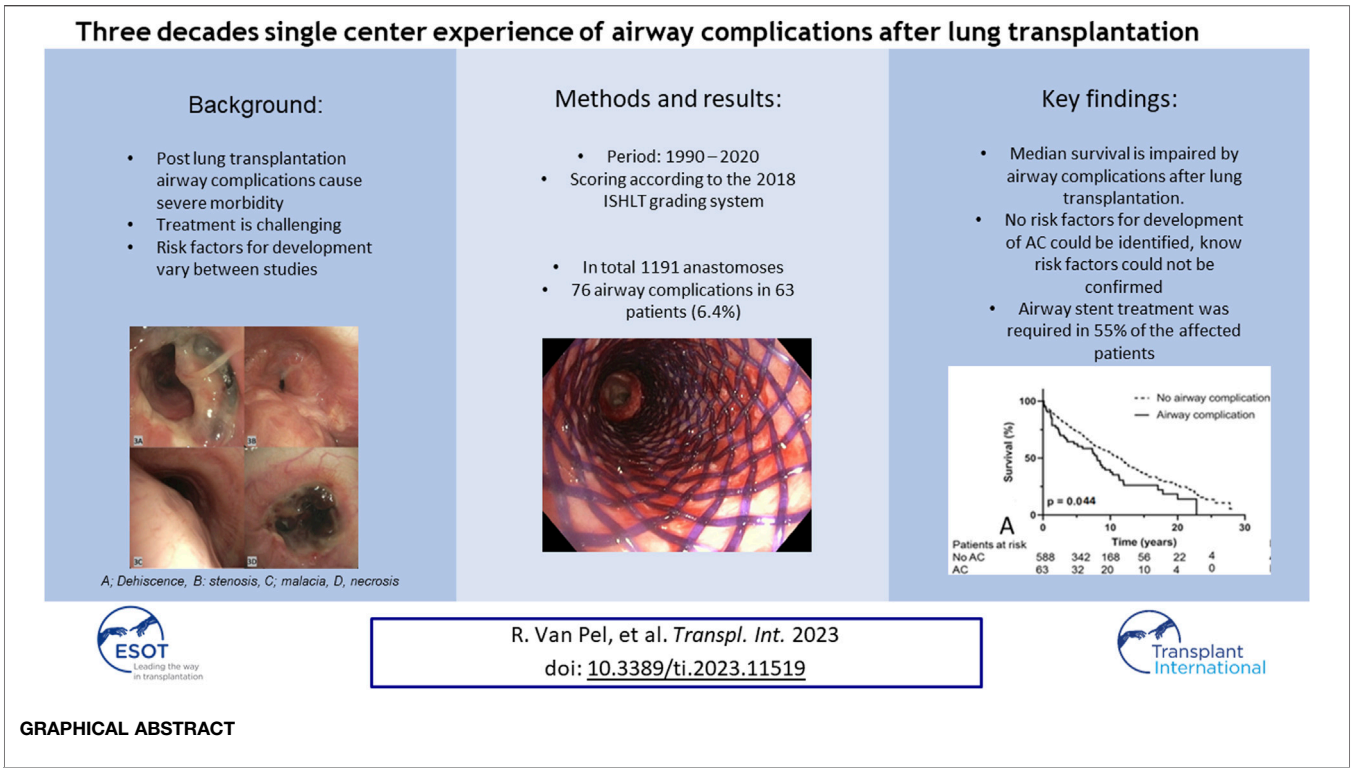
INTRODUCTION

Since the first lung transplantation, anastomotic airway complications (AC) have been a major cause of morbidity and mortality in lung transplant recipients [1, 2]. Broncho-arterial blood supply is not restored during transplantation [3, 4] and viability of the donor bronchus depends on retrograde blood flow. Development of AC can thus be attributed to hypoperfusion of the donor bronchi [5] and is subdivided in stenosis, malacia, dehiscence and necrosis [6]. Multiple grading systems have been developed for scoring AC [6, 7] Data from mainly retrospective cohort studies show AC incidence ranging from 2% to 18% [8–11].



GRAPHICAL ABSTRACT

Revascularization of the blood supply can take up to 4 weeks. In this period, ischemia can cascade an inflammatory response with remodeling and risk of both stenosis and malacia [12–15]. To prevent ischemia the donor and recipient bronchus are kept as short as possible [16] to acquire the shortest distance for retrograde bronchial perfusion and have the anastomosis within mediastinal tissue. Risk factors for development of AC are mainly associated with compromised blood flow, such as post-operative hypo perfusion [13] or acute cellular rejection [17]. Risk factors have also been suggested that cannot be related to hypoperfusion directly, for instance right sided anastomosis [9], prolonged ventilation of >48 h of donor [18] and height difference between donor and recipient [11, 18].

Management of AC is diverse and depends on etiology and severity [12]. Endoscopic interventions range from balloon dilatation, electrocautery debridement, laser treatment, cryoablation to endobronchial stent placement [19]. In case of stenosis necessitating treatment, the first approach is (repeated) balloon dilatation which can be sufficient in up to 26% [20]. Recurrent or persistent stenosis, malacia and dehiscence can be treated with endobronchial stent [21] after careful consideration given the potential complications such as sputum statis, infections, stent migration or granulation formation with re-stenosis [19, 22].

In this study, we evaluated known risk factors and try to identify new risk factors for the development of AC. Furthermore, survival data within different treatment modalities of AC were analyzed in our cohort.

MATERIALS AND METHODS

Study Design

Patients with a unilateral or bilateral lung transplantation with an age >18 years transplanted at the University Medical Center Groningen in The Netherlands between 1990 and 2020 were analyzed for AC. Patients gave written informed consent for transplant-related research and this analysis was approved by the local medical ethics committee (METc 2021.00408).

AC was defined as any airway problem necessitating bronchoscopic intervention or follow up. In this study, we graded all AC according to the 2018 ISHLT grading system at the time of detection based on bronchoscopy images and reports as far as possible [6]. With airway malacia defined as >50% reduction in luminal caliber with expiration and clinical impairment. The clinical characteristics of donor and recipient (pre- and post-transplant) were analyzed. Patients who died within 30 days after lung transplantation of causes not related to AC were excluded. From 2005 onward, donation after circulatory death (DCD) donor lungs were accepted besides donation after brain death (DBD) donor lungs.

AC treatment was subdivided into expectative with debridement at most, conservative treatment (balloon dilatation, electrocautery, laser therapy, cryotherapy, mitomycin C application), stent placement or surgical intervention.

The practiced surgical technique has been an end-to-end anastomosis, with telescoping technique in case of anatomical necessity. Since the publication of Aigner et al in 2003 [16], a running suture for the cartilaginous part was introduced. In addition we adopted the practice of a short as possible donor

bronchus in 2010 [23]. In case of bilateral transplantation, primary implantation of the right lung is preferred, depending on anatomical variation. According to local protocol, routine bronchoscopy is performed for inspection of the anastomosis during transplantation, prior to extubation and before hospital discharge. Surveillance bronchoscopy was standard at 6, 12, 18, and 24 months until 2008 and is adjusted to bronchoscopy on clinical indication or decline of lung function since. Acute cellular rejection was treated with pulse 1,000 mg methylprednisolone for 3 days. Immune suppression regime evolved over time from rATG, cyclosporine, azathioprine, and prednisolone in the beginning (1990–2001) of the program to Basiliximab, tacrolimus, azathioprine, prednisolone (2001–2009) and Basiliximab, tacrolimus, mycophonolate mofetil and prednisolone since 2009.

Intervention bronchoscopy for stent placement was performed under general anesthesia with rigid or flexible bronchoscope depending on individual case characteristics. Commercially available self-expandable metallic stents (SEMS) were used as standard. From 2019 and on biodegradable stents (ELLA-CS Ltd, Czech Republic) were used.

Statistical Analysis

Continuous data are expressed as median + range. Nominal variables are expressed as percentages. Because data did not fulfill conditions for normal distribution, non-parametric tests were used. To test for significance in categoric data Pearsons Chi squared test was used or if necessary, Fishers exact test. Continuous data was analyzed with the Mann-Whitney U test. Survival was analyzed with Kaplan-Meier analysis with Log-rank testing. All analyses have been conducted with IBM SPSS Statistics version 23 (IBM, Chicago, USA) and Graphpad Prism 9 (GraphPad software, Inc., La Jolla, USA).

RESULTS

Between November 1990 and September 2020, 758 lung transplantations were performed. After exclusion criteria 651 patients for our analysis remained. This cohort contained 540 bilateral, 40 unilateral left and 71 unilateral right transplantations resulting in 1191 airway anastomoses. Seventy-six AC occurred in 63 patients, with an AC prevalence of 6.4% per anastomosis and 9.6% per patient. Thirty-eight AC were on the left side and 36 on the right side ($p = 0.278$). The median age for lung transplantation was 52 years for the non-AC population and 50 years for the AC population ($p = 0.893$), 51% of the non-AC population was male compared to 57% of the AC patient group ($p = 0.264$).

The 76 cases of AC were subdivided into airway stenosis: 51 (67%), malacia: 11 (15%), ischemia/necrosis: 9 (12%) and dehiscence: 5 (7%). **Figure 1** shows an example of all four types of AC with corresponding ISHLT grading. Median time until detection of AC was 12 weeks. See **Table 1** for the further grading of all AC. 32 of the 51 stenoses consisted of an anastomotic location with >50% but <100% reduction in cross-sectional area. The 11 malacia occurred perianastomotic in 4 cases (36%%) and diffuse in 7 (64%) of the cases. Three of the 5 dehiscence could not be specified besides being partial. **Figure 2** shows the prevalence of AC per anastomosis throughout the years.

Risk Factors for Development of Airway Complications

Donor Characteristics

Median donor age was 45 years for non-AC patients versus 46 for AC patients ($p = 0.896$). Median donor age increased from 36 (range 12–55) years in the first 5 years of the program to 51 (range 11–78) in the most recent 5 years. Median ventilation time was 2 days for both AC affected, and non-AC affected patients ($p = 0.872$). Median donor packyears was 0 years for AC patients and non-AC patients alike ($p = 0.693$) (**Table 2**). Donation after circulatory death (DCD) was introduced in 2005 and performed in 32% of the transplants since and totals 21% of the entire cohort. AC occurred in 13 out of 126 (10%) of the DCD patients and 50 out of 462 (11%) of the DBD patients ($p = 0.884$). Donor ventilation time >48 h was not a risk factor for development of AC ($p = 0.992$). Median donor age was 30 years in the period from 1990 to 1995 and 51 in the period from 2015 to 2020. Forty patients were pretreated with *ex vivo* lung preservation, incidence of AC in this group was comparable to the cases without this treatment ($p = 0.714$).

Recipient Characteristics

The most common indication for lung transplantation was chronic obstructive pulmonary disease $n = 301$ (42%) followed by interstitial lung disease $n = 132$ (20%), infectious pulmonary disease including cystic fibrosis and bronchiectasis $n = 116$ (18%), and pulmonary vascular disease $n = 52$ (8%). Median donor-recipient size mismatch was −1 cm for both the AC patients and the non-AC patients ($p = 0.980$, ns). After introduction of the running suture for the membranous part in 2003, occurrence of AC was stable (7.4% vs. 12%, $p = 0.129$). Median recipient age raised from 45 years (range 19–64) in the first 5 years of the program to 58 (range 19–68).

Median intensive care unit (ICU) admission was 6 days for both AC patients and non-AC patients ($p = 0.209$). Median time to extubation was 2 days for both AC patients and non-AC patients ($p = 0.095$). Sixty seven of the 76 (88%) AC were identified after ICU discharge.

Surgical Ischemic time for implantation of the first lung was 313 min for patients with AC and 314 min for patients without AC ($p = 0.814$). Lung transplants were performed by twenty five surgeons, with a median of 28 transplants per surgeon (range 1–98). No difference in incidence of AC was observed between high volume (N > 28) and low volume (N < 28) surgeons ($p = 0.515$).

238 (40%) of the non-AC patients were treated for acute rejection within 30 days post-transplant compared to 31 (49%) of the AC patients ($p = 0.115$). Development of primary graft dysfunction of any severity whatsoever occurred in 313 (53%) of the non-AC patients and 34 (54%) of the AC patients ($p = 0.734$). The risk for development of AC was not influenced by the immune

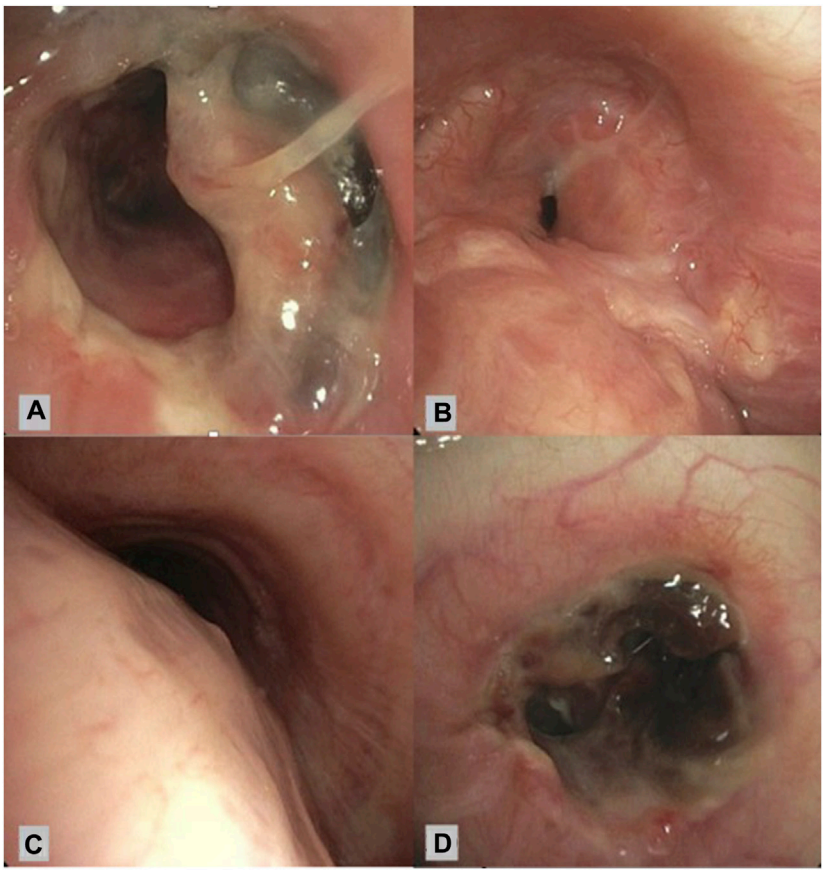


FIGURE 1 | Bronchoscopic view of different airway complications. **(A)**: Partial airway dehiscence at anastomosis ISHLT grading: DLaEb, **(B)**: Airway stenosis at anastomosis ISHLT grading SLaEc, **(C)**: Airway malacia proximal of anastomosis ISHLT grading Mb, **(D)**: Airway necrosis at anastomosis ISHLT grading NLaEd [6].

suppression regime (p = 0.162). See **Table 3** for further recipient characteristics.

Treatment; **Table 1** shows the treatment strategy of all 76 AC. Fifteen (20%) were approached expectative with follow up bronchoscopy or debridement at most without further intervention. AC was treated with conservative therapy in 17 (22%) of the cases.

Treatment with airway stent was required in 42 (55%) of the AC in 36 patients. Twenty-six airway (65%) stents were placed in the left main bronchus, fourteen (35%) in the right main bronchus and bronchus intermedius. Six cases were treated with biodegradable stent, 5 after prior treatment with SEMS, which were removed before biodegradable stent placement.

Three surgical interventions were required. One for a partial dehiscence, one pneumonectomy for stent therapy refractory stenosis and one anastomosis revision for stenosis without prior treatment with airway stent.

Survival; **Figure 3A** shows the overall survival between the 63 AC and the 588 non-AC lung transplant recipients. Occurrence of AC led to significantly shorter survival with median survival of 101 months versus 136 months (p = 0.044). Forty one of the 63 patients with AC died in the follow up. Cause of death could not be related to AC or its complications in 35 (85%) of the patients including 7 patients dying of chronic allograft dysfunction and 8 patients dying of a malignancy. One patient died because of a dehiscence of the right main bronchus without further treatment options, one patient with airway dehiscence died due to multi organ failure. Five patients with SEMS *in situ* died of pulmonary infection with *Aspergillus Fumigatus* and/or *Pseudomonas aeruginosa*.

In the 5 patients with airway dehiscence the median survival was 1 month (range 0–88 months). When these cases are excluded from survival analyses, median survival was not significantly different, but still showed a trend in favor of the non-AC group, with a median survival of 105 months for AC patients and 136 months for non-AC patients (p = 0.142).

Within the patient group suffering from AC, necessity of endobronchial airway stent placement shows overall median survival of 102 months compared to median survival of 91 months in AC patients without stent placement (p = 0.627) (**Figure 3B**). The 36 patients receiving an airway stent had a median survival of 102 months compared to 132 months in the remaining 615 patients in the total cohort (p = 0.346).

TABLE 1 | Distribution according to 2018 ISHLT grading, time until detection and treatment of AC.

Grading			Median time to detection in weeks (range)	Treatment			
				E	C	AS	S
All (N = 76)			**12 (1–630)**	**15**	**17**	**41**	**3**
Ischemia and Necrosis (I) (N = 9)			5 (1–14)	**0**	**5**	**4**	**0**
Location							
A	Perianastomotic – within 1 cm of anastomosis	1 (11%)	14	0	0	1	0
B	Extending > 1 cm from anastomosis to major airways	6 (67%)	5 (1–8)	0	4	2	0
C	Extending > 1 cm for anastomosis into lobar or segmental airways	2 (22%)	9 (1–14)	0	1	1	0
Extent							
A.	<50% circumferential ischemia	0		0	0	0	0
B.	50%–100% circumferential ischemia	0		0	0	0	0
C.	<50% circumferential necrosis	0		0	0	0	0
D.	>50–100% circumferential necrosis	9 (100%)	5 (1–14)	0	5	4	0
Dehiscence (D) (N = 5)			**2 (1–4)**	**4**	**0**	**0**	**1**
Location							
A.	Cartilaginous	2 (40%)	2 (1–3)	1	0	0	1
B.	Membranous	0		0	0	0	0
C.	Both	0		0	0	0	0
	Unknown	3 (60%)	2 (2–4)	3	0	0	0
Extent							
B.	>25–50% circumference	2 (60%)	3 (3–4)	1	0	0	1
	Unknown	3 (60%)	2 (1–2)	3	0	0	0
Stenosis (S) (N = 51)			**13 (1–630)**	**6**	**11**	**32**	**2**
Location							
A.	Anastomotic	41 (80%)	10 (1–467)	5	6	28	2
B.	Anastomotic plus lobar/segmental	4 (8%)	328 (4–630)	0	2	2	0
C.	Lobar/segmental only	6 (12%)	13 (2–35)	1	3	2	0
Extent							
A.	0%–25% reduction in cross-sectional area	1 (2%)	5	0	0	1	0
B.	>25%–50% reduction in cross-sectional area	8 (16%)	26 (2–467)	3	3	2	0
C.	>50%–100% reduction in cross-sectional area	40 (78%)	11 (2–630)	3	8	28	1
D.	100% obstruction	2 (4%)	4 (1–7)	0	0	1	1
Malacia (M) (N = 11)			**36 (9–340)**	**5**	**1**	**5**	**0**
A.	Perianastomotic – within 1 cm of anostomosis	4 (36%)	36 (25–280)	1	0	3	0
B.	Diffuse – involving anastomosis and extending beyond 1 cm	7 (64%)	44 (9–340)	4	1	2	0

E, Expectative treatment include debridement at most; C, conservative treatment consisting of balloon dilatation, incision, laser therapy, cryotherapy and mitomycin application; AS, airway stent; S, surgery.

DISCUSSION

In our retrospective cohort study investigating our entire 30 years' experience of lung transplantation, the incidence of clinically relevant AC post lung-transplant was 6.4% per anastomosis. No significant risk factors were identified of development of AC. However, occurrence of AC was associated with worse survival.

Incidence of AC was comparable to similar studies [8, 14, 17, 18] and surprisingly the incidence of AC did not decrease over time. Despite advances in surgical techniques, organ preservation and decrease in rejection. A recent study from the Vienna lung transplant center [24], which has a very high transplant volume, did show a low incidence of 1.56%. This indicates that experience might be beneficial, though the incidence of AC in our cohort was similar between experienced and less experienced surgeons.

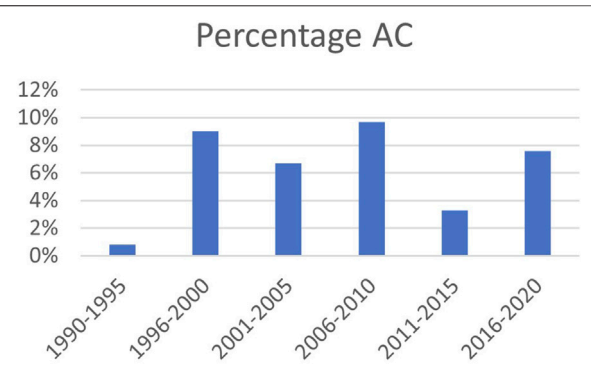


FIGURE 2 | Prevalence of AC over time; percentage of total number of anastomoses. AC: airway complication.

Another explanation can be the increased acceptance of recipients with more comorbidities and higher age. In our series, the median donor age increased from 30 years in the first 5 years of the program to 51 years in the most recent 5 years.

The AC have been scored according to the most recent ISHLT grading system [6]. What argues for the use of this grading is the rapid detection of necrosis. Necrosis often predisposes stenosis and malacia [6], and early recognition and debridement might prevent development of stenosis and malacia. However, structural recognition of early onset of necrosis asks for structural and periodic endobronchial inspection. This is not standard care in our- and most-institutions, which raises the question how feasible the classification systems are in day-to-day clinical setting. Given the fact that median detection of AC occurred at 12 weeks post-transplant and most AC have already been developed to a significant stenosis or malacia at the time of detection this is often after the period of standard periodic inspection. This is emphasized by the fact that all clinically significant cases of necrosis/ischemia had a 50%–100% circumferential necrosis. It is plausible that necrosis and ischemia occur much more frequently, but that this does not cause clinical complaints and may only become apparent after organization to stenosis.

Risk Factors Associated With Airway Complications

There are conflicting data on the relation between acute rejection and development of AC [11, 13, 17, 25]. In the cyclosporine era (1990–2001) a high number of patients were treated for acute rejection leading to 41% off all lung transplant patients treated in the first 30 days post transplantation. This is high compared to other studies [13, 17]. But did not result in more AC (p = 0.115).

TABLE 2 | Lung transplant donor related characteristics.

	All N = 651	AC N = 63	No AC N = 588	Sign
Donor age	46 11–78)	46 (18–71)	45 (11–78)	p = 0.896
Donor ventilation in days	2 (0–41)	2 (0–11)	2 (0–41)	p = 0.872
Donor height in centimeters	175 (120–196)	176 (158–190)	174 (120–196)	p = 0.281
Donor packyears	0 (0–50)	0 (0–25)	0 (0–50)	p = 0.693
Donation after circulatory death	139 (21%)	13 (21%)	126 (21%)	p = 0.884

AC, Airway complication. Continuous variables are expressed as median (range).

TABLE 3 | Lung transplant recipient related characteristics.

Recipient characteristics	All = 651	AC N = 63	No AC N = 588	Sign
Age lung transplant	52 (19–69)	50 (19–66)	52 (19–69)	p = 0.893
Male	328 (51%)	36 (57%)	292 (50%)	p = 0.264
Underlying disease:				
Obstructive pulmonary disease	301 (42%)	29 (43%)	282 (42%%)	p = 0.410
Infectious pulmonary disease	116 (18%)	10 (16%)	106 (18%)	
Vascular pulmonary disease	52 (8%)	2 (3%)	50 (9%)	
Insterstitial pulmonary disease	132 (20%)	16 (26%)	116 (18%)	
Other	44 (7%)	6 (10%)	38 (6%)	
Length (centimeter)	172 (149–198)	174 (160–194)	172 (149–198)	p = 0.914
Treatment for acute rejection first 30 days post-transplant	269 (41%)	31 (49%)	238 (40%)	p = 0.115
Any primary graft dysfunction in first 72 h	347 (53%)	34 (54%)	313 (53%)	p = 0.734
Time of ischemia of first implanted lung	314 (93–1,137)	313 (93–861)	314 (105–1,137)	p = 0.814
Time of ischemia second implanted lung (if applicable)	444 (158–1,271)	457 (158–989)	443 (197–1,271)	p = 0.877
ICU length of stay	6 (0–158)	6 (1–126)	6 (0–158)	p = 0.209
Time to extubation in days	2 (0–158)	2 (0–52)	2 (0–158)	p = 0.095
Size mismatch donor-recipient cm	−1 (-29–35)	−1 (-13–16)	1 (-29–35)	p = 0.980

AC, airway complication; ICU, intensive care unit. Continuous variables are expressed as median (range).

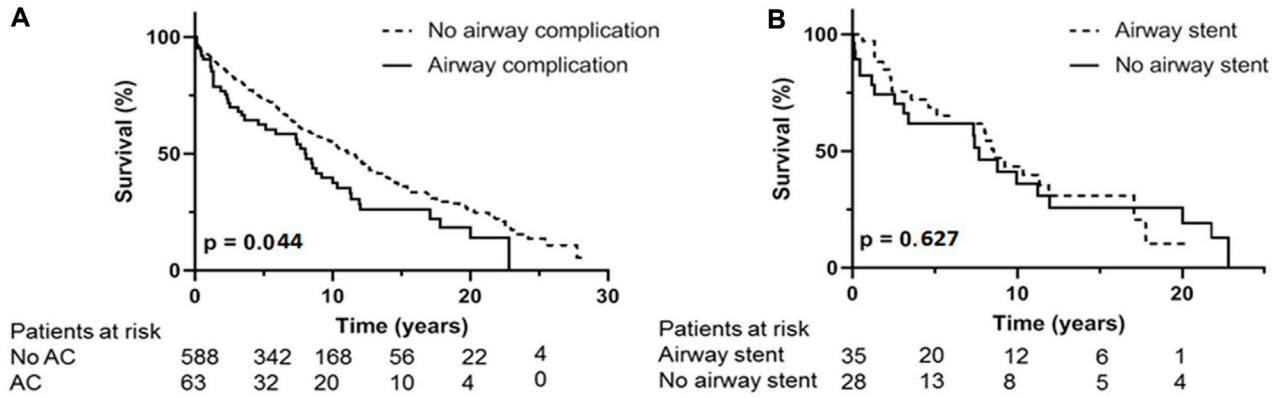


FIGURE 3 | (A): Kaplan Meijer estimates for survival. **(A)** occurrence of airway complication compared to no airway complication. **(B)** analysis within AC group, difference in survival between treatment with airway stent compared to treatment without with airway stent. AC: airway complication.

The median ischemic time in this study is 314 min for the first implanted lung and 444 min for the second if applicable. We did not find a relation between ischemia time and development of AC in contrast to recent studies. However, these are studies with average longer ischemia time compared to our cohort, ranging from 354 min for single and 516 for double lung transplantation [26, 27]. This study does not confirm previous reports that right sided anastomosis [9] is a risk factor for development of AC, previously attributed to bronchial artery anatomy. A possible explanation could be that in our center right lung implantation is preferably done first leading to a shorter time of ischemia, although this is common practice in most lung transplantation centers.

Findings in this study argue against previous studies that prolonged mechanical donor ventilation time is associated with higher incidence of AC [18]. Height mismatch between donor and recipient neither showed to be a risk factor for development of AC.

From the start of the transplant program, surgical technique has been the same with the end-to-end technique with separate single sutures for the membranous part with introduction of a running suture for the cartilaginous part after 2003. Therefore, no comparison can be made between surgical techniques. Heart-lung transplantations have been excluded because of the tracheal anastomosis and the possible decreased risk of development of AC attributed to collateral vessels from the coronary arteries [5].

In our institution we have a high percentage (31%) of DCD lungs since introduction in 2005 compared to other comparable studies [9]. Yet, this did not lead to a higher risk for development of AC. conform the aforementioned study [9].

Airway stent placement was required in 54% of the patients compared to 12%–44% in comparable studies [8, 18]. The high incidence of endobronchial stent placement strengthens the hypothesis of a more severe affected patient population. Traditionally, mainly SEMS are used [19] with silicone stents as alternative [28]. Endobronchial stent placement is associated with complications as sputum stasis, stent migration, in stent stenosis and infectious complications [29]. Recently, biodegradable stents have been developed and small case series have proven feasibility [30, 31]. Considering that the need for endobronchial stent is often temporary and stent removal is associated with possible complications [19, 28] the use of biodegradable stents could be a less invasive alternative.

This study showed significant impact on survival for patients affected with AC. With median survival significantly reduced from 136 to 101 months. This in contrast with data from previous studies [8, 14, 17] which show no influence on survival. Dehiscence seems to play an important role in this finding because when these cases are excluded median survival only shows a non-significant trend to worse survival (105 vs. 136 months, $p = 0.142$). It is known that dehiscence is associated with worse outcome [6, 24], though in previous literature these cases are systematically counted to the AC and are included in survival analyses.

Within the group of patients treated with a SEMS. Five patients died secondary to pulmonary infection with *Staphylococcus Aureus* and/or *Aspergillus fumigatus*. In 2009, Gottlieb et al. [22], already showed a strong association between SEMS and bacterial colonization and, in their analysis, there was a significant effect on survival. In this cohort, treatment with airway stent did not show impaired survival when compared to the AC group ($p = 0.489$) or the overall post lung-transplant cohort ($p = 0.200$). In the clinical practice, particularly these AC will be identified when symptoms as dyspnea and loss of lung function occur, and it is impossible to predict which patient will be affected without clear risk factors. Therefore, new developments of endobronchial treatment of AC are even more important, for instance with biodegradable airway stents to both avoid granulation and infection issues.

Limitations of this study are the retrospective character. Furthermore, the endobronchial treatment techniques have developed over the years with more treatment options. Nonetheless, this study provides a good reflection of the clinical reality and challenges associated with AC.

CONCLUSION

In our study we could not confirm any of the previous described risk factors for development of AC and found no new risk factors.

Assuming risk factors for development of airway complications are unclear, and therefore virtually impossible to influence. Future research should focus on improving treatment of AC, for example with pro-active bronchoscopic maintenance of the anastomosis region to avoid stent necessity and if biodegradable endobronchial stents prevent colonization at the stent site in comparison to SEMS.

DATA AVAILABILITY STATEMENT

Requests to access the datasets analyzed in this study should be directed to r.pel@erasmusmc.nl.

ETHICS STATEMENT

Patients gave written informed consent for transplant-related research and this analysis was approved by the local medical ethics committee (METc 2021.00408).

AUTHOR CONTRIBUTIONS

RvP, CG, and DJS contributed to the study design. CG, JG, WB, and DS contributed from the pulmonology perspective CW and ME contibuted from the cardiothoracic perspective. 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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Absolute Quantification of Donor-Derived Cell-Free DNA in Pediatric and Adult Patients After Heart Transplantation: A Prospective Study

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In this prospective study we investigated a cohort after heart transplantation with a novel PCR-based approach with focus on treated rejection. Blood samples were collected coincidentally to biopsies, and both absolute levels of dd-cfDNA and donor fraction were reported using digital PCR. 52 patients (11 children and 41 adults) were enrolled (NCT03477383, clinicaltrials.gov), and 557 plasma samples were analyzed. 13 treated rejection episodes >14 days after transplantation were observed in 7 patients. Donor fraction showed a median of 0.08% in the cohort and was significantly elevated during rejection (median 0.19%, $p < 0.0001$), using a cut-off of 0.1%, the sensitivity/specificity were 92%/56% (AUC ROC-curve: 0.78). Absolute levels of dd-cfDNA showed a median of 8.8 copies/mL and were significantly elevated during rejection (median 23, $p = 0.0001$). Using a cut-off of 7.5 copies/mL, the sensitivity/specificity were 92%/43% for donor fraction (AUC ROC-curve: 0.75). The results support the feasibility of this approach in analyzing dd-cfDNA after heart transplantation. The obtained values are well aligned with results from other trials. The possibility to quantify absolute levels adds important value to the differentiation between ongoing graft damage and quiescent situations.

Keywords: heart transplantation, rejection, prospective follow-up, cell free DNA, surveillance

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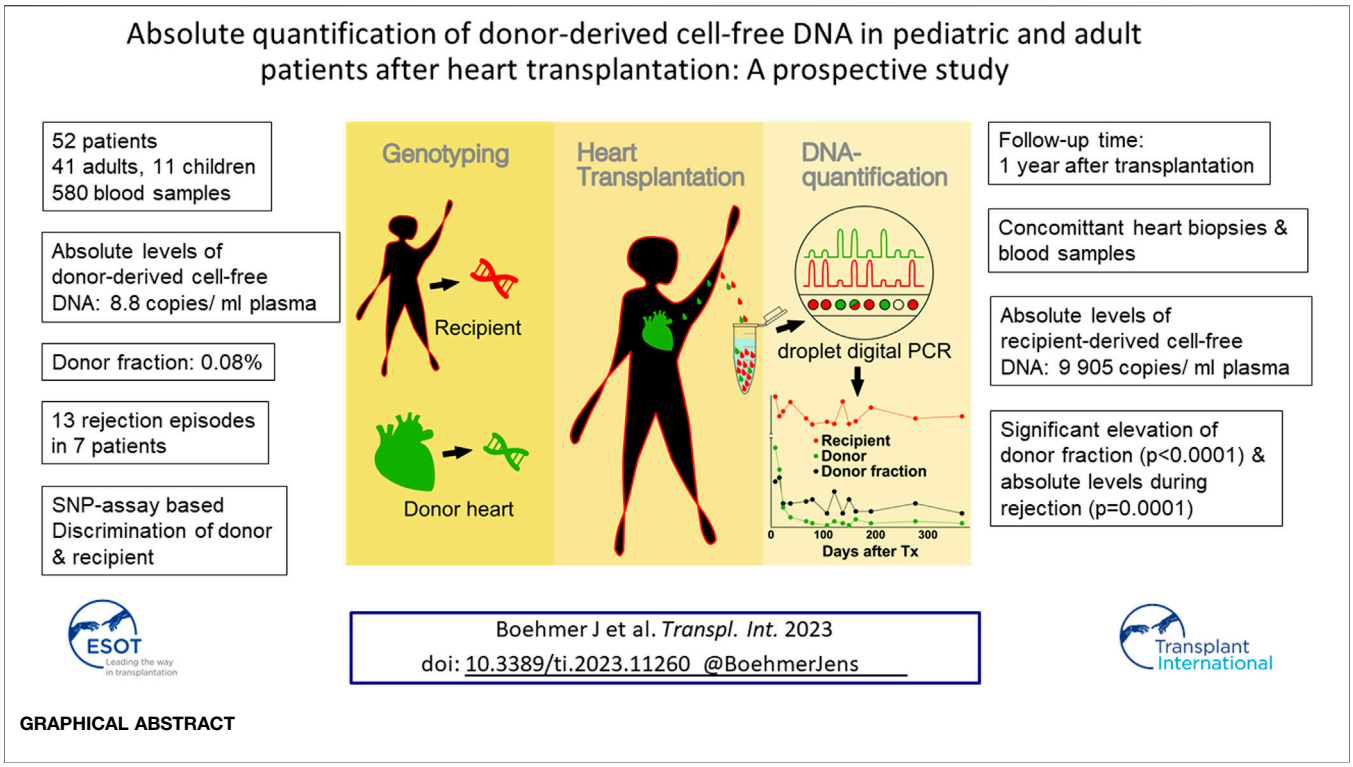
INTRODUCTION

Patients with advanced heart failure can undergo heart transplantation (HTx) as a definite treatment option. Acute and chronic rejection are major factors contributing to limited survival after HTx [1–4]. The diagnosis of rejection requires surveillance with endomyocardial biopsies and histopathological studies [5–7], which show a high interobserver-variability [8]. A less invasive

Abbreviations: cfDNA, cell-free; DNAdd, donor-derived; DF, donor fraction; dPCR, digital PCR; gDNA, genomic DNA; HTx, heart transplantation; NGS, next generation sequencing; NTC, no template control; PA, preamplification; PCR, polymerase chain reaction; qPCR, quantitative real-time; PCR rd, recipient-derived; SNP, single nucleotide polymorphism.



GRAPHICAL ABSTRACT

and less costly approach by reliable biomarkers is thus desirable, ideally with the possibility of timely diagnosis.

Cell-free DNA (cfDNA) is released into the bloodstream after cell apoptosis or necrosis and is mostly of hematopoietic origin [9–13]. Levels of cfDNA show a large inter- and intraindividual variability and vary between 0 and 5 ng/mL to >1,000 ng/mL; elevations are seen during both physiological and pathological situations (exercise, cancer, sepsis etc.) [14, 15]. Donor-derived cell-free DNA (dd-cfDNA) can be differentiated from recipient-derived cell-free DNA (rd-cfDNA) and has been correlated to rejection [16–19]. The quota of dd-cfDNA to total cfDNA, termed the donor fraction (DF), has been used for graft surveillance as the sole reported measure. Recent studies, however, have advertised the addition of absolute levels of dd-cfDNA for this purpose [20–22] considering not only DF, but also the high variability of rd-cfDNA. A steady state of 0.1% DF has been a consistent finding after HTx [18, 19, 23–25], which is the lowest among all solid organ transplantations [26–28]. The low abundance makes the use of highly sensitive quantification techniques mandatory. Digital PCR (dPCR) offers an absolute quantification of cfDNA in combination with quick turn-around time and high sensitivity [29–32].

In this non-interventional prospective cohort study, we describe a novel approach for the analysis of dd-cfDNA after HTx, using a technique including dPCR, and SNP (single nucleotide polymorphism) genotypes with target-specific preamplification. We aimed to establish a standardized protocol and then use the technique on patients after HTx. The primary objective of the study was to show if the use of donor fraction DF in HTx-patients can differentiate rejection from the absence of rejection, compared to the results of endomyocardial biopsies.

Secondary goals were: the investigation of absolute levels of dd-cfDNA with treated rejection events, differences between early and later samples after HTx as well as differences between female versus male recipients and adults versus children, respectively.

PATIENTS AND METHODS

Patient Recruitment

Patients were recruited from Sahlgrenska University Hospital, Gothenburg, Sweden as a part of the prospective BIODRAFT-trial (NCT03477383). All patients or caregivers provided informed consent and the study was approved by the institutional review board (no 014-16). Patients were eligible if they underwent HTx between 2016 and 2018. Blood samples were drawn coincidental to endomyocardial biopsies (EMB) during the first year after HTx. Access to donor blood samples was granted. Clinical data were extracted from medical records.

Sample Preparation

Blood was collected immediately before catheterization in 10 mL Cell-Free DNA® BCT (Streck, La Vista NE, USA). The samples were agitated for 10 s, shipped, and stored at room temperature for no longer than 7 days before plasma isolation. Plasma was separated from cells by centrifugation at 2,000 g for 15 min. The plasma fraction was transferred to a new tube followed by a second centrifugation at 16,000 g for 10 min. Centrifugations were performed at 20°C. The plasma was transferred to a

collection tube and frozen at −80°C. Genomic DNA was prepared from the leukocyte fraction of the blood samples using the DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany). cfDNA was extracted using the QIAamp® Circulating Nucleic Acid Kit (Qiagen) on the QIAvac 24 Plus vacuum manifold (Qiagen). cfDNA was eluted in 20 µL AVE buffer per ml plasma. Samples were stored at −20°C until analysis. Concentrations of cfDNA were quantified with the Qubit® 3.0 Fluorometer (Thermo Fisher Scientific, Waltham MA, USA), fragment sizes were analyzed with the 4200 TapeStation (Agilent technologies, Santa Clara CA, USA).

Discrimination of Donor and Recipient

35 previously published SNP assays [29] were selected for this study. For detection of the Y-chromosome the Human Y-Chromosome Specific Assay (TATAA Biocenter, Gothenburg, Sweden) targeting the TSPY1 gene was used. Probe and primer sets (Integrated DNA Technologies Inc., Carolville, IA, USA) were designed using HEX (Hexachlorofluorescin) and FAM (Carboxyfluorescin).

Genotyping of Recipient and Donor

Genomic DNA extracted from white blood cells was used. The donor was investigated with respect to the homozygous alleles found in the recipient. In sex mismatched HTx (female recipient, male donor), the Y-chromosome was used.

Target-Specific Preamplification

Preamplification using pooled primers for all 35 SNP and the Y-chromosome was conducted on cfDNA corresponding to 2 mL of patient plasma. 40 µL cfDNA, 45 µL Q5 Hot Start High-Fidelity 2x Master Mix (New England BioLabs, Ipswich, MA, USA), 3.6 µL primerpool (0.04 µmol) and 1.4 µL water were used in a total volume of 90 µL. Amplification was applied on a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA): 98°C for 3 min, followed by 10 cycles (98°C for 20 s, 63°C for 3 min and 72°C for 30 s). After the final extended (10 min) elongation step, the samples were stored at −20°C until analysis.

dPCR on Non-Amplified cfDNA

dPCR on non-amplified cfDNA was conducted on all patient samples using a targeted SNP assay. 10 µL of eluted patient cfDNA, corresponding to 0.5 mL of blood plasma, were used with 11 µL ddPCR Supermix for Probes (No dUTP) (Bio-Rad), 0.5 µL primer/probe-mix (900 nmol of each primer and 250 nmol of each probe) and 0.5 µL water in a total volume of 22 µL. Negative control with a no template control (NTC) with water was included as well as a positive control with a sample of genomic DNA (gDNA). Amplification was applied on a T100 Thermal Cycler (Bio-Rad): 95°C for 10 min, followed by 40 cycles (95°C for 30 s, 59°C/61°C for 1 min), 98°C for 10 min. Analysis was performed using the QX200 AutoDG Droplet Digital PCR System (Bio-Rad). Using negative and positive controls for cluster detection, manual fluorescent thresholds were placed. Analysis was conducted using QuantaSoft Analysis Pro v1.0 (Bio-Rad) to calculate absolute droplet counts as well as target DNA-concentration for rd-cfDNA. Target concentrations were expressed as copies per ml plasma.

dPCR on Target Specific Preamplified cfDNA (PA-dPCR)

dPCR on the preamplified cfDNA (PA-dPCR) was conducted using dilutions, based on the target concentrations of non-amplified cfDNA. All identified SNP were used. 10µL amplified cfDNA was diluted in purified water to acquire the desired concentration. All experiments were conducted as triplets, according to the protocol in 2.6. Target concentrations were expressed as copies per µl PCR-reaction, DF was calculated as dd-cfDNA/(rd-cfDNA + dd-cfDNA).

Calculation of cfDNA (cp/mL Plasma)

The initial dPCR is conducted on known concentrations directly corresponding to the amounts of isolated plasma (3 mL plasma is eluted in 60 µL, 4 mL plasma in 80 µL), see **Supplementary Figure S1**). This allows for the determination of absolute copy numbers for the recipient (cp/mL plasma). Using the donor fraction from the PA-PCR, by multiplying with absolute copy numbers for the recipient, total copy number for the donor is calculated (cp/mL plasma).

Determination of Assay Performances

The efficiency of target-specific preamplification was determined using a cfDNA control from normal donor plasma, in the range of 0.5–32 ng. Preamplification was performed in 30 µL reactions, using Q5 Hot Start High-Fidelity 1x Master Mix, 40 nM of each primer and template cfDNA at seven different concentrations (32, 16, 8, 4, 2, 1, and 0.5 ng/µL) in triplicate. The same amplification protocol was used as above. After the final extended (10 min) elongation step, the samples were immediately frozen on dry ice, slowly thawed on ice, diluted 1: 20 in 1x TE buffer (ThermoFisher Scientific, Waltham, MA, USA) and stored at −20°C until analysis. qPCR (real-time quantitative PCR) was performed in 10 µL reactions utilizing 1x TATAA SYBR GrandMaster Mix (TATAA Biocenter) with 400 nM of each primer (Integrated DNA Technologies) and 2 µL diluted preamplification product as template. qPCR was performed in triplicates using the CFX384 Touch Real-Time PCR Detection System (Bio-Rad): 95°C for 10 min, followed by 50 cycles of amplification (95°C for 15 s and 60°C for 1 min). Melting curve analysis was performed in the range of 65°C to 95°C, 0.5°C per 5 s increments. Cycles of quantification (Cq) values were determined by the second derivative maximum method. Limit of blank (LOB), limit of detection (LOD) and limit of quantification (LOQ) were defined according to Armbruster et al [33] and determined as published by our group [34]: LOB 0.016% DF, LOD 0.055% DF, with LOQ = LOD.

Statistics

Quantification of cfDNA in the dPCR-experiments is embedded in the software Quantasoft Pro (Bio-Rad) [35]. Continuous data are reported as means with standard deviation (SD) or as medians with interquartile range (IQR, q1-q3). Range is also reported when appropriate. In the boxplots, the horizontal line represents the median value, the top and bottom of each box show the upper and lower limit of the IQR, and the whiskers represent the range.

Receiver-operating characteristic (ROC) curves were used to assess the sensitivity and specificity of DF and dd-cfDNA to predict treated rejection. Correlations (Pearson and Spearman) report the correlation coefficient r, CI_{95} of r and R^2. Early (7–14 days) and late (>14 days) samples were compared using a linear mixed effects (LME) model. Other groups were also compared using a LME model, with adjustment for days from transplantation. A two-tailed p-value of <0.05 was considered statistically significant. The LME models were computed using R Statistical Software (v4.3.1) [36]. All other statistical analysis was performed using the GraphPad Prism Software (GraphPad Software Inc., version 10.0.2, GraphPad Software, Boston, Massachusetts USA).

RESULTS

Study Population

The 52 patients generated 580 venous samples during their 1 year follow-up. 23 samples were excluded (hemolytic sample, too little plasma yield, high technical error rate, too few droplets generated). The population consisted of 41 adults and 11 children who were aged 1–68 years, (median 52.5), 69% of patients were male, and the median BMI was 24.9. The indication for transplant was dilated cardiomyopathy in 58%, and ventricular assist device was used in 29%. Median donor age was 49.5 years, median donor BMI was 23.8, median donor heart ischemic time was 183.5 min. More detailed patient and donor specifications can be seen in the **Supplementary Tables S1, S2**. Median time to first biopsy was 10.5 days (IQR 9–12). Blood samples taken during the first 14 days after HTx (n = 48) were analyzed by dPCR but excluded from general statistical analysis except for when otherwise stated.

Study Protocol and General Results

The detailed workflow and calculation of cfDNA-levels from recipient, donor and DF can be seen in **Supplementary Figure S1**. After sample collection, cfDNA was extracted within a time frame of 7 days [37]. cfDNA-analysis was conducted on bundled samples after the patients had left the study. Results were available with 48 h. A mean of 4 mL plasma was obtained from each sample (SD 0.57, range 1.25–5.70). The median for the fluorometrically determined cfDNA-concentration was 34.20 ng/mL plasma (range 5.16–2,856, IQR 20.45–57.60). The 509 samples showed a median of 9,905 copies/mL plasma rd-cfDNA concentration (range 1,245-219,754, IQR 5,137-17,596), the median for dd-cfDNA was 9.31 copies/mL plasma (range 0.43–348.5, IQR 5.06–21.71, mean 18.9). Donor fraction showed a median of 0.09% (range 0.003–3.34, IQR 0.05–0.21). See **Supplementary Figure S2**.

Rejection and Levels of cfDNA

Of the 557 samples, 48 samples were excluded as early samples (<15 days) and 18 due to reasons that impaired interpretation (malignancy, severe infections). This resulted in 491 samples that were suitable for evaluation. Acute reaction was seen in 13 biopsy-matched samples from 7 patients, see **Figure 1**. One sample showed rd-cfDNA levels of 220,000 copies/mL, >20 times the median (see Study Protocol and General Results), falsely lowering the DF, and was thus excluded from analysis. Median levels were significantly higher during rejection episodes: absolute levels of dd-cfDNA showed a median of 23 copies/mL (IQR 10.6–49.8) during rejection compared to 8.8 (IQR 4.7–19.8) during quiescence (p = 0.0001). Using a cut-off of 7.5 copies/mL, sensitivity was 92% and specificity was 43% (AUC 0.75; 95% CI 0.63–0.87, PPV = 0.04, NPV = 0.99). DF was also elevated in rejection, with a median level was 0.19 (IQR 0.13–0.56) compared to 0.08 (IQR 0.05–0.19) during quiescence (p < 0.0001). Using a cut-off of 0.1%, sensitivity was 92% and specificity was 56% (AUC 0.78, 95% CI 0.68–0.88, PPV = 0.05, NPV = 0.99). See **Figure 2** and **Supplementary Figure S7**.

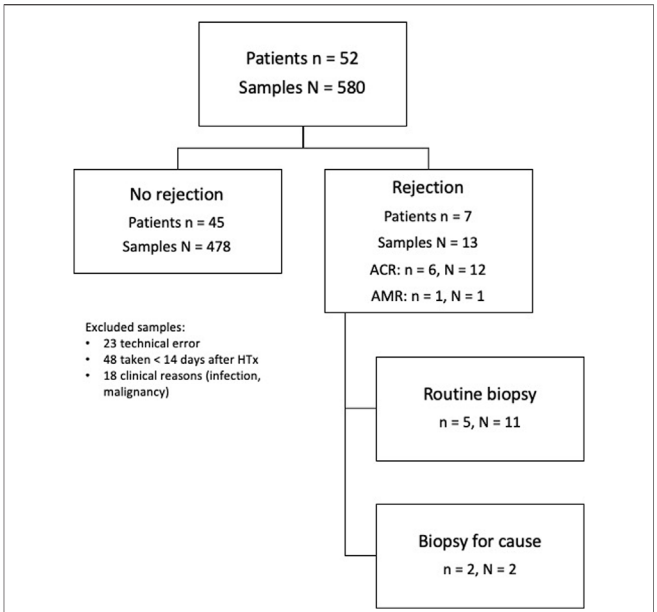


FIGURE 1 | Study flow chart. ACR, acute cellular rejection; AMR, antibody-mediated rejection; HTx, heart transplantation.

Group Comparisons

Early samples (day 7–14, n = 48) were compared to later samples (day 15–400, n = 509). The early samples showed significantly higher levels (p < 0.0001) for both rd-cfDNA (median 18,135 versus 9,905 copies/mL), dd-cfDNA (median 48.7 versus 9.3 copies/mL) and DF (median 0.31% versus 0.09%), see **Supplementary Figure S3**.

Samples from adults (n = 475) were compared to samples from children (n = 82), including early samples. Levels of rd-cfDNA did not differ significantly (adults median 11,091 copies/mL versus 7,177, p = 0.053). Levels of dd-cfDNA did not differ significantly (adults median 11.0 copies/mL versus children 9.8, p = 0.99). DF was significantly lower in adults (median 0.09 versus 0.13, p = 0.03), see **Supplementary Figure S4**.

Female patient samples (n = 171) were compared to samples from male patients (n = 386). As compared with females, males had significantly lower levels of rd-cfDNA

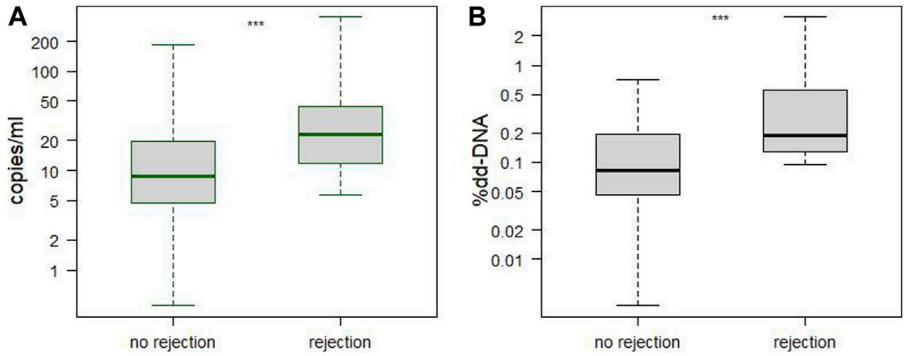


FIGURE 2 | Comparison of samples taken during episodes of acute rejection versus no rejection. Panel **(A)** shows absolute levels of dd-cfDNA in copies/mL, Panel **(B)** shows DF in percent. Dd-cfDNA was elevated in rejection (median 23.0, IQR 10.6–49.8) versus no rejection (median 8.9, IQR 4.7–20.3), $p = 0.0001$. DF was also elevated in rejection (median 0.19, IQR 0.13–0.56) versus no rejection (median 0.08, IQR 0.05–0.19), $p < 0.0001$. No rejection: N = 45 patients, $n = 479$ samples; rejection: N = 7, $n = 13$. cfDNA, cell-free DNA; dd-cfDNA, donor-derived cfDNA; DF, donor fraction; IQR, interquartile range; rd-cfDNA, recipient-derived cfDNA.

(median 8,888 copies/mL versus 15,943, $p = 0.0004$). There were no significant differences for dd-cfDNA (males median 9.8 copies/mL versus 13.2, $p = 0.13$) and DF (males median 0.12% versus 0.09%, $p = 0.08$), see **Supplementary Figure S5**.

Correlations and Validation of Assay Performance

The rd-cfDNA results of the initial dPCR (copies/mL) and the PA-dPCR (copies/µL) were compared including results from samples taken during the first 14 days after HTx ($n = 557$). The results showed a very high correlation (Pearson r = 0.97, CI_{95} (0.97; 0.98), $R^2 = 0.95$, $p < 0.0001$; Spearman r = 0.95, CI_{95} (0.94; 0.96), $p < 0.0001$). The levels of rd-cfDNA from PA-dPCR were also correlated with the fluoroscopic measurements of DNA-concentration in the initial plasma samples using Qubit. The results showed a very high correlation (Pearson r = 0.95, CI_{95} (0.94; 0.95), $R^2 = 0.90$, $p < 0.0001$; Spearman r = 0.93, CI_{95} (0.92; 0.94), $p < 0.0001$).

The efficiency of preamplification was determined using a cfDNA standard and qPCR to monitor individual SNP assays as previously published by our group (XX). The qPCR profiles for all SNP assays including the Y-chromosome are seen in **Supplementary Figure S6**. No changes in allelic distribution for the SNP assays could be detected within the range of cfDNA concentrations.

Patient Examples

Patient 1: A 27 year-old patient underwent uneventful HTx. The clinical course was unremarkable except for suspected bacterial infection on day 3 and day 20 as well as Influenza A infection on day 270. A total of 11 scheduled endomyocardial biopsies were obtained, none of which showed signs of rejection warranting treatment. The results are shown in **Figure 3**.

Patient 2: A 28 year-old patient underwent uneventful HTx. Scheduled routine biopsy on day 137 showed a 3R rejection and

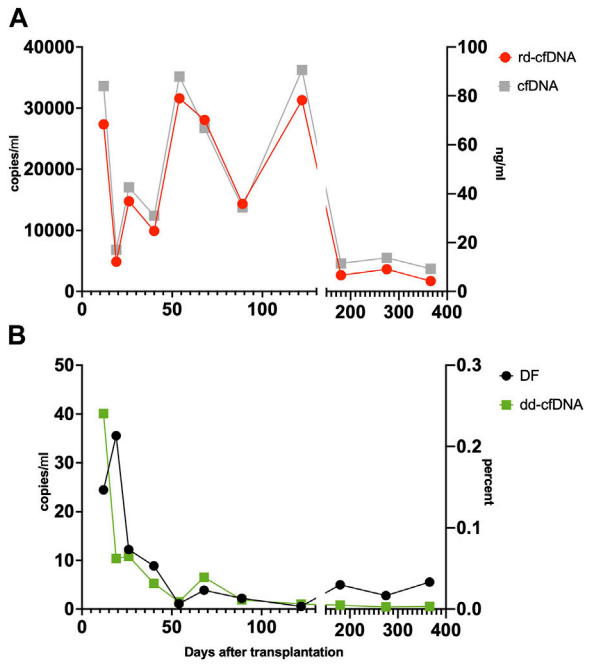


FIGURE 3 | Patient 1. Time course of rd-cfDNA and cfDNA-concentration (Panel **(A)**) as well as dd-cfDNA and DF (Panel **(B)**). rd-cfDNA and dd-cfDNA results are displayed on the left Y-axis, respectively, expressed in copies/mL. DF is expressed in percent, on the right lower Y-axis. Results of rd-cfDNA, dd-cfDNA and DF are based on three SNP [16, 21, 25] displayed as means. Results of cfDNA are the fluoroscopic measurements of patient plasma (Qubit), expressed in ng/mL on the right upper Y-axis. The time course of this rejection-free patient shows large variation of rd-cfDNA without clinical correlates. Dd-cfDNA declines after HTx and remains low. The quick fall of rd-cfDNA leads to a rise in DF in the second sample, despite absence of rejection. There is a very close relation between the fluoroscopically determined DNA-concentration and the results for rd-cfDNA as determined by dPCR. cfDNA, cell-free DNA; dd-cfDNA, donor-derived cfDNA; dPCR, digital PCR; DF, donor fraction; rd-cfDNA, recipient-derived cfDNA.

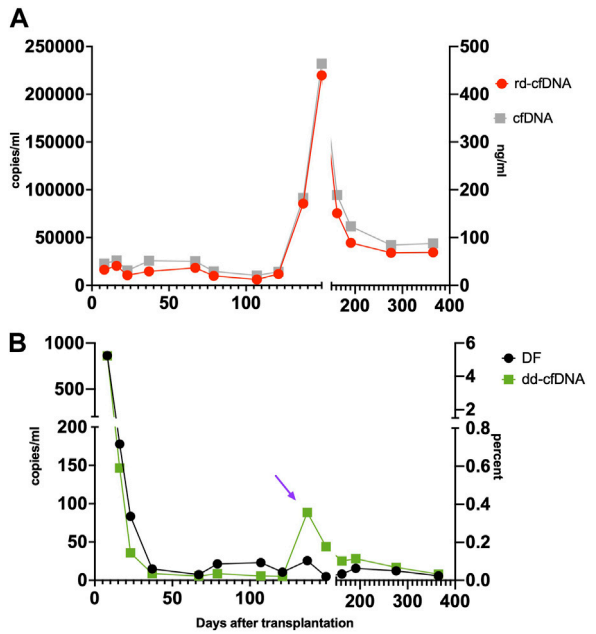


FIGURE 4 | Patient 2. Time course of rd-cfDNA and cfDNA-concentration (Panel **(A)**) as well as dd-cfDNA and DF (Panel **(B)**). The time point of a treated rejection (IHLT-2R) as warranted by the biopsy is marked with a purple arrow. rd-cfDNA and dd-cfDNA results are displayed on the left Y-axis, respectively, expressed in copies/mL. DF is expressed in percent, on the right lower Y-axis. Results of rd-cfDNA, dd-cfDNA and DF are based on three SNP [14, 16, 25], displayed as means. Results of cfDNA are the fluoroscopic measurements of patient plasma, expressed in ng/mL on the right upper Y-axis. The rejection is reflected by a marked rise in dd-cfDNA, as well as treatment response by a decline. This is, however, masked by the coincidental rise in rd-cfDNA, which thus prevents DF to rise significantly. cfDNA, cell-free DNA; dd-cfDNA, donor-derived cfDNA; DF, donor fraction; rd-cfDNA, recipient-derived cfDNA.

treatment was initiated. Further biopsies revealed resolution of the rejection. Infection episodes occurred on day 4 and day 94 (respiratory infections), day 125 (infection with enterovirus) and day 164 (urinary tract infection). The results are shown in **Figure 4**.

DISCUSSION

In the present study, a median DF levels of 0.09% is well aligned with that observed in previous studies in the field. Special attention should be given to the fact that the results are comparable despite the different technical approaches such as sequencing [24], massive multiplexed PCR [19], and PCR with a preselected SNP-set [38, 39], as used in our study. As has been shown before, DF is significantly higher in samples taken coincidental with biopsies showing acute rejection. Even absolute values of dd-cfDNA are significantly higher during rejection, a result shown in kidney transplantation [20–22], and in HTx recipients as shown by Kim et al. [25], even though Kim et al. did not reveal how absolute levels were quantified. It must be noted that absolute levels of cfDNA in our cohort showed very high intra- and interpatient variability (up to the factor 180), and the influence of this on DF is well illustrated in **Figure 3**: On the second sample, DF rises, and rejection can be suspected. This can, however, be explained by the kinetics of the rapid decline of rd-cfDNA compared to the modest decline in dd-cfDNA. Clinically, the peaks seen in some patients could be correlated to infection and bleeding, but sometimes no obvious reason could be found. Also, the differences seen between male and female patients regarding rd-cfDNA levels remain to be explained. Similarly, large variations in rd-cfDNA after transplantation have been noted by others [21]. Interestingly, total levels of dd-cfDNA seem to correlate as well between the different studies, and also between the different organs: Even though no range or IQR is given, Kim et al. propose a threshold of 13 copies/mL as being superior to DF in diagnosing rejection, which can be compared to the median of 9.3 copies/mL in our study. In stable kidney transplants, the dd-cfDNA levels showed a median of 25 copies/mL [20], which, however, must be viewed on the background of the higher median for DF (0.29%). Biologically, this similarity seems logical, given the similar organ sizes of heart and kidney (both around 300 g in males) and reflecting the higher cell-turn around in kidneys compared to the heart.

The technical robustness of our approach is supported by two findings: the comparison of our results to other studies and the very high correlation when comparing total cfDNA-concentration measured by Qubit with rd-cfDNA results from PA-dPCR. Even the very high correlation between rd-cfDNA levels from the first and second dPCR supports this assumption.

In our opinion, technical approaches solely reporting DF may be unreliable as variations in rd-cfDNA are not being accounted for. For example, an increase in dd-cfDNA, suggesting acute organ rejection, may not be detected if rd-cfDNA is simultaneously increased. This is clearly seen in **Figure 4**, where the high levels of rd-cfDNA mask the ongoing rejection if only focusing on DF. Contrary to just delivering DF, the method described by our group allows to separately monitor rd- and dd-cfDNA. This approach has been postulated in kidney transplantation with promising results [20, 21]. In a recent review [40], the authors stressed the advantages of quantifying absolute levels of dd-cfDNA to overcome the large variability of rd-cfDNA.

The use of target-specific preamplification enables the repeated analysis of multiple targets and has been thoroughly discussed by Jackson and Andersson [41, 42]. Quantifying DNA always introduces the problem that assays can have different efficiencies. Using several, averaged assays is one way of minimizing this technical challenge. The different and complex technical approaches are one of the reasons why a systematic review on the use of cfDNA after organ transplantation [28], initially planned to perform a diagnostic test accuracy (DTA) meta-analysis, failed to do so.

In conclusion, we established a robust and fast method to quantify cell-free DNA as an indicator of rejection in cardiac recipients, which is less invasive and less costly than endomyocardial biopsy. The results from 52 patients, for

whom DF was measured repeatedly, are in concordance with previous studies. Our method also allows for the measurement of cfDNA from the recipient and the donor, separately, providing more information than DF alone. Thus, our technique can be a promising tool for rejection-surveillance after HTx. Its usefulness will be examined by the BIODRAFT-trial (NCT03477383) comparing cfDNA-levels to clinical data of the patient cohort.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by Swedish Ethical Review Authority, Gothenburg, Sweden. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JB: Conceptualization and conduction of study, development of the methodology, project administration, funding acquisition, formal and statistical analysis, data curation, visualization, writing of the original draft. CW: Conceptualization and conduction of study, development of the methodology, investigation of the samples, visualization, review and editing of the draft. DA: development of the methodology, review and editing of the draft. AS: development of the methodology, review and editing of the draft. MJ: conduction of study, development of the methodology, investigation of the samples, review and editing of the draft. HW: Conceptualization and conduction of study, formal and statistical analysis, review and editing of the draft. KK: Conduction of study, formal and statistical analysis, writing of the original draft. JS: Conceptualization of the study, project administration, funding acquisition, review and editing of the draft. JA: Development of the methodology, review and editing of the draft. GD: Conceptualization and conduction of study, project administration, funding acquisition, data curation, visualization, writing of the original draft. AR: Conceptualization and conduction of study, development of the methodology, investigation of the samples, project administration, funding acquisition, data curation, visualization, writing of the original draft. SN: formal and statistical analysis, data curation, visualization, reviewing and editing of the draft.

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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.2023.11260/full#supplementary-material

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ORIGINAL RESEARCH
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Impact of Previous Conventional Cardiac Surgery on the Clinical Outcomes After Heart Transplantation

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The impact of the type, purpose, and timing of prior surgery on heart transplantation (HT) remains unclear. This study investigated the influence of conventional cardiac surgery (PCCS) on HT outcomes. This study analyzed HTs performed between 1999 and 2019 at a single institution. Patients were categorized into two groups: those with and without PCCS. Short-term outcomes, including post-transplant complications and mortality rates, were evaluated. Cox proportional and Kaplan–Meier survival analyses were used to identify risk factors for mortality and assess long-term survival, respectively. Of 368 patients, 29% had PCCS. Patients with PCCS had a higher incidence of post-transplant complications. The in-hospital and 1 year mortality rates were higher in the PCCS group. PCCS and cardiopulmonary bypass time were significant risk factors for 1 year mortality (hazard ratios = 2.485 and 1.005, respectively). The long-term survival rates were lower in the PCCS group, particularly in the first year. In sub-analysis, patients with ischemic cardiomyopathy and PCCS had the poorest outcomes. The era of surgery and timing of PCCS in relation to HT did not significantly impact outcomes. In conclusion, PCCS worsen the HT outcomes, especially in patients with ischemic etiology. However, the timing of PCCS and era of HT did not significantly affect this concern.

Keywords: heart transplantation, previous cardiac surgery, survival, resternotomy, ventricular assist device

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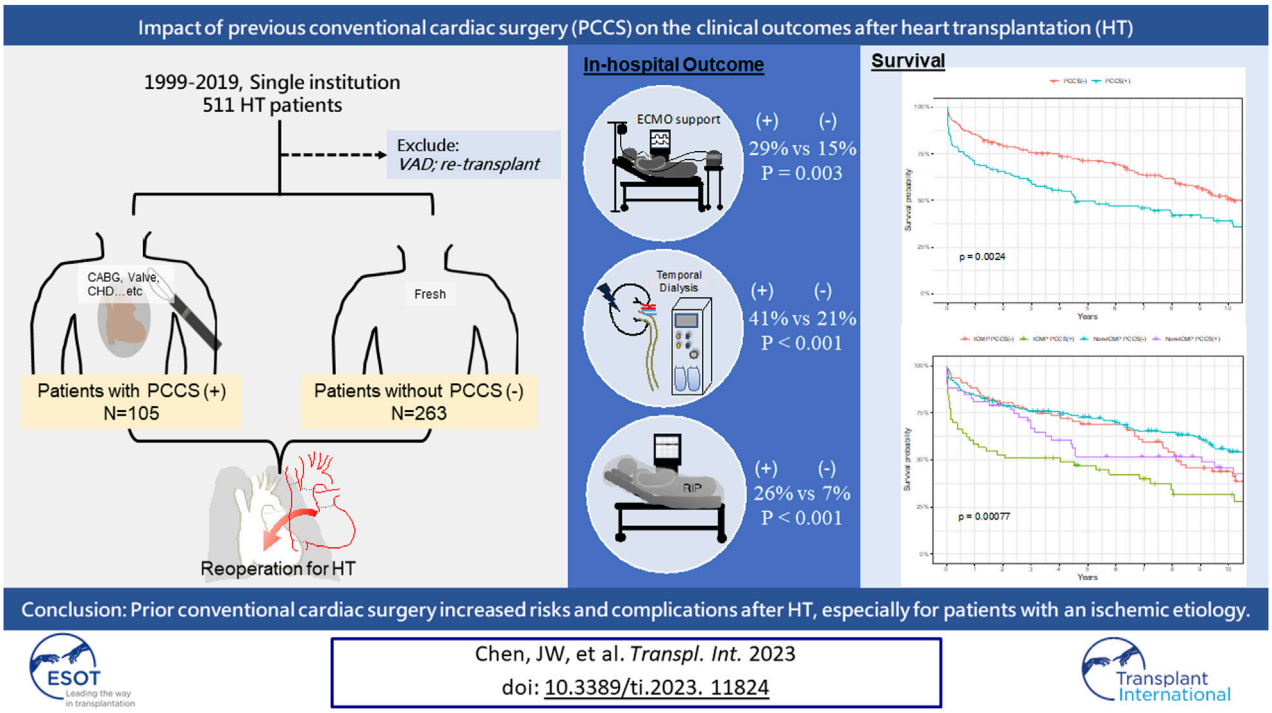
INTRODUCTION

Previous cardiac surgery is a well-known risk factor for increased morbidity and mortality after heart transplantation (HT) [1–5]. Re-sternotomy prolonged the duration of cardiopulmonary bypass (CPB), thereby increasing post-transplant complications, such as coagulopathy, bleeding, infection, acute kidney injury, and acute rejection [3, 6]. Before transplantation, high-risk conventional cardiac surgery cannot be completely avoided, as it still serves as an alternative strategy in cases where the organ is unavailable [7]. However, with advancements in ventricular assist devices (VADs), up to 45% of HTs are performed in recipients who have received mechanical circulatory support before transplantation, and the outcomes have been satisfactory [8–10]. Although VAD implantation also

Abbreviations: CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass; ECMO, extracorporeal membrane oxygenation; HT, heart transplantation; ICM, ischemic cardiomyopathy; PCCS, previous conventional cardiac surgery; UNOS, United Network for Organ Sharing; VAD, ventricular assist device.



GRAPHICAL ABSTRACT

requires an open chest and increases the complexity of subsequent HT, its benefits can mitigate the negative impact of re-sternotomy [11–14]. Moreover, studies have shown that patients who underwent conventional cardiac surgery and subsequently received VAD implantation before proceeding to HT had comparable survival outcomes to those who underwent their first cardiac surgery during HT [13]. With the increased durability of VADs, it remains uncertain whether critically ill patients with heart failure require life-saving conventional cardiac surgery and which type of surgery is warranted. Furthermore, it is unclear whether the impact of prior cardiac surgery on HT has changed, given advancements in perioperative care and growing experience with re-sternotomy. This study exclusively focused on investigating the impact of different types, timings, and operative eras of previous conventional cardiac surgery (PCCS) on the outcomes of patients undergoing HT within our hospital.

PATIENTS AND METHODS

All HTs performed between January 1999 and December 2019 at the National Taiwan University Hospital were included in the study. This study was approved by our Institutional Review Board, and the requirement for informed consent was waived (202208017RINB). Data were collected through a retrospective chart review of a prospectively observed patient cohort. Our hospital conducted the initial HT in 1987, followed by the first VAD implantation as a bridge to HT in 1997. Taiwan's national health insurance has covered paracorporeal VAD since 2011 and intracorporeal durable VAD since 2018. In this study, we excluded patients who were bridged to HT with a VAD and those who underwent heart re-transplantation because of allograft dysfunction (**Figure 1**). Other cardiac surgeries, apart from those stated earlier, were recognized as conventional cardiac surgeries. The rationale behind this exclusion is that VADs serve as alternative tools to stabilize patients and potentially improve the outcome of HT, which introduces selection bias [8, 9]. Furthermore, re-transplantation for allograft dysfunction is known to have a poor prognosis because of immune sensitization [15, 16]. All patients were categorized into "with PCCS" and "without PCCS," based on whether they had undergone conventional cardiac surgery before HT. The primary outcomes assessed were the short- and long-term survival rates. Secondary outcomes included postoperative morbidities, such as re-exploration or delayed sternum closure, renal dialysis, early bloodstream infection (within 30 days), and post-transplant hospital stay. The etiology of heart failure and the type of cardiac surgery are highly associated. To evaluate the impact of the initial operation, both groups were further divided into two subgroups based on the purpose of surgery: PCCS for ischemic cardiomyopathy (ICM) and non-ICM. Patients were divided into two subgroups to investigate the timing of PCCS in relation to HT: over 2 years or within 2 years, depending on the patient distribution. Additionally, both groups were divided into two subgroups based on the year of HT in our hospital (1999–2009 and 2010–2019) to examine the impact of the new

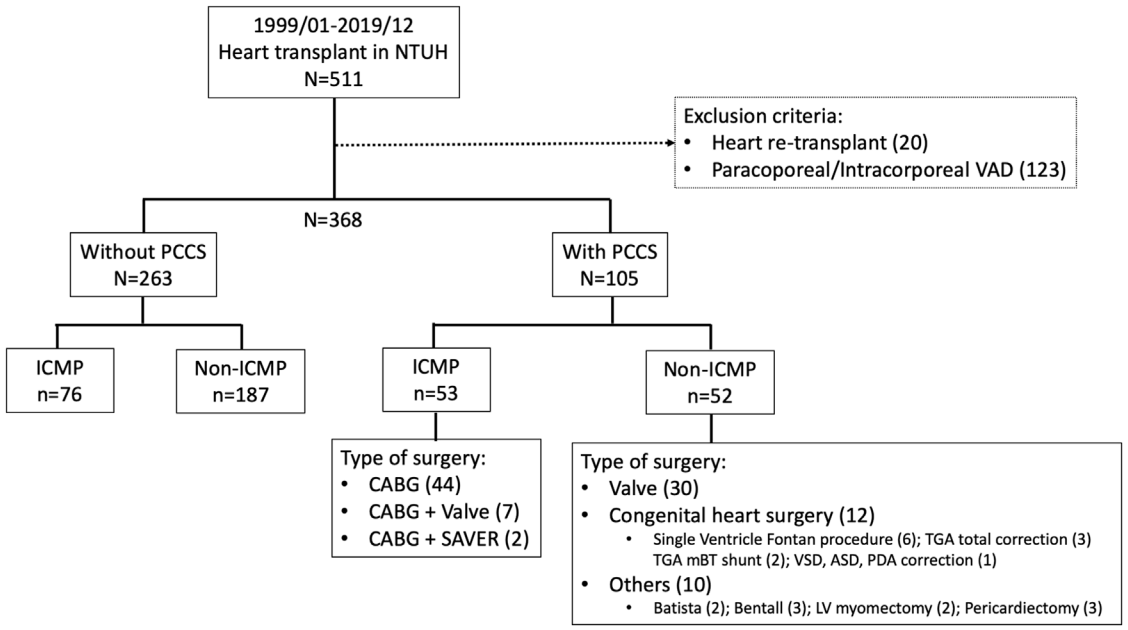


FIGURE 1 | Details regarding the group and type of previous conventional cardiac surgery in study patients. CABG, coronary artery bypass surgery; ICMP, ischemic cardiomyopathy; PCCS, previous conventional heart surgery; SAVER, surgical anterior ventricular endocardial restoration.

era compared with the old era. While the allocation system in the United States was expanded in 2018 to include seven statuses, designed to address the diverse situations of VAD-supported HT candidates, our study retained the prior allocation framework. We specifically focused on United Network for Organ Sharing (UNOS) statuses 1A, 1B, and 2, as we excluded VAD patients from our study.

Management of Patients With PCCS Receiving HT

When enrolling patients who have undergone PCCS and are currently receiving HT, several important points need to be considered. We routinely performed pericardial closure during the initial surgery or used anti-adhesive patches when pericardial tissue was insufficient. Preoperative CT for re-sternotomy risk assessment was always performed. Although it may not be possible to cease anticoagulation and antiplatelet medications before HT owing to the unpredictable timing of organ availability [17], we promptly evaluated the candidate's medication profile and initially suspended any potentially harmful drugs. As a preparatory measure before re-sternotomy, we routinely exposed the femoral artery and vein as an emergency route for CPB setup. To avoid the need for emergent CPB, it is crucial to allow an adequate amount of time for the surgeon to perform dissection. Continuous communication between the donor organ harvest team and the recipient preparation team is necessary to minimize CPB and allograft ischemia time. After confirming the suitability of the donor heart, the recipient team performed re-sternotomy. In urgent situations such as unexpected bleeding or changes in the donor's condition, rapid CPB is established via femoral access for quick heart decompression and re-entry. After surgery, sternal closure may be delayed for 24 h if adequate hemostasis is not achieved. Furthermore, immunosuppressant and desensitization protocols were followed as usual, based on previous publications [18–21].

Statistical Analysis

All statistical analyses were performed using the R software (version 4.1.0; R Foundation for Statistical Computing, Vienna, Austria). For descriptive statistics, means and standard deviations were calculated for nonparametric data. For categorical variables, numbers and frequencies were described. Mann–Whitney U, Fisher's exact, and chi-square tests were used to compare the clinical characteristics and outcomes between patients with and without PCCS. Cox proportional analysis was used to identify independent factors associated with 1 year mortality and included all significant predictors in the multivariate analysis, with a p-value <0.05. Survival curves were plotted using Kaplan–Meier analysis; survival rates between patients with and without PCCS were compared using the log-rank test, and p-values <0.05 were considered statistically significant.

RESULTS

Patient Characteristics

A total of 368 patients were included in this study, of whom 105 (29%) had PCCS and 263 (71%) did not. In the PCCS group, 53 patients (50%) underwent surgery for ICM and 52 (50%) for non-ICM. The range of timing between prior cardiac surgery and

TABLE 1 | Demographic data of recipients with and without PCCS before heart transplantation.

Variate	Overall	Without PCCS	With PCCS	*p*-value
N (%) or mean (±SD)	**N = 368**	**N = 263**	**N = 105**	
Age	45.9 (±16.7)	45.3 (±16.2)	47.5 (±17.9)	0.036
Sex, female	58 (15.8%)	37 (14.1%)	21 (20%)	0.160
Body weight (kg)	62.36 (±17.60)	63.96 (±17.33)	58.34 (±17.70)	0.002
Blood type				0.480
O	101 (27.5%)	67 (25.5%)	34 (32.4%)	
A	121 (32.9%)	86 (32.7%)	35 (33.3%)	
B	109 (29.6%)	83 (31.6%)	26 (24.8%)	
AB	37 (10.1%)	27 (10.3%)	10 (9.5%)	
Etiology				<0.0001
Congenital heart disease	18 (4.9%)	4 (1.5%)	14 (13.3%)	
Ischemic cardiomyopathy	129 (35.1%)	76 (28.9%)	53 (50.5%)	
Dilated cardiomyopathy	184 (50%)	171 (65%)	13 (12.4%)	
Restrictive cardiomyopathy	10 (2.7%)	8 (3%)	2 (1.9%)	
Valvular heart disease	23 (6.3%)	3 (1.1%)	20 (19.1%)	
Others	4 (1.1%)	1 (0.4%)	3 (2.9%)	
UNOS status				0.590
1A	85 (23.1%)	57 (21.7%)	28 (26.7%)	
1B	94 (25.5%)	68 (25.9%)	26 (24.8%)	
2	189 (51.4%)	138 (52.5%)	51 (48.6%)	
Cardiopulmonary resuscitation history	42 (11%)	24 (9%)	18 (17%)	0.044
Pretransplant support				
Ventilator	63 (17%)	39 (15%)	24 (23%)	0.068
IABP	61 (17%)	44 (17%)	17 (16%)	1
ECMO	48 (13%)	28 (11%)	20 (19%)	0.039
Renal dialysis	37 (10%)	23 (9%)	14 (13%)	0.190
Diabetes mellitus	92 (25%)	63 (24%)	29 (28%)	0.510
Hyperlipidemia	80 (22%)	51 (19%)	29 (28%)	0.084
Creatinine	1.4 (±0.8)	1.4 (±0.8)	1.4 (±0.8)	0.880
BUN	30.1 (±17.6)	29.8 (±16.5)	31.0 (±20)	0.980
T-Bil	2.2 (±3.2)	2.3 (±3.5)	2.0 (±2.2)	0.170
In-hospital waiting, days	14.4 (±28.8)	13.1 (±24.0)	17.9 (±38.5)	0.720
Donor				
Age	35.0 (±14.0)	35.5 (±13.9)	33.8 (±14.4)	0.290
Sex, female	106 (29%)	76 (29%)	30 (29%)	1.000
Body weight (kg)	65.0 (±34.6)	63.7 (±14.9)	68.2 (±60.2)	0.640
Allograft ischemia time (min)	161.4 (±63.4)	160.6 (±61.9)	163.39 (±67.4)	0.910
Cardiopulmonary bypass time (min)	155.1 (±66.8)	136.1 (±49.3)	202.3 (±80.2)	<0.0001

PCCS, previous conventional cardiac surgery; IABP, intra-aortic balloon pump; VAD, ventricular assist device.

HT in patients with PCCS varied from 1 week to 44 years, with a median of 45 weeks (IQR 10–132 weeks). Detailed information on the PCCS type is shown in **Figure 1**.

The demographic data of the patients are shown in **Table 1**. Most heart failure cases in the PCCS group were due to ICM (50%), whereas dilated cardiomyopathy accounted for 65% of the cases in the non-PCCS group. Patients with PCCS were older, had a lower body weight, and had a higher incidence of cardiopulmonary resuscitation history (17% vs. 9%, $p = 0.044$), pre-transplant ventilator use, and extracorporeal membrane oxygenation (ECMO) support (19% vs. 11%, $p = 0.039$). During surgery, patients with PCCS had significantly longer CPB times than those without PCCS (220 ± 80 vs. 136 ± 49 min, $p < 0.001$). There were no significant differences between the groups regarding sex, blood type, UNOS status, pre-transplant intra-aortic balloon pump use, pre-transplant dialysis, diabetes, renal and liver function, age and body weight of donors, or allograft ischemic time.

Short-Term Outcomes

Patients with PCCS had a higher incidence of post-transplant ECMO support (29% vs. 15%, $p = 0.003$), renal dialysis (41% vs. 21%; $p < 0.001$), and postoperative re-exploration or delayed sternal closure (31% vs. 18%; $p = 0.004$) (**Table 2**). Although not statistically significant, patients with PCCS also showed a higher incidence of early bloodstream infection and a longer post-transplant hospital stay (18% vs. 11%, $p = 0.059$; 49 ± 55 vs. 38 ± 23 days, $p = 0.085$, respectively).

The in-hospital mortality rate was significantly higher in patients with PCCS than in those without (26% vs. 7%,

TABLE 2 | Short-term outcomes between recipients with and without PCCS.

Post-transplant	Overall	Without PCCS	With PCCS	*p*-value
N (%) or mean (±SD)	**N = 368**	**N = 263**	**N = 105**	
ECMO support	70 (19%)	40 (15%)	30 (29%)	0.003
Renal dialysis[a]	97 (26%)	54 (21%)	43 (41%)	<0.0001
Re-exploration or delayed sternum closure	80 (22%)	47 (18%)	33 (31%)	0.004
Early bloodstream infection (30-day), n (%)	47 (13%)	28 (11%)	19 (18%)	0.059
Hospital stay, days	40.9 ± 35.5	37.7 ± 23.1	48.8 ± 55.0	0.085
In-hospital death	45 (12%)	18 (7%)	27 (26%)	<0.0001
Cause of death, n (% of in-hospital death)				
Primary graft failure	9 (20%)	4 (22%)	5 (19%)	
Infection, sepsis	19 (42%)	8 (44%)	11 (41%)	
Acute rejection	5 (11%)	3 (17%)	2 (7%)	
Aortic rupture	1 (2%)	0	1 (4%)	
Ischemic bowel	3 (6%)	1 (6%)	2 (7%)	
Cerebrovascular event	5 (11%)	0	5 (19%)	
Limb ischemia	2 (4%)	1 (6%)	1 (4%)	
Pulmonary embolism	1 (2%)	1 (6%)	0	

HT, heart transplantation; PCCS, previous conventional cardiac surgery.

[a]*The incidence of renal dialysis included temporal dialysis; 97% of the patients were discharged without dialysis.*

$p < 0.001$), and the 1 year mortality rate was also higher in the PCCS group (30% vs. 14%, $p < 0.001$). The leading cause of 1 year mortality in both groups was infection, with cerebrovascular events accounting for a higher proportion in the PCCS group (15.6% vs. 2.6%).

Risk Factors for One-Year Mortality

Table 3 presents the results of the Cox regression analysis conducted to identify risk factors for 1 year mortality. Univariate analysis revealed several variables associated with 1 year mortality, including recipient age, previous cardiopulmonary resuscitation, UNOS status, pre-transplant ventilator use, pre-transplant intra-aortic balloon pump support, pre-transplant ECMO support, creatinine level, pre-transplant renal replacement therapy, PCCS, donor age, and CPB time. However, in multivariate analysis, only PCCS (hazard ratio (HR) = 2.485, 95% confidence interval (CI) = 1.241–4.975, $p = 0.01$) and CPB time (HR = 1.005, 95% CI = 1.000–1.009, $p = 0.044$) emerged as significant risk factors for 1 year mortality.

In examining the influence of etiology on heart failure and the various types of surgery, univariate analysis revealed that ICM posed a significant risk factor for 1 year mortality compared to dilated cardiomyopathy (HR = 1.737, 95% CI = 1.037–2.911, $p = 0.036$). Additionally, coronary artery bypass grafting (CABG) was a substantial risk factor for 1 year mortality when compared to patients without prior cardiac surgery (HR = 3.391, 95% CI = 1.934–5.946, $p < 0.001$). As there was a strong correlation between the etiology of heart failure and the type of prior cardiac surgery, a multivariate analysis was conducted, categorizing patients into four groups based on the presence or absence of prior cardiac surgery and the etiology of ICM or non-ICM. After adjusting for other significant factors, the multivariate analysis demonstrated that patients with prior cardiac surgery for ICM had a 4.848-fold increased risk (95% CI = 1.644–14.299, $p = 0.004$), while patients with prior cardiac surgery for non-ICM had a 3.554-fold increased risk (95% CI = 1.016–12.439, $p = 0.047$) compared to those without prior cardiac surgery and non-ICM as the etiology.

Long-Term Survival

All patients had complete follow-up data, with a mean follow-up duration of 7.1 ± 5.6 years. Kaplan–Meier survival analysis revealed lower 1, 5, and 10 years survival rates in the PCCS group than in the non-PCCS group (69.5% ± 4.5% vs. 85.6% ± 2.1%, 49.5% ± 5.0% vs. 71.7% ± 2.8%, and 39.2% ± 5.1% vs. 51.8% ± 3.3%, respectively; log-rank test, $p = 0.0024$, **Figure 2A**). However, excluding patients who died within the first year, the conditional Kaplan–Meier survival curve did not show a significant difference between the PCCS and non-PCCS groups (log-rank test, $p = 0.33$, **Figure 2B**).

Subgroup Analysis for Long-Term Survival

The results of subgroup analyses for long-term survival are shown in **Figure 3**.

DISCUSSION

The present study investigated the impact of PCCS on the outcomes of patients undergoing HT, excluding those bridged with a VAD and those who underwent heart re-transplantation. The findings of this study demonstrated that patients with PCCS had significantly poorer short- and long-term outcomes than those without PCCS. In the short term, patients with PCCS had higher rates of post-transplant complications, including the need for renal dialysis, postoperative re-exploration or delayed sternal closure, and post-transplant bloodstream infection. The study revealed that the major survival difference between these two groups occurred in the first year, with PCCS and CPB time during the operation being significant risk factors for 1 year mortality. However, the survival outcome of PCCS did not differ between

TABLE 3 | Cox regression for risk factors associated with 1-year mortality.

	Univariate			Multivariate		
	Hazard ratio	**95% CI**	***p*-value**	**Hazard ratio**	**95% CI**	***p*-value**
Age, +1	1.022	(1.005–1.039)	0.011	1.009	(0.987–1.031)	0.441
Sex, male	0.985	(0.518–1.876)	0.964			
BW, + 1 kg	1.000	(0.987–1.013)	0.978			
Blood type (References: AB = 1)						
A	2.151	(0.639–7.240)	0.216			
B	2.869	(0.862–9.557)	0.086			
O	2.504	(0.810–10.876)	0.053			
Smoking	1.272	(0.732–2.210)	0.393			
Hyperlipidemia	1.304	(0.708–2.402)	0.394			
Diabetes	1.627	(0.993–2.665)	0.053			
Previous CPR	3.963	(2.357–6.665)	<0.001	2.539	(0.928–6.947)	0.070
UNOS status (References: 1A = 1)						
1B	0.269	(0.139–0.520)	<0.001	0.380	(0.103–1.402)	0.146
2	0.265	(0.157–0.447)	<0.001	0.597	(0.152–2.347)	0.460
Pre-transplant ventilator	3.751	(2.317–6.074)	<0.001	1.571	(0.396–6.226)	0.520
Pre-transplant IABP	2.869	(1.741–4.727)	0.011	0.925	(0.349–2.452)	0.876
Pre-transplant ECMO	3.582	(2.147–5.976)	<0.001	0.536	(0.144–1.985)	0.350
Pre-transplant dialysis	3.139	(1.773–5.557)	<0.001	2.410	(0.939–6.182)	0.067
Creatinine, + 1 mg/dL	1.295	(1.019–1.646)	0.034	0.963	(0.653–1.418)	0.847
BUN, + 1 mg/dL	1.012	(1.000–1.025)	0.056			
Total bilirubin, + 1 mg/dL	1.019	(0.941–1.103)	0.645			
PCCS (yes)	2.372	(1.482–3.797)	<0.001	2.485	(1.241–4.975)	0.010
Donor age, +1	1.034	(1.016–1.053)	<0.001	1.021	(0.996–1.046)	0.102
Donor sex, male	1.008	(0.600–1.693)	0.975			
Donor BW, + 1 kg	0.999	(0.992–1.007)	0.837			
Allograft ischemic time, + 1min	1.002	(0.998–1.005)	0.376			
CPB time, + 1min	1.006	(1.003–1.009)	<0.001	1.005	(1.000–1.009)	0.044
Etiology of heart failure						
Dilated cardiomyopathy	1					
Congenital heart disease	1.173	(0.356–3.866)	0.794			
Ischemic cardiomyopathy	1.737	(1.037–2.911)	0.036			
Restrictive cardiomyopathy	1.381	(0.328–5.808)	0.66			
Valvular heart disease	2.005	(0.828–4.855)	0.123			
Others	1.826	(0.248–13.443)	0.554			
Etiology and PCCS[a]						
ICM, PCCS (–)	1			1		
ICM, PCCS (+)	4.329	(1.992–9.406)	<0.001	4.848	(1.644–14.299)	0.004
Non-ICM, PCCS (–)	1.347	(0.638–2.845)	0.435	2.447	(0.824–7.267)	0.107
Non-ICM, PCCS (+)	1.739	(0.707–4.280)	0.228	3.554	(1.016–12.439)	0.047

CI, confidence interval; HT, heart transplantation; VAD, ventricular assist device.

[a]*Multivariate analysis was adjusted for age, blood type, diabetes, cardiopulmonary resuscitation, United Network for Organ Sharing status, ventilator use, mechanical circulatory support, donor age, and cardiopulmonary bypass time.*

the new and old eras, and the timing of PCCS and HT did not affect survival. Notably, patients who underwent PCCS due to underlying ICM etiology had significantly poorer 1 year survival, but PCCS did not affect the early survival of patients with non-ICM etiology.

Early Mortality After HT in Patients With PCCS

The UNOS database report shows that 30% of HTs were performed in recipients with prior cardiac surgery and that prior surgery increased the 1 year and 5 years mortality rates by 1.192 times and 1.104 times, respectively [12, 13, 22]. However, not all types of prior cardiac surgery have the same impact on HT outcomes. Studies indicate that VAD implantation does not affect the subsequent HT outcome, whereas re-transplantation for prior allograft failure exhibits a lower survival rate than other surgery types [11–14]. This study excluded patients with prior VAD implantation or heart re-transplantation to specifically examine the influence of PCCS on HT outcomes. Remarkably, patients with PCCS had a significantly higher 1 year mortality rate (30% vs. 14%) than those without PCCS. PCCS increased mortality risk by 2.485 times within the first year. Notably, the findings of this study highlight the amplified effect of prior cardiac surgery on HT outcomes when exclusively focusing on conventional cardiac

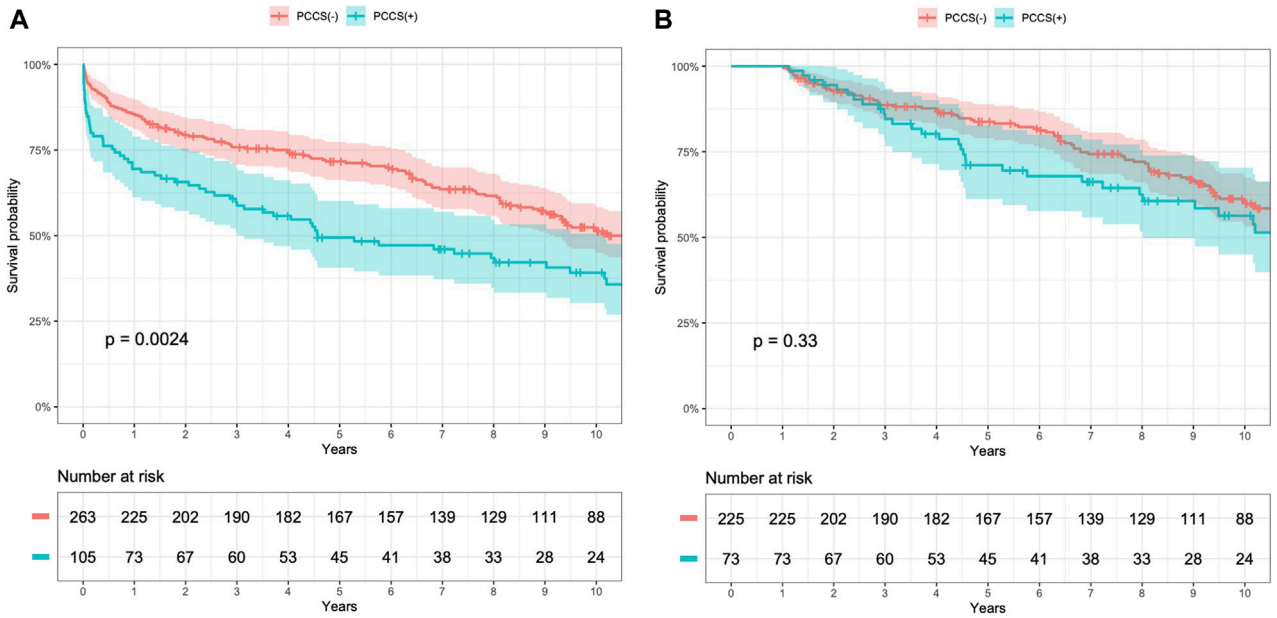


FIGURE 2 | Kaplan–Meier survival curves for patients with and without previous conventional cardiac surgery (PCCS) before heart transplantation (HT) **(A)** and conditional 1 year survival **(B)**.

surgery, emphasizing the necessity of meticulously considering this factor when selecting HT candidates.

Cause of Early Death in Patients With PCCS

Infection remained the leading cause of mortality in both groups in this study. Our previous research found that post-transplant dialysis and early bloodstream infection contributes to a 5.5-fold and 3.43-fold increase in early mortality, respectively [18, 21]. In the present study, the PCCS group exhibited a higher incidence of certain factors, namely, post-transplant ECMO support (29%), delayed sternum closure (31%), early bloodstream infection (18%), and dialysis (41%).

While previous studies have shown a correlation between prior cardiac surgery and an increased incidence of acute rejection after HT [6], immune sensitization presents a challenge for patients who have undergone prolonged VAD support or prior HT, which can negatively affect transplant outcomes [15, 16, 23]. However, after excluding these two high-risk groups, our study findings suggest that PCCS may not significantly elevate the risk of acute rejection following HT (7.4% vs. 16.7% in the groups with and without PCCS, respectively).

Interestingly, a significantly higher incidence of cerebrovascular event-related death, including hemorrhagic and ischemic stroke, was observed in the PCCS group (18.5% vs. 0% in the without PCCS group). This finding is consistent with a recent UNOS report that showed a significant increase in post-HT stroke in patients with prior cardiac surgery [24]. Of the five post-transplant strokes observed in this study, two were hemorrhagic, and three were ischemic. Three ischemic strokes occurred in recipients with ICM and prior CABG, who also received ECMO support before HT. ICMP, ECMO support, and re-sternotomy are recognized as the risk factors for post-HT stroke [24–26]. In this study, 50% of patients with PCCS received HT for ICMP, and 19% of patients with PCCS required preoperative ECMO support, which could explain the high incidence of stroke in this group. These findings suggest the importance of further cerebrovascular evaluation before HT, especially in patients with PCCS who require ECMO support and have an underlying ICMP.

Long-Term Survival in Patients With PCCS

In this study, the 10 years survival rate of patients with PCCS was 39%, significantly lower than that of the patients without PCCS. However, after excluding those who died within a year, the conditional survival analysis showed no significant difference in long-term survival outcomes between the groups. This indicates that the elevated mortality risk associated with PCCS primarily affects the early post-transplant period, consistent with previous studies [3–6, 27].

Subgroup Analysis for Survival Outcomes

To evaluate the etiology and related surgery on HT outcome, the subgroup analysis revealed that patients with ICM and PCCS had significantly worse outcomes than those in other subgroups during the early postoperative period (**Figure 3C**). Interestingly, patients with ICM as the etiology but without PCCS showed good short-term survival; however, their long-term outcomes were as poor as those with PCCS. This finding aligns with UNOS reports indicating that ICM, as the etiology of heart failure itself, is a significant risk factor for poor survival after HT [22]. In the context of end-stage heart failure due to ICM, it remains debatable whether patients would benefit more from

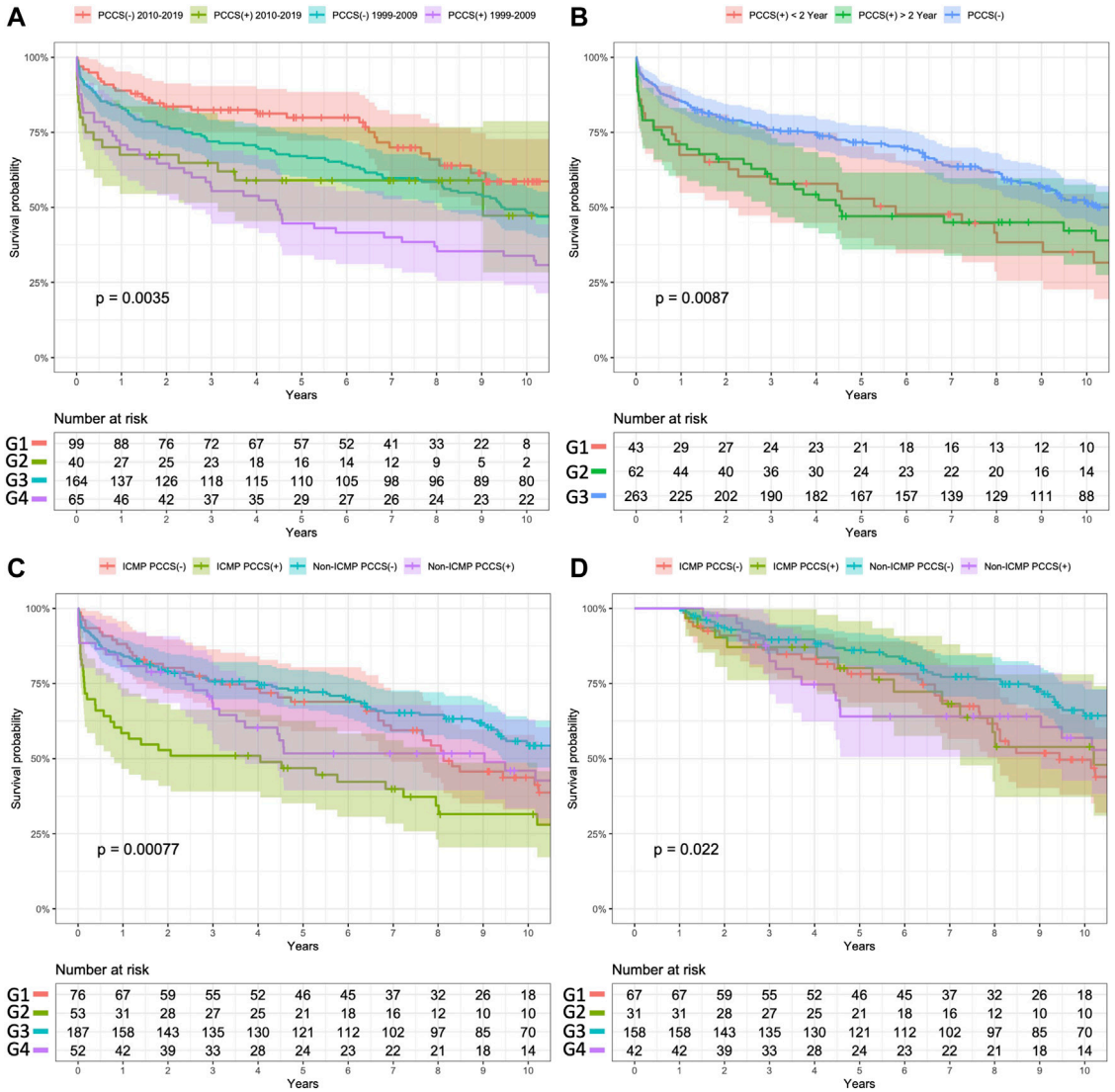


FIGURE 3 | Subgroup analysis of Kaplan–Meier survival curves for study patients. **(A)** Surgery in different eras **(B)**. Prior cardiac surgery within or before 2 years. **(C)** Different purposes, with or without a PCCS. **(D)** Conditional 1 year survival in subgroup analysis. Panel **(A)** Comparison of the survival outcomes between the two groups based on the era of HTs. Patients without PCCS who underwent transplantation after 2010 exhibited better survival outcomes than those in the other three groups (log-rank *p* values for comparisons of G1 vs. G2, G1 vs. G3, and G1 vs. G4 were 0.096, 0.130, and <0.001, respectively). Among patients with PCCS, there was no significant difference in survival outcomes between those who underwent surgery before 2010 (G4) and after 2010 (G2) (log-rank *p*-value = 0.269). Panel **(B)** Segregation of patients with PCCS into two groups based on the timing of PCCS relative to HT. The survival outcome did not differ significantly between these two groups (G1 vs. G2, log-rank *p*-value = 0.103), but their survival was significantly worse than that of patients without PCCS (log-rank *p*-values = 0.026 and *p* = 0.041, respectively). Panel **(C)** Segregation of patients into four subgroups based on the etiology of heart failure and the purpose of PCCS. Patients who underwent PCCS for ICM (G2) had the worst survival outcomes among those in the other subgroups (overall log-rank *p*-value <0.001). Patients who received PCCS for non-ICM (G4) did not show significantly different survival outcomes than those without PCCS (G1 and G3, log-rank *p*-values = 0.485 and 0.346, respectively). As shown in Panel **(D)**, after excluding patients who died within 1 year, the conditional Kaplan–Meier survival analysis showed that even patients without PCCS who had ICM had significantly lower survival outcomes than those without PCCS (G1 vs. G3, log-rank *p*-value = 0.013).

high-risk conventional bypass surgery for complete revascularization, or from medical treatment with VAD bridging to HT. Further research is needed to resolve this issue. Furthermore, although not statistically significant, patients with PCCS of non-ICM etiology showed worse survival outcomes in the mid-term follow-up (between 3 and 5 years after HT). It is important to consider the impact of different etiologies of heart failure, such as restrictive cardiomyopathy and rheumatic heart disease, on post-transplant outcomes [28–30]. Although our study attempted to address this impact, the limited number of cases prevented us from conducting a comprehensive analysis. Future studies utilizing large databases, such as UNOS reports, could provide more insights into this matter.

Our study also found that both PCCS and extended CPB time were significant risk factors of 1 year mortality. Despite no significant difference in cold ischemic time, patients with PCCS had a total CPB time that was an hour longer. Prolonged CPB can increase micro-emboli formation and the occurrence of renal and neurological complications [31, 32]. The longer duration between PCCS and re-sternotomy may reduce the difficulty of performing re-sternotomy [33]. We found a negative correlation between CPB time and the timing of PCCS and HT ($R = -0.19$, $p = 0.05$). However, the timing of PCCS to HT did not demonstrate a significant risk reduction in HT outcomes according to the univariate Cox survival analysis. Subgroup analysis also demonstrated that PCCS within or after 2 years did not significantly impact survival outcomes based on Kaplan–Meier analysis (**Figure 3B**). We found that the era in which HT took place significantly affected survival rates, with patients undergoing HT after 2010 exhibiting better survival outcomes (**Figure 3A**), thus reflecting advancements in perioperative care and growing HT experience. However, there were no significant differences in survival outcomes between patients with PCCS across different eras. It is important to note that re-sternotomy and prolonged CPB continue to pose challenges for HT in patients with PCCS. Allowing the surgeon sufficient time to perform a demanding re-sternotomy without needing CPB is crucial for improving HT outcomes.

The limitations of this study include the small sample size and the nearly three-decade span it covers, during which significant changes in cardiogenic shock, AKI, and HT management have occurred. While we conducted a thorough examination within our center, it is important to recognize that the findings may have a more localized impact, potentially being more applicable to a single-center scenario rather than offering a comprehensive analysis suitable for a wider range of centers. To better understand the effects of various subgroups, such as the timing of PCCS, type of surgery, and effect of mechanical support (e.g., durable and non-durable VAD support or re-transplantation), a larger number of patients would need to be recruited within a shorter timeframe. This could be achieved through multicenter collaboration or by analyzing a national registry.

Despite these limitations, our study underscores the increased risks and complications faced by patients with PCCS undergoing HT. Therefore, meticulous patient selection and management strategies, including preoperative assessment of re-sternotomy risks, are vital for improving the outcomes. Further research is required to investigate whether the use of VAD in patients with PCCS would improve outcomes after HT and to determine any potential benefits it may offer.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by National Taiwan University Hospital Review Board, and the requirement for informed consent was waived (202208017RINB).

AUTHOR CONTRIBUTIONS

J-WC and R-BH conceived of the presented idea. J-WC collected the clinical data and performed the analysis. H-WC, N-KC, C-HW, N-HC, S-CH, H-YY, Y-SC, and R-BH contributed data. R-BH supervised the findings of this work. J-WC wrote the paper. All authors discussed the results and contributed to the final manuscript.

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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Adherence to Pharmacotherapies After Heart Transplantation in Relation to Multimorbidity and Socioeconomic Position: A Nationwide Register-Based Study

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No studies have examined the impact of multimorbidity and socioeconomic position (SEP) on adherence to the pharmacological therapies following heart transplantation (HTx). Using nationwide Danish registers, we tested the hypothesis that multimorbidity and SEP affect treatment patterns and adherence to pharmacological therapies in first-time HTx recipients. Pharmacological management included cost-free immunosuppressants and adjuvant medical treatment (preventive and hypertensive pharmacotherapies; loop diuretics). We enrolled 512 recipients. The median (IQR) age was 51 years (38–58 years) and 393 recipients (77%) were males. In recipients with at least two chronic diseases, prevalence of treatment with antihypertensive pharmacotherapies and loop diuretics was higher. The overall prevalence of adherence to treatment with tacrolimus or mycophenolate mofetil was at least 80%. Prevalence of adherence to preventive pharmacotherapies ranged between 65% and 95% and between 66% and 88% for antihypertensive pharmacotherapies and loop diuretics, respectively. In socioeconomically disadvantaged recipients, both the number of recipients treated with and adherence to cost-free everolimus, lipid modifying agents, angiotensin-converting enzyme/angiotensin II inhibitors, calcium channel blockers, and loop diuretics were lower. In recipients with multimorbidity, prevalence of treatment with antihypertensive pharmacotherapies and loop diuretics was higher. Among socioeconomically disadvantaged recipients, both number of patients treated with and adherence to cost-free everolimus and adjuvant pharmacotherapies were lower.

Keywords: heart transplantation, pharmacological management regime, immunosuppression, socioeconomic position, multimorbidity

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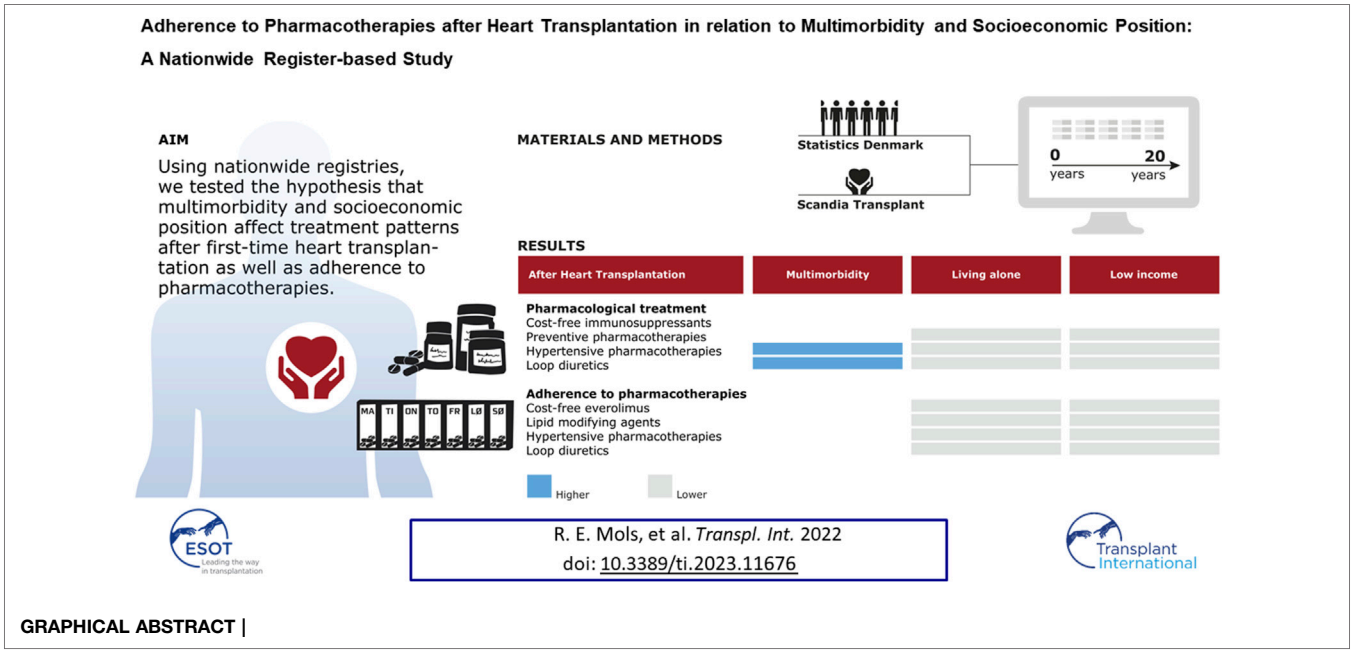
Abbreviations: ATC, anatomical therapeutic chemical; CRS, civil registration system; DNPR, Danish national patient registry; HTx, heart transplantation; MACE, major adverse cardiovascular event; NPR, Danish national prescription registry; PCRR, psychiatric central research registry; SEP, socioeconomic position; STD, Scandiatransplant database.



GRAPHICAL ABSTRACT |

INTRODUCTION

Heart transplantation (HTx) is the ultimate treatment for end-stage heart failure [1, 2]. HTx recipients require life-long pharmacological treatment [2, 3]. Improvements in immunosuppressive and adjuvant medical treatment to avoid graft rejection has improved survival in HTx recipients [4–6]. Thus, pharmacological treatment has become more complex to prevent or treat post-transplant complications and comorbidities [5, 7, 8]. Polypharmacy including up to sixteen pharmacotherapies is seen in one-third of recipients 5 years after HTx [7]. A single-center study suggested that especially regular and accurate intake of immunosuppressants is vital for organ survival [9].

Previous studies have reported sub-optimal self-reported adherence to medical therapies following HTx [10–13]. A cross-sectional study described that the medication complexity score, and the rate of new onset multimorbidity were alarmingly high in Spanish HTx recipients [14]. Addressing long-term non-adherence to medical therapies is crucial to achieve optimal post-HTx outcomes [9, 12, 15].

We previously described the patterns of multimorbidity and socioeconomic position (SEP) concerning the overall pharmacological services utilization after HTx. The study reported a higher number of prescriptions in recipients with three or more comorbidities, and a lower number of prescriptions in recipients within the lowest income group or among those living alone [16]. In the United States and the United Kingdom, lower SEP is documented to be associated with poorer HTx outcome [17–20] and it could be hypothesized that a plausible explanation may be found in a socioeconomic gradient in non-adherence to pharmacotherapies [9, 17]. However, no studies have examined the impact of multimorbidity and SEP on adherence to the pharmacological therapies in post-HTx recipients. Moreover, the majority of earlier studies of adherence to post-transplant pharmacotherapy have utilized self-reported measures of adherence [21] in countries without universal healthcare systems. Using nationwide registries, we tested the hypothesis that multimorbidity and SEP affect treatment patterns after first-time HTx as well as adherence to pharmacotherapies.

MATERIALS AND METHODS

Design and Setting

We conducted a nationwide cohort study in first-time HTx recipients in Denmark between 1 January 1995 and 31 December 2018. Denmark has two HTx centers at University Hospital of Copenhagen and Aarhus University Hospital. The Danish healthcare system is primarily tax-financed with free access to both in-hospital and general practice healthcare services for all Danish citizens. The Danish Civil Registration System (CRS) records vital status using a unique ten-digit identifier assigned to all citizens at birth or immigration. The personal identifier enables access to individual-level data across health and administrative registers [22]. General reimbursement is given for prescription medicine at community pharmacies apart from a minor co-payment, and immunosuppressants are provided free of charge from hospital pharmacies (independent of multimorbidity and SEP) [22]. HTx recipients in this study were followed until 31 December 2018, migration or the date of all-cause mortality, whichever came first.

Study Cohort and Characteristics

We used the complete Scandiatransplant Database (STD) [23] to construct a cohort of first-time HTx recipients identified by the

International Classification of Diseases system Revision (ICD-10 code: DZ94.1). The index date was the date of surgery in the STD. Information regarding recipient age and gender at index date was extracted from the CRS [24].

We identified morbidities from the Danish National Patient Registry (DNPR) [25] and in the Psychiatric Central Research Register (PCRR) [26]. Diagnoses are coded according to ICD-8/10 [25, 26] and somatic and mental morbidities 10 years prior to index date were defined (**Supplementary Table S1**). To address multimorbidity, we used an algorithm applied in previous Danish studies [16, 27] including a high number of specific physical and mental chronic morbidities, divided into 11 comprehensive chronic disease groups: cardiovascular disease, hypertension, diabetes, obstructive pulmonary disease, cancer, neurological disorder, arthritis, bowel disease, liver disease, kidney disease, and mental illness. This Danish algorithm defined multimorbidity as the co-occurrence of two or more chronic conditions included in the 11 comprehensive chronic disease groups. We summarized the number of chronic diseases, excluding cardiovascular diseases (**Supplementary Table S1**).

Four different individual-level SEP indicators were applied from Statistic Denmark and CRS: cohabitation status, highest attained educational degree, employment status, and personal income [22, 24]. Information on cohabitation status at index date was defined as living alone or cohabitation. We used the highest attained educational degree in the calendar year before the index date and grouped recipients into four categories: low (no formal education, primary and lower secondary education); medium (upper secondary education and academy profession degree); high (bachelor and above); not completed any education (recipients under age of 16 years). Employment status the year before index date was divided into working, not working, early retirement, state pension, and under education. Based on the annual percentiles in the Danish population, we classified income into percentiles and used the 25th percentile as a cut-off point for low (≤25th percentile) and medium-high (>25th percentile) income (**Supplementary Table S2**).

Pharmacological Management Regime

The pharmacological management regime [3] was defined by used treatment with cost-free immunosuppressants such as ciclosporin, tacrolimus, everolimus, mycophenolate mofetil, and prophylactic anti-infectious medication such as valganciclovir, and sulfamethoxazole with trimethoprim. Glucocorticoids were not totally cost-free and were generally tapered down during the first year and stopped after 12–18 months depending on biopsy history. Patients also had to pay a minor part of the costs of adjuvant medical treatment such as preventive pharmacotherapies (antiplatelet agents, lipid lowering agents) as well as antihypertensive pharmacotherapies (ACE/AT [Angiotensin-converting enzyme/angiotensin] II inhibitors, aldosterone antagonists, calcium channel blockers, thiazides) and loop diuretics (furosemide or bumetanide) (**Supplementary Table S3**). Lipid modifying agents (primarily Pravastatin) was given as a standard to all recipients. In case of statin intolerance, ezetimibe was prescribed. Antiplatelet therapy was not routinely given to all recipients but only on specific indications.

Data on reimbursed pharmacotherapies were provided by the Danish National Prescription Registry (NPR) [28]. Records include Anatomic Therapeutic Chemical (ACT) code, date of reimbursement, strength and formulation, and number of tables reimbursed. However, no information on prescribing indication or prescribed daily dose is available in the DNPR [28]. We defined treatment with medical therapies (ACT code) as one or more reimbursed prescription within 180 days intervals after HTx. The hospital pharmacy at Aarhus University Hospital has electronically recorded use of cost-free immunosuppressants by date of dispensing, strength and formulation, and the number of tablets dispensed. We used this information in a sub-analysis including HTx recipients from Transplant Center Aarhus from 1 January 1995 to 31 December 2018 [16].

Prevalence of medical treatment within the pharmacological management regime was estimated by 180 days intervals during follow-up. We only included HTx recipients with a complete follow-up of a least 365 days and prescriptions redeemed (≥1) in the first and/or second 180 days interval after index date.

Polypharmacy before HTx (baseline) was defined as at least one reimbursed prescription related to ≥5 agents within the Cardiovascular ACT index 180 days prior to index date.

Adherence to Pharmacotherapies

To describe adherence to used pharmacotherapies, we estimated the proportion of days covered (PDC) [29] within 180 days intervals in recipients treated with medical therapies. The first 180 days after index date were considered as a blanking period to allow breaks, change, or up-titration of medical therapies. We applied 80% of days covered as the threshold for adherence and PDC < 80% as non-adherence [29]. Since data on prescribed daily dose is not available in neither the NPR nor in pharmacy records at Aarhus University Hospital, we calculated the gold standard for prescribed daily dose of immunosuppressants and adjuvant medical treatment by two different methods: a) a fixed dosing regimen or b) an estimated dosing regimen. Based on clinical guidelines [3] and local practice, a fixed daily dose of two tablets or one tablet per day was used for cost-free immunosuppressants. In line with preventive guidelines [2], a fixed daily dose of one tablet per day was chosen as the gold standard in glucocorticoids and preventive pharmacotherapies. In antihypertensives and loop diuretics, we calculated the median daily dose (MDD) by all prescriptions in the period 180–360 days after index date (**Supplementary Table S4**). This individual MDD-1 was used as the gold standard daily dose during the next five 180 days intervals. Next, a new individual MDD (MDD-2) was estimated using all prescriptions in the period 1,081–1,260 days after index date. The MDD-2 was used as the gold standard daily dose in the period 1,261–2,160 days after index date (**Supplementary Figure S1**).

In case of a break in reimbursed prescriptions of more than 365 days in HTx recipients, we defined this as a +365 days break if recipients survived or did not emigrate in this period. The HTx recipients were followed to end of pills within this break of 365 days. We allowed a 7 days grace period to account for short discontinuations. As the DNPR and the pharmacy at Aarhus University Hospital do not capture pharmacotherapies

dispensed during hospitalizations, HTx recipients were assumed to receive medical therapies in-hospital if readmitted for more than 7 days. If HTx recipients had pills left within the 365 days of follow-up, we pragmatically decided that maximum 90 pills were included in the next follow-up period (several pharmacotherapies have 3 months of durability). This was decided as recipients collect prescriptions lasting longer than the follow-up interval.

Statistical Analysis

We characterized HTx recipients according to baseline characteristics by presenting median and interquartile range (25th–75th percentile [IQR]) or numbers (n) and percentage (%).

To assess the potential influence of multimorbidity and socioeconomic disadvantage, we also dichotomized educational degree (low education [low] versus medium-high education [medium + high]) and employment status (unemployed [not working, early retirement] versus employed [working, state pension, under education]) (**Supplementary Table S2**). The categorization was based on general epidemiological assumptions used in Denmark. Recipients with missing data were not included (<0.01%).

Over time, used treatment within the pharmacological management regimes after HTx was examined. First, we graphically displayed prevalence curves for immunosuppressants and adjuvant pharmacotherapies by 180 days intervals during follow in recipients still alive and not emigrated. Next, we described the influence of multimorbidity and SEP on the used pharmacotherapies by graphically depicted prevalence curves stratified by the dichotomized variables of multimorbidity and SEP. Similarly, we followed recipients still alive or not emigrated within 180 days periods. We evaluated the prevalence of non-adherence (PDC < 80%) for used pharmacotherapies by descriptive illustrations. HTx recipients were followed by 180 days intervals until censoring event (+365 days break, mortality, emigration) or end of follow-up. Then, graphical curves were stratified according to the dichotomized variables of multimorbidity and SEP to illustrate the influence of these baseline variables. Sensitivity illustrations were performed as distributed plots for PDC outcomes during follow-up. According to the Danish Data Protection Agency and IRB (Institutional Review Boards) approval, scientists are not allowed to report numbers less than five or aggregated results based on less than five observations. These are thus marked as NA (not available) in the manuscript or ended follow-up in graphical displays. Moreover, if prevalence was less than 20% of pharmacotherapies during 180 days follow-up intervals, adherence outcomes stratified by multimorbidity and socioeconomic disadvantage were not presented.

Analyses were conducted using the SAS Statistical Software version 9.4 (SAS Institute, Cary, NC) and R version 4.1.0 (2021-05-18).

RESULTS

We enrolled 512 Danish HTx recipients during the study period. **Table 1** shows baseline characteristics of the recipients included.

TABLE 1 | Baseline characteristics of heart transplant recipients.

	Total
	N = 512
Gender	
Male	393 (77)
Female	119 (23)
Age	
Median (IQR)	51 (38–58)
Age groups	
0–20	50 (10)
21–40	93 (18)
41–60	301 (59)
+61	68 (13)
Follow-up time in years	
0–5	146 (29)
5–10	141 (28)
+10	225 (44)
Alive at end of follow-up	334 (65)
Cardiovascular morbidities (10 years prior to the index date)	
Myocardial infarction	175 (34)
Angina Pectoris	223 (44)
Heart failure	439 (86)
Heart valve diseases	59 (12)
Cardiac arrhythmias	245 (48)
Congenital heart disease	46 (9)
Cardiomyopathy	347 (68)
Cardiac inflammation	55 (11)
Aortic disease	NA
Peripheral arterial disease	33 (6)
Cerebrovascular disease	47 (9)
Cardiogenic shock and pulmonary edema	50 (10)
Hyperlipidemia	72 (14)
Other morbidities (10 years prior to the index date)	
Hypertension	62 (12)
Diabetes	59 (12)
Chronic obstructive pulmonary disease	58 (12)
Cancer	18 (4)
Chronic neurological disease	9 (2)
Chronic arthritis	NA
Chronic bowel disease	NA
Chronic liver disease	8 (2)
Chronic kidney disease	24 (5)
Chronic mental disease	NA
Mental disorder	NA
Multimorbidity (10 years prior to the index date)	
Number of chronic diseases, median (IQR)	1 (1–2)
Cardiovascular polypharmacy (180 days prior to the index date)	293 (57)
Cohabitation status	
Living alone	228 (45)
Cohabitation	284 (55)
Highest obtained educational degree	
Low (primary and lower secondary education)	165 (32)
Medium (upper secondary education and academy profession)	217 (42)
High (bachelor and above)	91 (18)
Not completed education (patients age ≤16 years)	28 (6)
Missing	11 (2)
Employment status	
Working	243 (48)
Not working	52 (10)
Early retirement	159 (31)
State pension	36 (7)
Under education	20 (4)
Missing	NA
Personal income group	
Low income (≤25th percentile)	103 (20)
Medium-high income (>25th percentile)	409 (80)

Values are n (%).
NA, not available (numbers less than five).

The median (IQR) age was 51 (38–58) and 393 recipients (77%) were males. The differences in age between the categories of multimorbidity and SEP were minor, except for cohabitation status and employment status (**Supplementary Figure S2**). We found no differences in the median number of multimorbidities between categories of SEP (**Supplementary Table S4**).

Prevalence of treatment within the cost-free immunosuppressive regime is shown in **Figure 1**; though, only including HTx recipients (n = 258) recorded by the pharmacy at Aarhus University Hospital (**Supplementary Table 6**). During the 7 years follow-up, 25% of the recipients were on treatment with ciclosporin and the use of tacrolimus ranged between 68% and 82%. More than 95% used mycophenolate mofetil after heart transplantation and the prevalence decreased to 75% after 6 years. Recipients on treatment with everolimus steadily increased from 25% to 35%–36% within follow-up. Among recipients with at least two chronic diseases, a higher prevalence of treatment with tacrolimus was observed, whereas a lower prevalence of recipients used ciclosporin. A lower prevalence of treatment with everolimus was seen in recipients living alone. In recipients with low income, we observed a lower prevalence of use with ciclosporin and everolimus in contrast to higher prevalence of treatment with tacrolimus (**Figure 1**).

Figure 2 illustrates the prevalence of treatment with glucocorticoids and adjuvant pharmacotherapies within 10 years of follow-up for all recipients (n = 512). Prevalence of treatment with glucocorticoids decreased from 75% to 30% during follow-up. The prevalence of use with antiplatelet agents increased from 20% to 50% and treatment with lipid modifying agents was approximately 75% during follow-up. During the 10 years follow-up, prevalence of treatment with ACE/AT II inhibitors increased from 30% to 65%. Approximately 50% of recipients

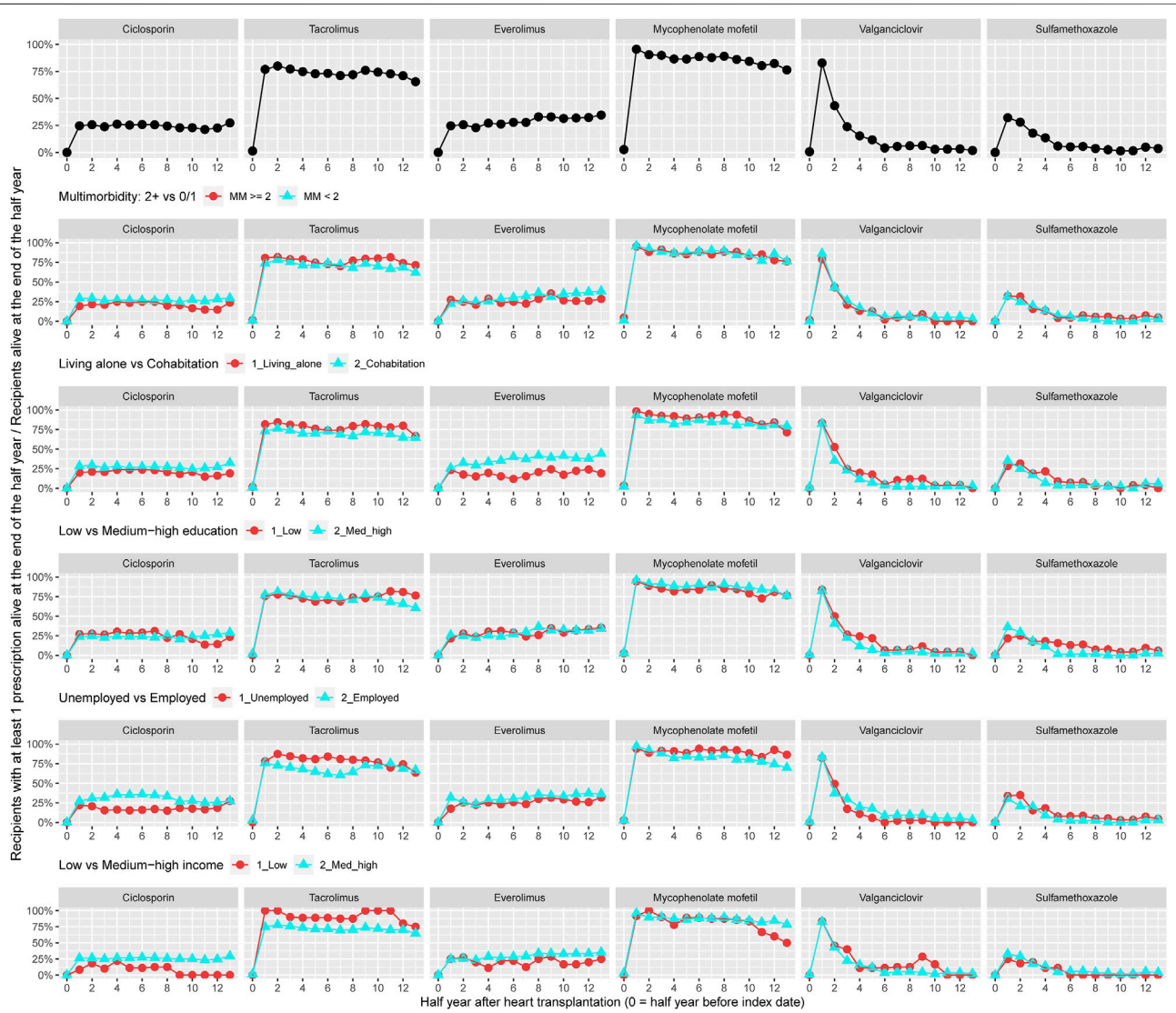


FIGURE 1 | Prevalence of treatment with cost-free immunosuppression overall and by categories of multimorbidity and socioeconomic position.

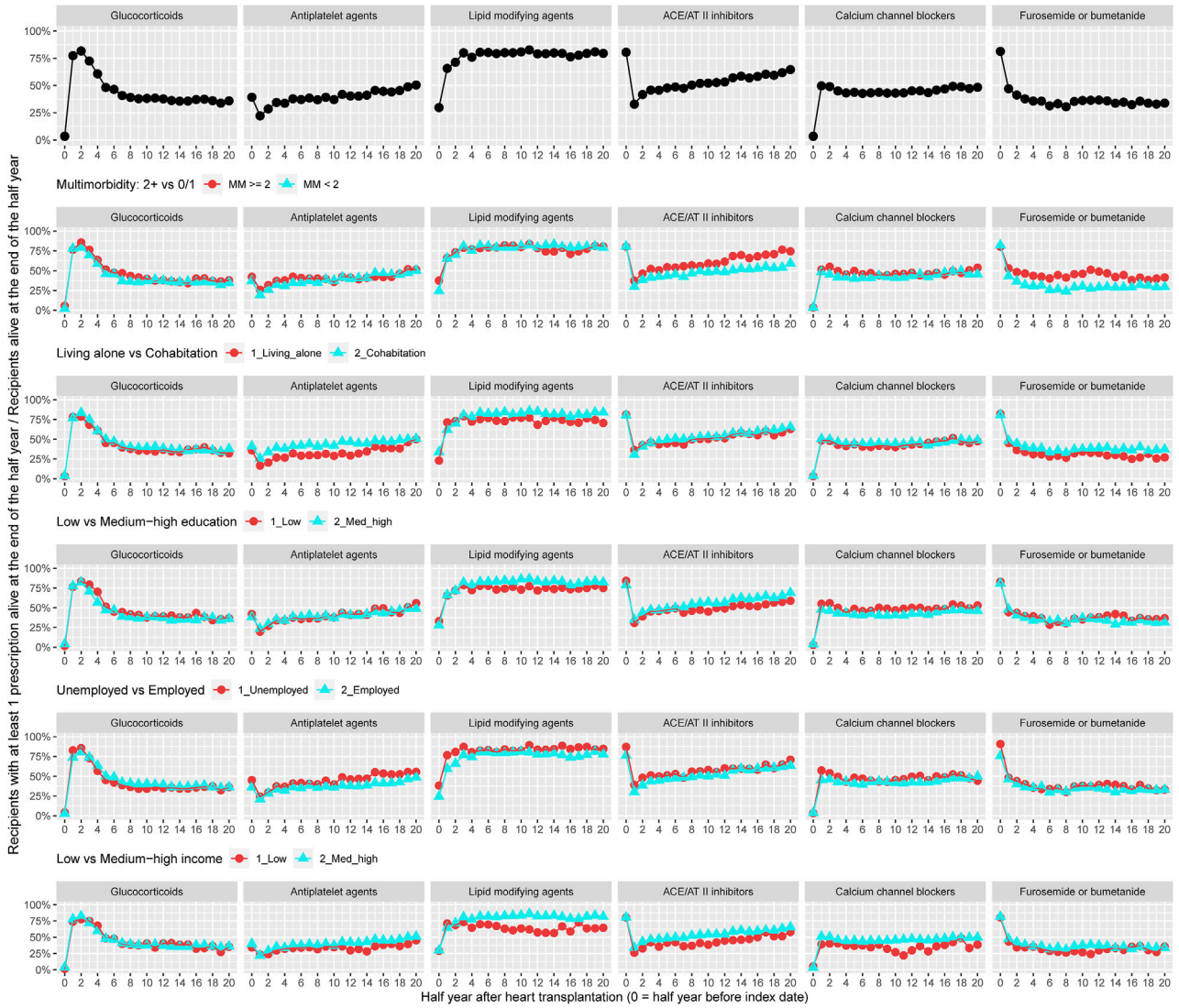


FIGURE 2 | Prevalence of treatment with glucocorticoids and adjuvant pharmacotherapies overall and by categories of multimorbidity and socioeconomic position. ACE, Angiotensin-converting enzyme; AT, Angiotensin.

were in treatment with calcium channel blockers and 35% used furosemide or bumetanide (**Figure 2**). The prevalence of treatment with aldosterone antagonists and thiazides, respectively, was lower than 5% and not presented in **Figure 2**. We observed higher prevalence of use with antihypertensive medical therapies and loop diuretics in recipients with at least two chronic diseases. Among recipients living alone, prevalence of treatment with antiplatelet agents, lipid modifying agents, and furosemide or bumetanide during follow-up was lower. A lower prevalence of use of lipid modifying agents and ACE/AT II inhibitors was seen in recipients with low educational degree. Prevalence of treatment with lipid modifying agents, ACE/AT II inhibitors, and calcium channel blockers was lower among recipients with low income (**Figure 2**).

Figure 3 shows the prevalence of adherence to cost-free immunosuppression therapy 1–7 years post-HTx. The overall prevalence for adherence was at least 80% for both treatment with tacrolimus or mycophenolate mofetil. Since less than 36% of the sub-recipients (n = 258) used ciclosporin or everolimus, we were not permitted as per IRB approval to present stratified adherence prevalence curves for these two medical therapies. We observed half-year periods with higher prevalence of non-adherence to both tacrolimus and mycophenolate mofetil in recipients with more than two chronic diseases. Half-year periods with higher prevalence of non-adherence to treatment with tacrolimus and mycophenolate mofetil, respectively, were seen in illustrations categorized by socioeconomic disadvantage; thus primarily observed for tacrolimus among recipients living alone, with a low educational degree, or unemployment (**Figure 3**). Due to data protection, variables for personal income were not included in the stratified illustrations.

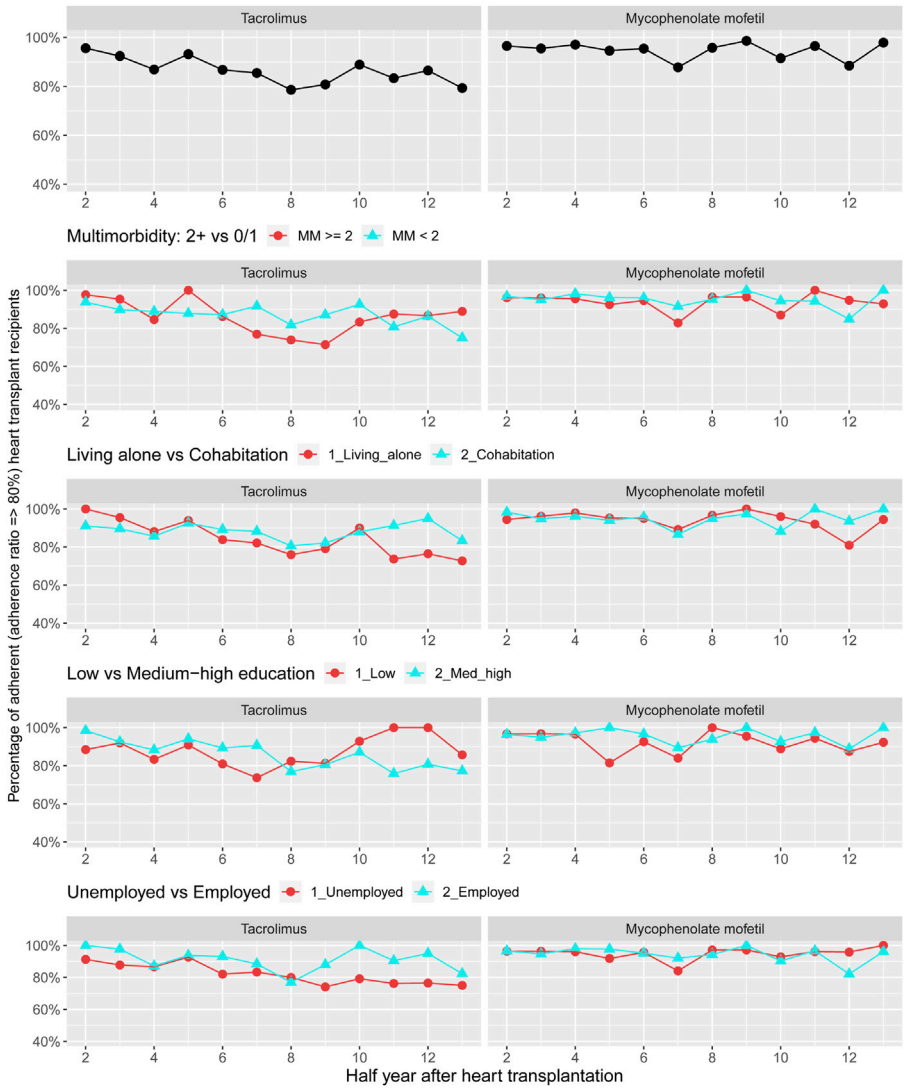


FIGURE 3 | Prevalence of adherence to cost-free immunosuppressants overall and by categories of multimorbidity and socioeconomic position. Due to data protection, the variable of personal income was not included in the stratified illustrations.

Figure 4 displays adherence curves regarding glucocorticoids and preventive pharmacotherapies 1–10 years after HTx. For glucocorticoids, we observed that the prevalence of adherence ranged between 65% and 92% during follow-up; prevalence of adherence to antiplatelet agents ranged between 75% and 95% and the prevalence of adherence to lipid modifying was approximately 85%–90%. We documented no pattern for adherence to glucocorticoids and preventive pharmacotherapies by multimorbidity. Among recipients with low income, we found half-year periods with higher prevalence of non-adherence to treatment with glucocorticoids. Half-year periods of higher prevalence of non-adherence were observed for lipid modifying agents in recipients living alone, with low educational level and low income (**Figure 5**) (**Supplementary Figure S3**).

Description of adherence to antihypertensive pharmacotherapies and loop diuretics 1–7 years after HTx are presented in **Figure 5**. We found that the overall prevalence of adherence to these medical therapies was 66%–88%. No pattern was observed in prevalence of adherence to antihypertensive pharmacotherapies and loop diuretics when categorized by multimorbidity. We observed that recipients living alone presented half-year periods of higher prevalence of non-adherence to ACE/AT II inhibitors. Half-year periods with a higher prevalence of non-adherence to calcium channel blockers and treatment with loop diuretics were seen in unemployed or low income group recipients (**Figure 4**) (**Supplementary Figure S4**).

DISCUSSION

This nationwide register study with longitudinal follow-up from 1995–2018 showed that in first-time HTx recipients with

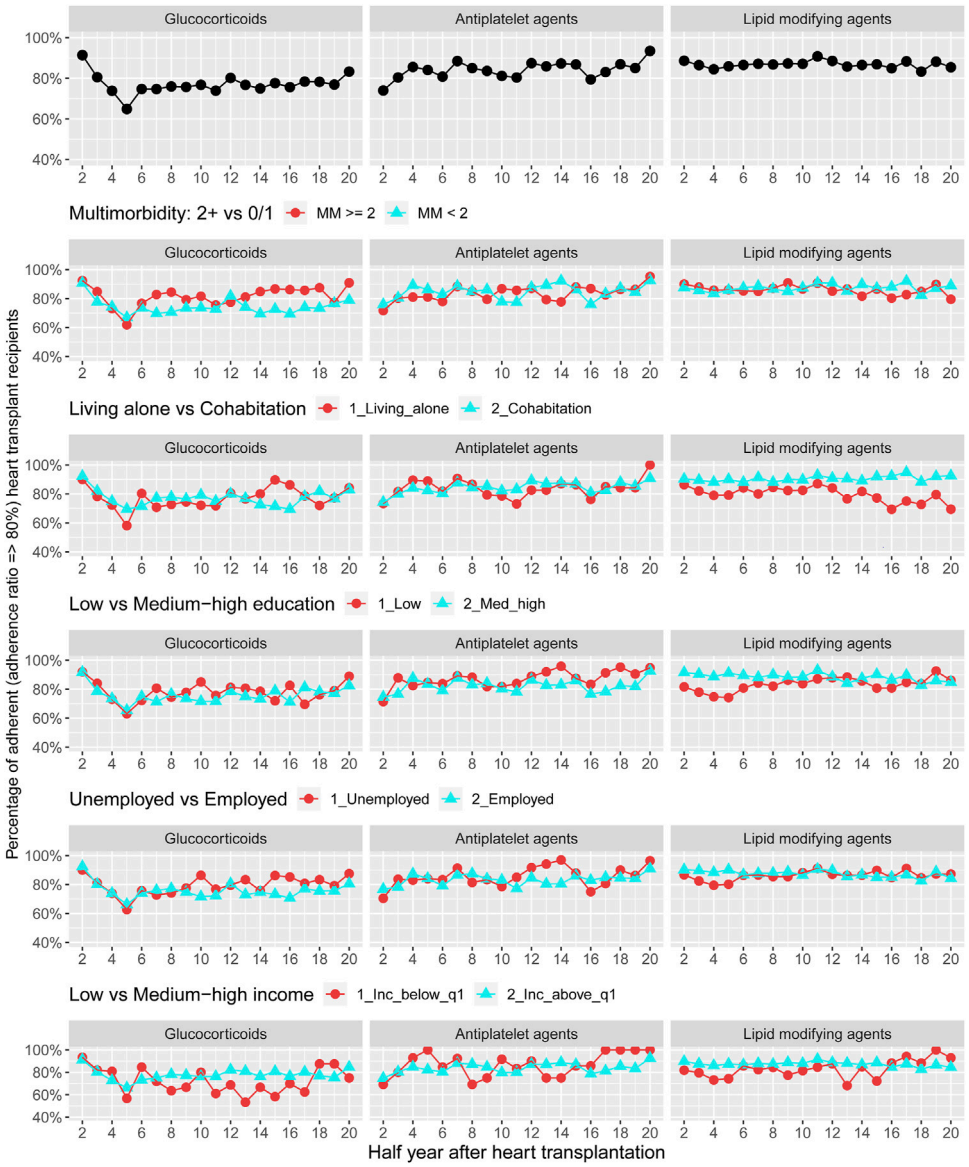


FIGURE 4 | Prevalence of adherence to glucocorticoids and adjuvant pharmacotherapies overall and by stratified variables of multimorbidity and socioeconomic position.

multimorbidity, the prevalence of treatment with antihypertensive pharmacotherapies and loop diuretics were higher. In socioeconomically disadvantaged recipients, both the number of patients treated with and adherence to cost-free everolimus, lipid modifying agents, ACE/AT II inhibitors, calcium channel blockers, and loop diuretics were lower. This was particularly pronounced in recipients living alone or with low income.

Multimorbidity is typically defined as the coexisting of two or more chronic conditions and has been shown to be associated to both the medical regime complexity as well as to non-adherence to the multiple medical therapies [30]. In accordance with our study, a small single-center study (n = 60) evaluating patient-level medication complexity over time showed that 5 years after surgery, HTx recipients were treated with increasing amounts of immunosuppressants, antihypertensives (81.8% used ACE/AT II inhibitors), and lipid modifying agents (98.3% used statins) to treat both existing and new-onset morbidities [7] as well as complications (allograft vasculopathy, graft failure, hypertension, cardiovascular diseases, and kidney disease, etc.) [3]. A smaller Spanish study [14] including adult chronic-stage (follow-up >1.5 years) HTx recipients (n = 135) demonstrated a relation between multimorbidity and worse patient-level Medication Regimen Complexity Index score (pMRCI). The pMRCI score was primarily influenced by the medical treatment of new-onset comorbidities [14]. This could indicate the need for strategies to reduce medication complexity and

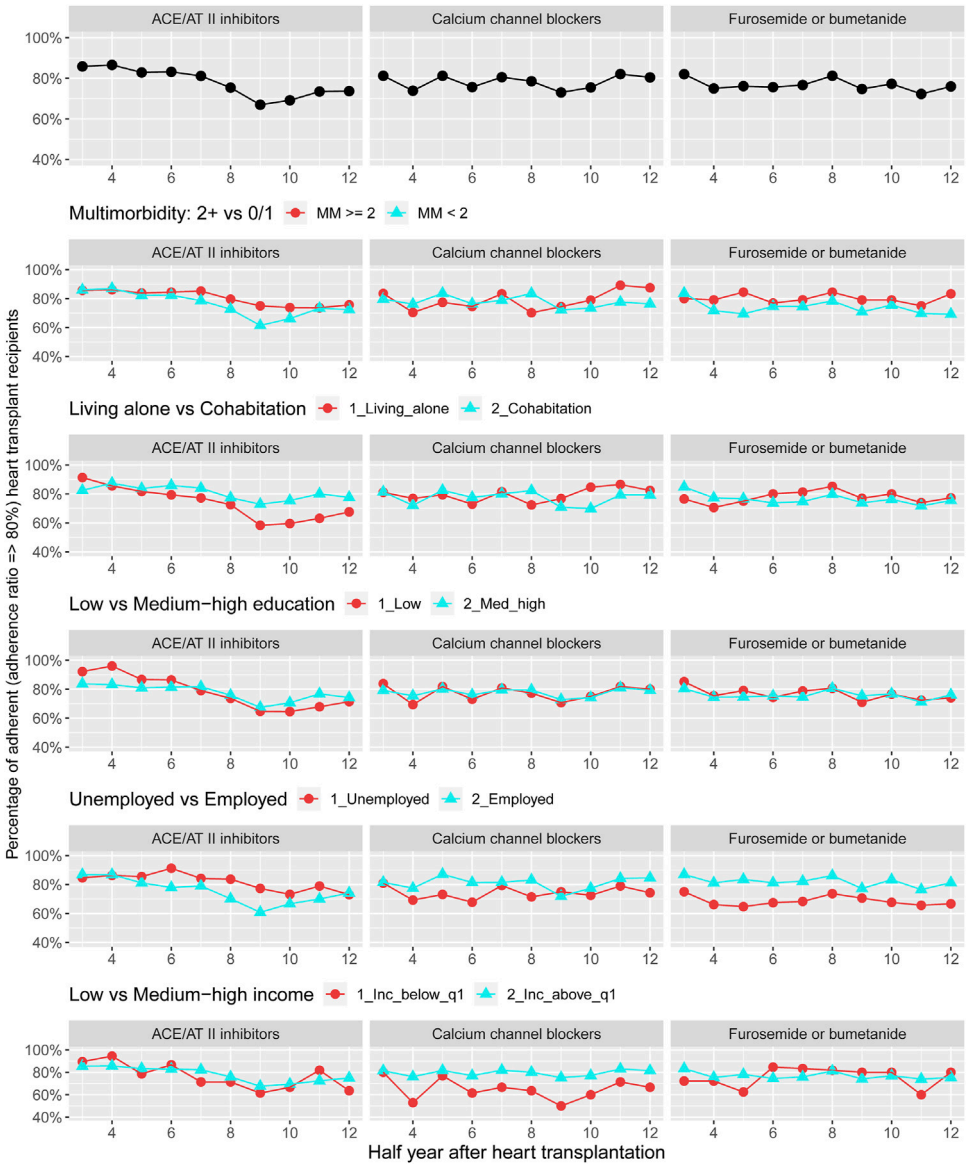


FIGURE 5 | Prevalence of adherence to antihypertensive pharmacotherapies and loop diuretics overall and by categories of multimorbidity and socioeconomic position. ACE, Angiotensin-converting enzyme; AT, Angiotensin.

support self-management in long-term HTx survivors with increasing multimorbidity.

To our knowledge, this is the first study to describe register-verified (as opposed to self-reported) initiation of pharmacotherapies after HTx by individual-level SEP indicators in a universal healthcare system. We found that socioeconomic deprivation seems to influence lower initiation of cost-free treatment with everolimus, antiplatelet agents, lipid modifying agents, ACE/AT II inhibitors, calcium channel blockers, and loop diuretics; however, this mainly applied to recipients living alone or in the low-income group. We can only speculate how mechanisms of socioeconomic disadvantage in HTx recipients may affect initiation of pharmacotherapies. A nationwide population-based study among Danish patients with incident heart failure and reduced ejection fraction (n = 15.290) investigated the association between socioeconomic factors and quality of care (guideline-recommended process performance measures) [31]. The authors demonstrated that living alone, low-level education, and income in the lowest tertile were associated with reduced number of prescriptions redeemed for recommended medical therapies [31]. Thus, life-long complex pharmacological regimen and rigorous follow-up to monitor graft function and prevent new-onset comorbidities after HTx, may require well-coordinated multidisciplinary care, recipient engagement, and self-management [3], which could be negatively affected in socioeconomic disadvantaged patients. However,

further studies are necessary to examine whether any interventions targeted against this socioeconomic imbalance can be of benefit.

Evidence is sparse regarding the association between multimorbidity and non-adherence to the life-long pharmalogical regime post-HTx. A study using meta-analytical methods reported an overall non-adherence rate of 14.5 cases per 100 recipients per year after HTx [10]. A review (2021) [21] documented that non-adherence to immunosuppressants in HTx recipients differed considerably (25%–40%), however, with adherence rates higher than 80% in several studies [21]. It should be noted that adherence is self-reported in most studies [10, 21]. Similarly, our study more accurately verified that adherence by pharmacy registrations of cost-free immunosuppressants was higher than 80%. Our sub-analysis among cost-free immunosuppression pharmacy data also implies that multimorbidity could impact periods of non-adherence to mainly tacrolimus and mycophenolate mofetil and not adjuvant pharmacotherapies.

In the international BRIGHT study [7] (n = 1,380), non-adherence to the pharmacological management regime post-HTx (1–5 years) has been reported to be 82.7% concerning immunosuppressive medical treatment and 76.1% to co-medical treatment (BASSIS scale). This self-reported non-adherence was significantly (α = 0.05) higher to co-medications than to immunosuppressants (adjusting for data clustering and center levels) [7]. Consistent with these studies, we observed periods of the lowest prevalence of adherence in adjuvant pharmacotherapies as ACE/AT II inhibitors (60%) during follow-up. In the present study, the documented register-verified description of higher prevalence of half-year periods of non-adherence to cost-free immunosuppressants compared with adjuvant pharmacotherapies when categorized by multimorbidity could be underpowered and results thus coincidental. Nonetheless, the observed differences in adherence between pharmacotherapies may be partly explained by differences in multimorbidity and the recipients expected efficacy versus side effects by multiple medical therapies. Thus, prioritizing of certain pharmacotherapies was reflected in recipients' self-management behavior [30].

Socioeconomic inequality in adherence to pharmacotherapies after HTx has been demonstrated in four previous studies. A study from the United States [17] using the UNOS register (n = 33.893) showed that low neighborhood socioeconomic status (index score) was associated with higher risk of non-compliance to immunosuppressive treatment (HR 1.76) [17]. A second analysis of data from the international BRIGHT study [32] examined cost-related medication adherence (CRMNA) to immunosuppressive pharmacotherapies in recipients undergoing HTx. Self-reported items in 1,365 recipients measured CRMNA on average 3.35 ± 1.38 years after surgery. CRISMA was positively associated with being single (OR 2.29, 95% CI 1.17–4.47) and costs being a barrier (OR 2.60, 95% CI 1.66–4.07) [32]. In a single-center Chinese study (n = 168), adherence to immunosuppressants (BAASIS scale) showed that monthly income (<3,000 Chinese Yuan) correlated with non-adherence (OR 3.11, 95% CI 1.58–6.12) [33]. Our findings that mainly recipients living alone and those with low income have half-year periods of higher prevalence of non-adherence to treatment with tacrolimus, mycophenolate mofetil, glucocorticoids, lipid modifying agents, ACE/AT II inhibitors, calcium channel blockers, and loop diuretics agree with these four previous studies. We also found that recipients living alone were younger than those cohabiting. Age-based differences in non-adherence to medical therapies post-HTx were demonstrated in a single-center study from Germany (n = 858) [11]. The overall prevalence of adherence by the ITAS scale was 72.4% and positively associated with age (p < 0.001) [11]. Furthermore, a meta-analysis [34] assessed the impact of social support on organ transplant outcomes (including HTx). Married compared to unmarried recipients experienced 1.46 higher odds of adherence to pharmacotherapies [34]. Our findings are in line with previous studies showing register-verified measures of non-adherence to pharmacotherapies and individual-level indicators of economic and social disadvantage that could facilitate inequalities in self-management ability.

We have not identified other studies investigating the impact of educational level or employment on adherence to the life-long pharmacological management regime after HTx. Our study described that lower prevalence of initiation with ACE/AT II inhibitors and lipid modifying agents as well as half-year periods with higher prevalence of non-adherence to tacrolimus and lipid modifying agents were seen in recipients with low educational degree. Half-year periods with higher prevalence of non-adherence to tacrolimus, calcium channel blockers, and furosemide or bumetanide treatment were observed among unemployed recipients. Unemployed recipients in our study were approximately 3 years older than those employed, and the differences could thus be the result of confounding from age. However, our results could reflect inequalities in both pharmalogical treatment and self-management according to degree of education and employment status. This indicates the need for more focus on these individual indicators of socioeconomic deprivation also in countries with universal healthcare systems. In the view of this, a Danish study in heart-transplant recipients (n = 649) suggested that non-adherence to pharmacotherapies in socioeconomic disadvantages recipients seems to lead to a poorer prognosis [27]. During 1–10 years after HTx, low educational level (adjusted HR 1.66, 95% CI 1.14–2.43) and low income (adjusted HR 1.81, 95% CI 1.02–3.22) were associated with a first-time MACE (composite of readmission due to heart failure, graft failure, percutaneous coronary intervention, and all-cause mortality) [27].

Overall, our findings highlighted that, even in countries with free access to healthcare services and free- or low cost pharmacotherapy, integrated life-long adherence assessment to both immunosuppressive pharmacotherapies and adjuvant pharmacotherapies requires awareness. In the BRIGHT study, multidisciplinary teams, specified patient-centered practice, and higher degree of chronic illness management was associated with higher prevalence of adherence [12, 35, 36]. Interestingly, recent reviews [21, 37] indicate it could be helpful to electronically monitor long-term adherence by validated self-reported adherence questionnaires. We suggest paying attention to the organization and delivery of healthcare services also in universal

healthcare systems, because some socioeconomically disadvantaged HTx recipients with multimorbidity may benefit from more individualized strategies to improve initiation and adherence to life-long pharmalogical management regime.

Data from Danish registers are validated for epidemiological research and have high completeness [22]. Using pharmacy records reduces potential recall bias, the risk of recipients changing behavior during observation, and "dose dumping" to appear more adherent. However, pharmacy records only account for pharmacotherapies dispensed and do not show if and how the medical therapies were used by recipients. We only included pharmacotherapies redeemed using at least one reimbursed prescription within 180 days. Thus, we cannot exclude potential misclassification of adherence to pharmacotherapies started late after transplant. Since the prescribed daily dose is not included in Danish Pharmacy records [28], surrogates for gold standards must be used. Consequently, the definition of adherence relies on assumptions and it may therefore be reasonable to assume that the variable used will involve some residual confounding caused by misclassification. We did not censor hospital stays as it is shown to have minor impact on PDC estimates [38]. We found no indication of any difference between the two Danish transplant centers and no indication of selection bias in the sub-analysis. Our inclusion of two centers working with very similar protocols and nationwide approach is a major strengths. The most pronounced limitation of the present study is the descriptive statistical approach due to small sample size; especially in the sub-study. As an example, description of the pharmacological regimes by decades is relevant due to changes in immunosuppressants and adjuvant medical treatment. However, the small number of recipients within the common time periods (1995–2001, 2002–2009, 2010–2019) makes the graphical illustrations too sensitive. Due to regulations, we are not allowed to present results stratified by decades [23]. Finally, we used independently collected individual-level information of multimorbidity and SEP of high validity only estimated at baseline.

In conclusion, our nationwide register study revealed that in first-time HTx recipients with multimorbidity, treatment with antihypertensive pharmacotherapies and loop diuretics were higher. Among socioeconomic disadvantages recipients, both treatment with and adherence to cost-free everolimus and adjuvant pharmacotherapies were lower.

DATA AVAILABILITY STATEMENT

Data underlying this study cannot be shared publicly because of Danish legislation. Study data, statistical plan, and log-files can be made available through proposal to the Project Database (ID: 707738) at Statistics Denmark. Requests to access the datasets should be directed to https://www.dst.dk/en/TilSalg/Forskningsservice. Foreign researchers can access data from Statistics Denmark through an affiliation with a Danish-authorized research institution.

ETHICS STATEMENT

Register-based studies in Denmark do not require ethics approval. The Danish Data Protection Agency (No. 1-16-02-656-18) approved this study and the Danish Patient Safety Authority authorized access to medical record data (No. 3-3013-3173/1).

AUTHOR CONTRIBUTIONS

RM, HE, and BL designed the study. RM collected the data. RM, HE, and BL directed data management and analysis, which were carried out by IB and EH-P. RM, HE, and BL organized the writing and RM wrote the initial draft. 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.2023.11676/full#supplementary-material

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ORIGINAL RESEARCH
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Performance of Scores Predicting Adverse Outcomes in Procurement Kidney Biopsies From Deceased Donors With Organs of Lower-Than-Average Quality

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Several scores have been devised for providing a prognosis of outcomes after kidney transplantation. This study is a comprehensive test of these scores in a cohort of deceased donors with kidneys of lower-than-average quality and procurement biopsies. In total, 15 scores were tested on a retrospective cohort consisting of 221 donors, 223 procurement biopsies, and 223 recipient records for performance on delayed graft function, graft function, or death-censored graft loss. The best-performing score for DGF was the purely clinical Chapal score (AUC 0.709), followed by the Irish score (AUC 0.684); for graft function, the Nyberg score; and for transplant loss, the Snoeijs score (AUC 0.630) and the Leuven scores (AUCs 0.637 and 0.620). The only score with an acceptable performance was the Chapal score. Its disadvantage is that knowledge of the cold ischemia time is required, which is not known at allocation. None of the other scores performed acceptably. The scores fared better in discarded kidneys than in transplanted kidneys. Our study shows an unmet need for practical prognostic scores useful at the time of a decision about discarding or accepting deceased donor kidneys of lower-than-average quality in the Eurotransplant consortium.

Keywords: kidney transplantation, pathology, transplant loss, marginal donor, procurement biopsies

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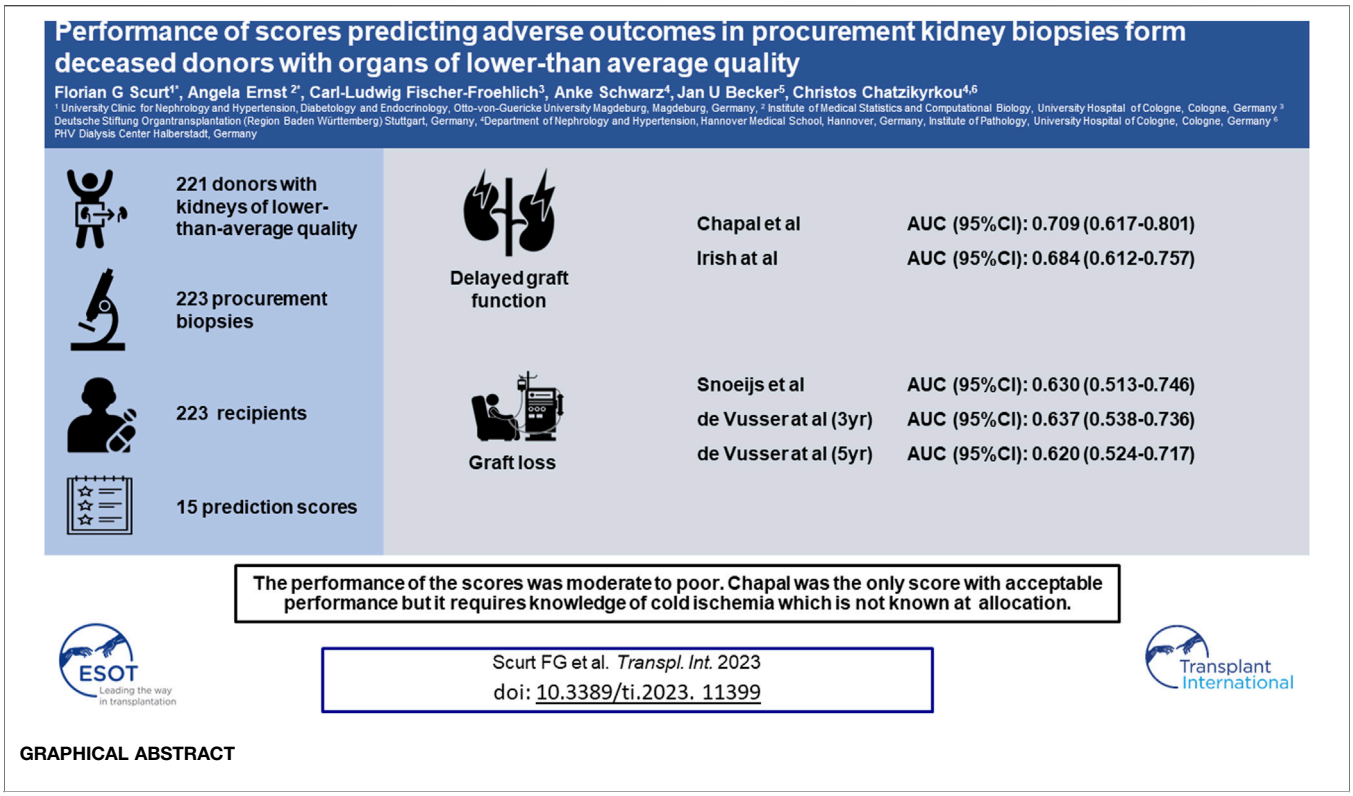
INTRODUCTION

For most patients with end-stage kidney disease, kidney transplantation is the best available treatment with better survival, quality of life and lower use of healthcare resources [1–3]. Despite the increasing use of living donation [4, 5], most patients on dialysis still have to wait on a deceased donor kidney transplant (DDK). Today, transplant physicians are facing the dilemma of how to best use the scarce pool of increasingly older DDKs while avoiding the risk of a poor outcome for the recipients which can be associated with delayed graft function (DGF), premature transplant loss or even endanger their lives [1, 3].

Several purely clinical [6–13], combined clinicohistological [14–16], or purely histological scores [17–20] have been devised for quality assessment of DDKs; the Nyberg score, is for practical purposes best considered clinical, as it does not requires histopathology [9]. The scores with a histology component have been developed on preimplantation but not the clinically decisive procurement biopsies from unselected



GRAPHICAL ABSTRACT

cohorts, reflecting the full spectrum of DDK quality, including those with the lowest risk. Some of these scores have been internally [9, 14, 16] or externally validated in the publications of subsequent scores from other authors or in separate studies. A recent publication has tested four scores [6–8, 12] for their performance in the prognostication of DGF in a large Dutch cohort of unselected preimplantation biopsies [21]. An earlier study from the United Kingdom evaluated the performance of four scores [9, 11, 22, 23] regarding mid-term transplant function [24], two of which have been updated since [7, 9]. A recent study from the United States (US) validated three scores [9, 25, 26] on a single-centre cohort of donors with kidneys of lower quality for the prognostic performance regarding two-year-transplant survival [27]. Similarly, in another study [28], four scores, including that proposed by Banff [16, 19, 25, 29] failed to predict graft survival and early graft function. The scores and their validation studies have helped to better understand and address the causes of DGF and premature transplant failure. However, these scores have never been validated regarding their usefulness for the decision about acceptance or discard of a DDK on a set of procurement biopsies, taken to assess organ quality before allocation. This is particularly important in view of recent data showing that procurement biopsies lead to discard of organs suitable of transplantation [30].

Primary aim of this study is to conduct the overdue comprehensive test of a variety of scores (listed in **Table 1**) for their performance on various end points, such as delayed graft function, graft function, or death-censored graft loss on a retrospective cohort of procurement biopsies specifically commissioned for DDK quality assessment by the Deutsche Stiftung Organtransplantation (DSO; German Foundation for Organ Transplantation), operating within the Eurotransplant consortium. As a secondary aim, we examined whether purely clinical scores perform as well as scores including a histopathology component. Lastly, we wanted to test their performance on the considerable proportion of the discarded kidneys in our cohort.

MATERIALS AND METHODS

Biopsies, Reporting, Donor, and Recipient Data

We extracted data from the "DSO Region Nord" and from the German transplant centers of kidneys allocated, between 1 January 2003, and 31 March 2012. The collection of recipient follow-up data was completed in December 2015. Data were analyzed between 1 January 2018, and 31 May 2020. Only adult recipients of deceased donor kidneys of lower quality were included. Recipients with dual kidney- and combined kidney transplantation were excluded. Our cohort consisted exclusively of brain death donors since donation after cardiac death is not allowed in Germany.

The allocation was under the auspices of Eurotransplant, an international non-profit organization responsible for the coordination and distribution of organs for transplantation between residents of eight European countries.[1]

The following donor data were collected: age, sex, weight, height, body mass index (BMI), length of hospital stay, cardiopulmonary

[1]www.Eurotransplant.org

TABLE 1 | Parameters used in the previously published scores for the quality assessment of DDKs.

Score		Donor								Transplant procedure						Donor kidney									Recipient						
Name	Type	Cause of death	Hypertension	DM	Kidney function	Age	Ethnicity	Weight	HCV	CIT	WIT	DKT	MM	CMV match	Induction therapy with ATG	Global GS	Banff ci	Banff ct	Banff cv	Banff cg	Banff i	Banff ah	Banff mm	Renal artery plaque	Age	Weight	Body mass index	Previous Tx	DM	Dialysis duration	PRA
Balaz[15]	C + P	x				x											x		x												
Chapal[6]	C				x	x				x					x												x				
Irish[7]	C				x	x		x		x	x		x													x				x	x
Jeldres[8]	C					x				x			x												x	x					x
Schold[11]	C	x	x	x		x	x			x			x	x																	
Navarro[17]	P															x	x	x	x			x									
Port[10]	C	x	x		x	x																									
Rao[26]	C	x	x	x	x	x	x		x	x		x	x																		
Snoeijs[20]	P															x	x	x	x	x		x									
de Vusser[16]	C + P					x											x	x													
Remuzzi[19]	P															x	x	x	x												
Anglicheau[14]	C + P		x		x											x															
Nyberg[9]	C	x	x	x	x					x														x							
Ortiz[18]	P															x	x	x	x		x	x	x								
Foucher[13]	C																								x			x	x	x	

Abbreviations: ah, arteriolar hyalinosis; ATG, anti-thymocyte globulin; BMI, body mass index; CIT, cold ischemia time; ci, interstitial fibrosis; cg, glomerular basement membrane splitting; CMV, cytomegalovirus; ct, tubular atrophy; cv, arterial intimal fibrosis; DDK, deceased donor kidney; DKT, double kidney transplantation; DM, diabetes mellitus; EPTS, Estimated post transplant survival score; HCV, hepatitis C virus; GS, glomerulosclerosis; i, interstitial infiltrates; KDRI, kidney donor risk index; MM, miss matches, mm mesangial matrix; PRA, panel-reactive antibodies; WIT, warm ischemia time.

The score designation and the reference are given in the first column; the type of score as in purely clinical (C), combined clinical and pathological (C + P) or solely pathological (P) is given in the second column. Subsequent columns list the parameters used in the respective scores. The parameters are organized as relating to the donor, to the transplant procedure, to the transplant itself or to the recipient. Note that although renal artery plaque as used in the Nyberg score is a pathological finding, it is not typically assessed by a pathologist (pathological and clinic-pathological scores are in italics; the numbers correspond to the references in the manuscript).

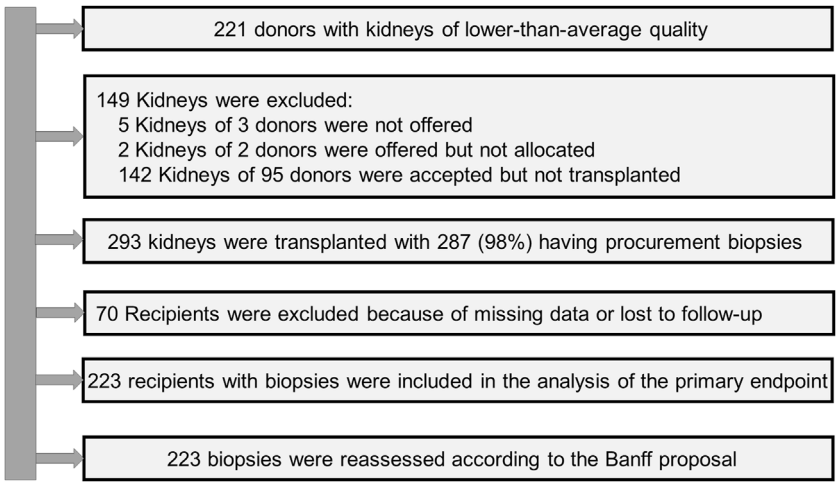


FIGURE 1 | Flow chart of the study (DGF, delayed graft function; m, months; PNF, primary non-function; yr, year).

resuscitation, cardiovascular comorbidities, history of smoking, cause of brain death, use of vasopressors, hemodynamic parameters such as, blood pressure and central venous pressure, creatinine at admission, peak creatinine and creatinine at organ recovery, diuresis volume 24 h and at the last hour before recovery, and urine dipstick test at recovery. Recipients' records were searched for medical history, immunologic risk, peritransplant data, and outcome.

The biopsies were evaluated at the Institute of Pathology in parallel to the transport of the DDK and the preparation for transplantation. Procurement biopsies were not performed in all kidneys but only in that deemed to be of lower quality to increase their chance of acceptance. The results were reported after rapid paraffin-embedding on multiple hematoxylin-eosin and periodic-acid-Schiff-stained sections within 4 h. The DSO oversaw DDK management after notification. The decision about use or discard of the DDK was then made by the transplant physician in the receiving centre. The first assessment was done by the pathologist on duty and included information on representativeness of biopsy, number of glomeruli and arteries, percentage of tubular atrophy, and grading of acute tubular injury. The recommendation was usually suitable/not suitable or partially suitable. The histopathological scores reported below were provided in a second, blinded reading by an experienced nephropathologist. A flowchart of the study is given in **Figure 1**.

Histopathological parameters included type of biopsy (needle or wedge), total number of glomeruli, ratio of globally sclerosed glomeruli, number of arteries (media ≥2 smooth muscle cell layers), presence of focal and segmental glomerulosclerosis (FSGS), Banff Lesion Scores i, t, v, g, ptc, ci, ct, cv, cg, ah, arteriolar fibrosis scored as absent, mild, moderate and severe, cortical tubular hypertrophy, epithelial cell flattening, brush border loss, vacuolisation and luminal detritus scored as 0 (absent), 1 (<25%), 2 (<50%) and 3 (≥50%), tubular nuclear loss scored as 0 (absent), 1 (1 quadrant), 2 (two quadrants), 3 (3 quadrants of the most affected tubular cross-section), pyelonephritis and thrombotic microangiopathy. The Banff meeting report 2011, the Banff consensus criteria for preimplantation biopsies, the german recommendations for procurement biopsies [29, 31, 32] and classification systems for glomerular diseases [33, 34], as well as scoring systems for calcification [35] and acute tubular injury [36, 37] were also considered. A summary of all histopathological parameters is provided in **Supplementary Material**.

Definitions

The definition of lower organ quality depended not on strict criteria but was based on clinical judgment considering the macroscopic appearance of the organ in combination with donor's clinical data. The macroscopic appraisal was done on the "back table," after removal of the perinephric fat and the clean dissection of the vessels from the surrounding tissues. It included organ quality as well as perfusion quality, both of which were rated as good, medium, or poor; likewise, atherosclerosis was characterized as no, mild, or severe. The decision was usually felt after discussion of each case between the senior surgeon of the harvesting team and the physician of the recipient's center. Senior surgeons were accredited by the DSO and had many years of experience in the transplant field.

Extended criteria donors (ECD) were classified as previously reported [38]. eGFR was calculated by means of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Admission, highest, lowest, and terminal eGFR were respectively estimated by using the first, the lowest, the highest and the last serum creatinine prior to organ recovery [39]. Primary non-function (PNF) was defined as the permanent lack of graft function from the time of transplantation [40] and delayed graft function (DGF) as the need for dialysis in the first week [41].

Scores

An overview of the parameters included in the respective scores is given in **Table 1**. Kidney Donor Profile and Risk Index (KDP, KDRI) were calculated according to the Organ Procurement and Transplantation Network (OPTN)[2] and estimated post transplant survival (EPTS) score by the web calculator provided by OPTN (EPTS calculator—OPTN).[3]

[2]https://optn.transplant.hrsa.gov/media/1512/guide_to_calculating_interpreting_kdpi.pdf

[3]hrsa.gov

Outcome Measures

The following outcomes were analyzed: PNF, DGF, graft function at 3 months, one- and 3 years, death censored graft failure and patient death at one, three and 5 years. All survival times were censored at the last date a patient was known to be alive. eGFR results were presented as 10 mL/min per 1.73 m^2 for ease of interpretation.

Statistics

Continuous variables were described as mean ± standard deviation (SD) and central trends between groups compared by Mann-Whitney-U-tests. Fisher's exact- and χ^2-tests were used to compare distributions of categorical variables, respectively. To estimate how well a risk-score discriminates the different endpoints, the area underneath the receiver operating characteristics curve (AUC) was calculated. AUCs range from 0% to 100%, with 0% suggesting perfect inaccuracy, 100% perfect accuracy, 50% suggesting no discrimination and 50%–70% suggesting poor discrimination, 70%–80% suggesting acceptable and 80%–90% excellent and finally 90% suggesting outstanding performance [42, 43]. A *p*-value below 0.05 was considered significant in all comparisons in two-sided tests; however, in this retrospective observational study, *p*-values can only be considered descriptive. Statistical analysis was performed with the use of SPSS software, v24 (IBM Corp, Armonk, NY, United States) and IBM SPSS Statistics Essentials for R.

Ethical Permission

All organ transplants were performed according the Declaration of Istanbul [44]; no transplants from prisoners were used. The study was conducted in accordance with the Declarations of Helsinki and approved by the local ethical review board of Hannover Medical School (No. 1519-2012).

RESULTS

Donors' and Recipients' Characteristics

From 442 kidneys recovered from 221 donors, 149 were discarded. In 287 (98%) of the 293 transplanted kidneys the tissue blocks were found. Follow-up data were available from 223 recipients (**Figure 1**). The KDRI was 1.48 and 107 (63.3%) were ECD. The average age was 61 years and 54% were males. Only 13% of donors had diabetes and 30% cardiovascular disease. The prevalence of hepatitis B and C was low (6.5% and 1.2%). Cerebrovascular accident was the most common cause of brain death (60%). The serum creatinine at recovery was 149 μmol/L. Approximately 50% of donors experienced acute kidney injury (AKI) (**Table 2**). The accepted kidneys showed macroscopically a good perfusion and organ quality at all, except for atherosclerosis which was severe in 46.5% of them. Biopsies were performed in 80% and the majority were needle biopsies with a representative number of glomeruli and arteries. Mean and minimal (<5%) global glomerulosclerosis were 10.4% and 50% respectively, whereas the majority of acute and chronic tubular, interstitial, and vascular Banff lesion scores were of low grade. On the contrary, acute tubular injury was, as expected, more severe (**Table 3**). The average age of recipients

TABLE 2 | Demographic data and ICU monitoring parameters of the donors.

Characteristic	Value
Donor characteristics (No. of donors = 169)	
Age, y	60.8 ± 16.2
Sex, n (%)	
Female	77 (45.6)
Male	92 (54.4)
BMI, kg/m^2	27.4 ± 5.8
Diabetes mellitus, n (%)	22 (13.0)
Hypertension, n (%)	96 (56.8)
Cardiovascular disease, n (%)	49 (29.2)
Smoker, n (%)	46 (27.2)
Hepatitis B Virus positive, n (%)	11 (6.5)
Hepatitis C Virus positive, n (%)	2 (1.2)
Cytomegalovirus positive, n (%)	110 (65.1)
Cerebrovascular accident (CVA), n (%)	101 (59.8)
Extended Criteria Donors (ECD), n (%)	107 (63.3%)
Kidney Donor Risk Index (KDRI)	1.48 ± 0.51
KDRI Grading, n (%)	
KDRI Grade I (0–20)	11 (6.5)
KDRI Grade II (21–40)	17 (10.1%)
KDRI Grade III (41–60)	29 (17.2)
KDRI Grade IV (61–80)	27 (16.0)
KDRI Grade V (81–100)	85 (50.3)
Donor ICU data	
Time ICU until confirmed brain death, h	118.1 ± 126.6
Time confirmed brain death until cross-clamp, h	13.2 ± 14.8
CPR at ICU stay, n (%)	30 (17.8)
Transfusion at ICU stay, n (%)	18 (10.7)
Units of RBC, n (%)	3.06 ± 8.13
Units of FFP, n (%)	3.28 ± 9.28
Volume expander at ICU stay, n (%)	25 (14.8)
Diuretics at ICU stay, n (%)	22 (13.1)
Antidiuretics at ICU stay, n (%)	57 (33.9)
Antibiotics at ICU stay, n (%)	90 (53.3)
AKI, n (%)	79 (46.7)
RIFLE criteria, n (%)	
No AKI	90 (53.3)
Risk	46 (27.2)
Injury	13 (7.7)
Failure	20 (11.8)
Serum creatinine, μmol/L	
At admission	111 ± 88
Minimum	101 ± 73
Peak	161 ± 129
Last	149 ± 119
Last blood and urine values before cross clamp	
Hemoglobin, g/dL	17.5 ± 3.4
White cell count, per cubic millimeter	13.58 ± 5.052
Platelet count, per cubic millimeter	176,868 ± 97,578
International normalized ratio	1.27 ± 0.51
Activated partial thromboplastin time, sec	39.4 ± 16.6
Aspartate transaminase, IU/L	123.4 ± 293.4
Alanine transaminase, IU/L	109.2 ± 357.4
Alkaline phosphatase, IU/L	98.3 ± 51.2
Lactate dehydrogenase, IU/L	456.9 ± 500.6
Total bilirubin, mg/dL	16.7 ± 16.6
C-reactive protein, mg/L	187.8 ± 191.6
Urine protein dipstick, % (neg/1+/2+)	64.1/29.9/6.0
Urine volume last 24 h, mL/kg	42.3 ± 32.4
Urine volume last hour, mL/kg	2.44 ± 5.93

(Continued on following page)

TABLE 2 | (*Continued*) Demographic data and ICU monitoring parameters of the donors.

Characteristic	Value
Donor data at cross-clamp period	
Time incision until cross-clamp, min	52.6 ± 31.0
Time cross-clamp until ectomy right	43.7 ± 16.6
Time cross-clamp until ectomy left	49.0 ± 17.9
Catecholamines, n (%)	130 (76.9)
Mean Arterial Blood pressure, mmHg	97.2 ± 15.8
Pulse,/min	96.4 ± 25.6
Central venous pressure, mmHg	9.74 ± 3.69
Temperature, °C	36.61 ± 1.17

Continuous parameters are given as mean ± standard deviation, numerical and ordinal parameters as count and percentage.
Abbreviations: AKI, acute kidney injury; BMI, body mass index; DDK, deceased donor kidney; DGF, delayed graft function; FFP, fresh frozen plasma; HBsAG, hepatitis B virus surface antigen; ICU, intensive care unit; IU, international units; RBC, red blood cells.

was 61 years. They showed a low immunologic risk profile, a cold ischemia time (13.8 h) which was at the lower range of that reported for Eurotransplant [45] and a high EPTS score (**Table 4**). PNF occurred in 26 (11.7%) and DGF in 109 (48.9%) patients. We observed 49 graft losses during a median follow-up of 43.8 months (IQR 19–68 months). Patient and death-censored graft survival at 1, 3, and 5 years after kidney transplantation were respectively 90.6% and 91.1%, and 86.1% and 82.9% and 83% and 81.6% (**Table 5**).

Donor and Organ Related Differences Between Discards and Transplantations

149 of the 442 available kidneys were discarded (33%). 45 were recovered from donors whose contralateral kidney was transplanted and 104 from donors whose both kidneys were discarded (**Figure 2**).

TABLE 3 | Macroscopic and histopathological parameters.

Characteristics	Transplanted kidneys (*n* = 223)
Macroscopic parameters[a]	
Perfusions quality, % (good/medium/bad)	94.6/3.1/2.2
Organ quality, % (good/medium/bad)	74.0/24.2/1.8
Atherosclerosis, % (no/mild/severe)	38.4/15.2/46.5
Organ localization, % (right kidney/left kidney)	48.4/51.6
Histopathological parameters	
Biopsy performed, n (%)	179 (80.3)
Art of biopsy, % (Needle/Wedge)	82.1/17.9
Renal cortex proportion of total parenchyma, %	66.1 ± 34.2
Glomeruli, *n*	36.2 ± 69.0
Arteries, *n*	8.1 ± 15.2
Global glomerulosclerosis, % of total glomeruli	10.4 ± 15.0
Global glomerulosclerosis < 5, %	50.3
Any FSGS, % of biopsies	2.5
Banff Lesion Scores (0/1/2/3), %	
Interstitial inflammation (i)	84.2/14.6/1.2/0.0
Tubulitis (t)	88.9/11.1/0.0/0.0
Intimal arteritis (v)	99.4/0.6/0.0/0.0
Glomerulitis (g)	86.0/12.3/1.1/0.6
Peritubular capillaritis (ptc)	100.0/0.0/0.0/0.0
Interstitial fibrosis (ci)	80.1/18.1/1.2/0.6
Tubular atrophy (ct)	61.4/36.8/1.2/0.6
Vascular fibrous Intimal thickening (cv)	41.5/33.9/21.1/3.5
Glomerular basement membrane splitting (cg)	97.1/2.9/0.0/0.0
Mesangial matrix expansion (mm)	81.9/14.0/1.8/2.3
Arteriolar hyalinosis (ah)	35.1/38.6/22.8/3.5
Interstitial fibrosis and tubular atrophy, % (0–10/10–25/25–50/>50)	70.4/14.3/13.9/0.9
Arteriolar wall fibrosis, % (no/mild/moderate/severe)	54.4/35.1/9.4/1.2
RPS diabetic nephropathy class ≥1, %	5.2
Thrombotic microangiopathy, %	6.4
Nephrocalcinosis, % (no/mild/moderate to severe)	88.9/4.7/6.4
Tubular hypertrophy, %	19.3
Epithelial cell flattening (0/1/2/3), %	3.5/40.4/32.7/23.4
Brush border membrane defect (0/1/2/3), %	1.2/26.9/46.8/25.1
Vacuolization (0/1/2/3), %	7.0/22.8/22.2/48.0
Loss of nuclear staining (0/1/2/3), %	1.8/27.5/38.0/32.7
Cellular detritus (0/1/2/3), %	15.8/40.9/23.4/19.9
Pyelonephritis, %	8.2

Continuous parameters are given as mean ± standard deviation, numerical and ordinal parameters as count and percentage.
Abbreviations: DDK, deceased donor kidney; FSGS, focal and segmental glomerulosclerosis; RPS, renal pathology society.
[a]*Of note, macroscopic parameters listed in this table were determined by the harvesting surgeon, and not by a pathologist while the histopathological parameters were determined retrospectively by an experienced nephropathologist.*

TABLE 4 | Clinical parameters of recipients.

	Recipients with follow-up data (*n* = 223)
Recipients' parameters	
Age, y	61.0 ± 13.5
Sex, n (%)	
Female	75 (33.6)
Male	148 (66.4)
BMI, kg/m^2	25.5 ± 4.4
Diabetes mellitus, n (%)	59 (26.5)
Hypertension, n (%)	191 (85.7)
Cardiovascular disease, n (%)	96 (43.0)
HBsAg positive, n (%)	49 (22.0)
Hepatitis C Virus positive, n (%)	6 (2.7)
Cytomegalovirus positive, n (%)	148 (66.4)
Dialysis vintage, months	166.9 ± 79.2
Prior organ transplant, n (%)	24 (10.8)
Estimated Post Transplant Survival (EPTS)	2.66 ± 0.62
Estimated Post Transplant Survival (EPTS) Groups, n (%)	
Group 1: 0%–20%	18 (8.1)
Group 2: 21%–40%	14 (6.3)
Group 3: 41%–60%	29 (13.0)
Group 4: 61%–80%	30 (13.5)
Group 5: 81%–100%	131 (58.7)
Transplant baseline parameters	
HLA-A mismatch (0/1/2), %	14.3/56.1/29.6
HLA-B mismatch (0/1/2), %	8.1/48.9/43.0
HLA-DR mismatch (0/1/2), %	14.3/55.2/30.5
Negative PRA at transplantation, n (%)	200 (89.7)
Average PRA at transplantation, %	2.4 ± 9.7
Historic Peak of PRA, %	7.5 ± 20.8
Origin of donor kidney (right/left/both), %	50.7/48.0/1.3
Cold ischemia time, h	13.8 ± 5.0
Warm ischemia time, min	40.6 ± 14.3
Maintenance therapy	
Calcineurin inhibitors, % (Cyclosporin/Tacrolimus/other)	74.8/24.5/0.7
Anti-metabolites, % (Azathioprine/Mycophenolate/other)	0.7/84.1/15.2
mTOR inhibitors, %	4.2
Steroids, %	91.0

Continuous parameters are given as mean ± standard deviation, numerical and ordinal parameters as count and percentage.
Abbreviations: b, both; BMI, body mass index; HBsAg, hepatitis b virus surface antigen; HLA, human leukocyte antigen; PRA, panel reactive antibodies.

Except for the higher prevalence of hepatitis C and the longer duration of brain death, there were no differences in the baseline characteristics between donors of transplanted and discarded kidneys (**Table 6**).

The discarded kidneys were of lower macroscopic organ quality (deemed to be bad in 9.2% vs. 1.7%, $p < 0.001$) and showed more chronic glomerular (FSGS: 9.4% vs. 2.1%, $p = 0.013$; cg3: 2% vs. 0%, $p = 0.03$), more severe acute tubular cell (cellular detritus score 3: 33% vs. 21%, $p = 0.036$), more chronic tubulointerstitial (ci, ct, IFTA $p < 0.001$) and more chronic macrovascular injury (cv ≥ 1: 75% vs. 62%, $p = 0.02$). There were no differences in the percentage of glomerulosclerosis at all (11% vs. 10%, $p = 0.305$) or other tubular cell injury features. Lastly, findings of thrombotic microangiopathy (TMA) were more often observed (14.9% vs. 5.6%, $p = 0.001$) (**Table 7**).

The following categories of reasons for discard were recorded: 1) Macroscopic organ damage, such as renal capsule fissure, cortical hemorrhage, large infarcts, large renal cysts, heavy aortic patch and/or renal artery atherosclerosis and mottled appearance after reperfusion. 2) findings of procurement biopsies. 3) concerns about a transmissible donor infection, 4) extrarenal malignancy known or detected during procurement or tumor of the contralateral kidney; 5) denial of the transplant center to finally accept the offer 6) non transplantability of the recipient.

47 kidneys were discarded due to macroscopic findings, 43 due to the results of biopsy and 27 due to one of the reasons belonging to categories 3 to 6. Unfortunately, for nearly every fifth discarded kidney (32/149, 21.5%) the exact reason remained unknown.

Score Performance in Transplanted Kidneys

The performance of the scores is shown in **Table 8**. Depending on missing data, up to 103 (46%) out of the 223 DDKs had to be excluded for the analysis of the endpoints.

TABLE 5 | Outcome data of recipients.

	Recipients with follow-up data (*n* = 223)
Primary non function, %	26 (11.7)
Delayed graft function, %	109 (48.9)
Patient survival at 1 year, %	202 (90.6)
Patient survival at 3 years, %	192 (86.1)
Patient survival at 5 years, %	185 (83.0)
Death-censored graft survival at 1 year, %	163 (91.1)
Death-censored graft survival at 3 years, %	141 (82.9) ($n_{missing}$ = 6)
Death-censored graft survival at 5 years, %	133 (81.6) ($n_{missing}$ = 6)
Kidney function at 3 months (creatinine), µmol/L	188.3 ± 77.9 ($n_{missing}$ = 1)
Kidney function at 3 months (eGFR), mL/min/1.73 m^2	34.6 ± 14.7 ($n_{missing}$ = 1)
Kidney function at 1 year (creatinine), µmol/L	166.9 ± 52.9 ($n_{missing}$ = 1)
Kidney function at 1 year (eGFR), mL/min/1.73 m^2	37.4 ± 13.6 ($n_{missing}$ = 1)
Kidney function at 3 years (creatinine), µmol/L	165.8 ± 59.8 ($n_{missing}$ = 61)
Kidney function at 3 years (eGFR), mL/min/1.73m^2	38.4 ± 15.2 ($n_{missing}$ = 61)
Rejections overall	0.65 ± 1.05 ($n_{missing}$ = 93)
Without Rejections (%)	57.7

Continuous parameters are given as mean ± standard deviation, numerical and ordinal parameters as count and percentage.

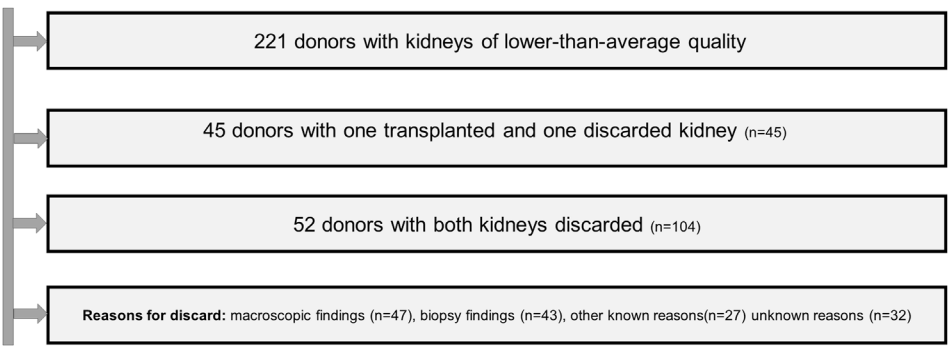


FIGURE 2 | Flow chard of the handling of discarded organs.

Chapal and Irish had the best predictability for DGF with an AUC of 0.709 and 0.684, respectively, whereas Jeldres had an AUC of 0.503, Balaz of 0.506/0.490, and Schold of 0.451. For the prognostication of graft survival, the best-performing scores were of Rao and Port for 1 year with a significant AUC of 0.699 and 0.662, followed by de Vusser for 3 years, Snoeijs and de Vusser for 5 years with respective AUCs of 0.637, 0.630 and 0.620. Regarding graft function the trend was similar. Here, Navaro was acceptable, whereas the performance of Anglicheau poor (AUC 0.649) and the significance of Ortiz marginal (Kendall's tau 1 year 0.157, p = 0.026). The predictive power of the EPTS score was poor (AUC 0.642).

Score Performance in Discarded Kidneys

In another approach we tested the scores for the prediction of discards (**Table 9**). The best results for the comparison between bilateral discard and bilateral transplantation (column A vs. column C of **Table 9**) showed Balaz (1.80 vs. 1.11, p = 0.034), Snoeijs (4.55 vs. 3.12, p = 0.028), Remuzzi (p = 0.013) and Ortiz (4.36 vs. 2.83, p = 0.029). For the comparison between unilateral discard and bilateral transplantation (column B vs. column C of **Table 9**), Balaz <1 (p = 0.030), Navaro (p = 0.010) and Remuzzi (p = 0.011) came out to be significant.

DISCUSSION

Primary aim of this retrospective study was to test the performance of scores previously devised for quality assessment of a DDK of lower quality for their value in supporting the decision about discard or acceptance. The rather dismal clinical outcome in our cohort with 48.9% and 15.8% of recipients respectively developing DGF or losing their graft within the first year shows that it was indeed a formidable real-life challenge for the scores.

For DGF we found an acceptable discrimination with an AUC of 0.709 for the Chapal score. The Irish score could have even performed better if we would have been able to provide the missing recipient parameter of "previous blood transfusion." Moreover, the applicability of the purely clinical and thus economical Irish score is

TABLE 6 | Comparison of baseline characteristics between donors with transplanted and discarded kidneys.

	Transplanted kidneys (*n* = 293)	Discarded kidneys (*n* = 149)	*p*-value
Donor characteristics			
No of Donors	169	97	
Age, y	60.8 ± 16.2	61.4 ± 15.2	0.999
Sex, n (%)			
Female	77 (45.6)	38 (39.6)	0.345
Male	92 (54.4)	58 (60.4)	
BMI, kg/m^2	27.4 ± 5.8	27.3 ± 5.4	0.874
Diabetes mellitus, n (%)	22 (13.0)	14 (14.6)	0.721
Hypertension, n (%)	96 (56.8)	56 (58.3)	0.809
Cardiovascular disease, n (%)	49 (29.2)	30 (31.6)	0.682
Smoker, n (%)	46 (27.2)	27 (28.1)	0.874
Hepatitis B Virus positive, n (%)	11 (6.5)	7 (7.3)	0.808
Hepatitis C Virus positive, n (%)	2 (1.2)	6 (6.3)	**0.021**
Cytomegalovirus positive, n (%)	110 (65.1)	65 (67.5)	0.665
Cerebrovascular accident (CVA), n (%)	101 (59.8)	50 (52.1)	0.225
Kidney Donor Risk Index (KDRI)	1.48	1.52	0.788
Time confirmed brain death until cross-clamp, h	13.2 ± 14.8	15.8 ± 17.1	**0.032**
AKI, n (%), Creatinine first, max	61 (36.1)	32 (33.3)	0.651
AKI, n (%), Creatinine min, max	79 (46.7)	40 (41.7)	0.424
Last serum creatinine, mg/dL	1.68 ± 1.35	1.80 ± 1.40	0.775
Last creatinine kinase	849 ± 1,046	1,608 ± 8,345	0.435
Last Sodium, mmol/L	148.0 ± 9.4	147.6 ± 7.9	0.847
Blood pressure, mmHg			
Systolic	126.1 ± 22.1	126.1 ± 25.0	0.918
Diastolic	68.0 ± 12.3	68.3 ± 14.9	0.870
Mean arterial	97.2 ± 15.8	97.3 ± 18.3	0.932
Pulse/min	96.4 ± 25.6	97.2 ± 24.1	0.889
Central venous pressure, mmHg	9.74 ± 3.69	9.5 ± 3.7	0.803
Central venous pressure—PEEP, mmHg	4.94 ± 4.60	4.7 ± 4.5	0.568
Temperature, °C	36.61 ± 1.17	36.7 ± 1.2	0.631
P_aO_2/FiO_2 Ratio	252.1 ± 108.8	266.6 ± 108.7	0.425
Last urine test strip, % (neg/+/++)			
Protein	64.1/29.9/6.0	63.8/33.0/3.2	0.570
Leukocytes	56.8/27.7/15.5	53.6/34.5/11.9	0.491
Red blood cells	36.8/40.1/23.0	29.4/40.0/30.6	0.351
Nitrite	81.3/18.7/0.0	85.1/14.9/0.0	0.458
Urine volume last 24 h, mL	3,347 ± 2,272	3,372 ± 2,053	0.657
Urine volume last 24 h, mL/kg	42.3 ± 32.4	43.075 ± 29.4	0.533
Urine volume last hour, mL	194 ± 499	196 ± 288.6	0.236
Urine volume last hour, mL/kg	2.44 ± 5.93	2.53 ± 3.87	0.247

Continuous variables are presented as mean ± standard deviation.

Abbreviations: AKI, acute kidney injury; BMI, body mass index; CPR, cardiopulmonary resuscitation; DGF: delayed graft function; dl, deciliter; g, gram; h, hours; IU, international units; kg, kilogram; L, liter; mL, milliliter; min, minutes; mmHg, Millimeter of mercury; mmol, millimole; m^2, square meter; sec; seconds; y, years; µg, microgram.

Bold values represent statistically significant parameters.

[a]*One kidney from one donor with missing data about transplantation status.*

limited because it requires the cold and warm ischemia time, both unknow at the time of allocation. Conversely, the Chapal score required donor- and recipient parameters, which, except for the cold ischemia time, are easily to obtain. The score of Chapal showed a lower AUC than that reported in the initial publication [6]. This may be explained by the higher incidence of DGF in our cohort (48.9% vs. 25.4% reported by Chapal).

Similarly poor results were seen for the Anglicheau and Ortiz scores to predict graft function. Their poor performance may be explained by the higher age of our recipients, compared with those in the cohorts of Anglicheau and Ortiz (61.0 vs. 50.6 vs. 48 years), as well as the higher ratio of our donors with hypertension (56.8% vs. 30.8%) and their higher creatinine levels before organ removal (149 vs. 101 µmol/L) compared with those in the cohort of Anglicheau. However, the better performing score of Nyberg, requires cold ischemia time, a parameter not known at the time of allocation.

None of the scores for graft survival reached an acceptable performance. The pathological scores of Navarro and Snoejjs and the clinicopathological of de Vusser outperformed the solely clinical Rao and Port's scoring systems. This suggests that there are aspects of donor organ quality that cannot be reliably determined from clinical data alone. Inclusion of pathologic data could allow for better assessment of overall organ quality, particularly in kidneys of lower-than-average quality and explain the better performance of the scores with histopathology. Still, this was not sufficient to push AUC into the acceptable range. The score of Navarro [17] has been adopted by the Spanish Society of Nephrology [46]. Here, kidneys with a score <8 are proposed for single transplantation. The very poor results obtained by

TABLE 7 | Comparison of macroscopic and histological characteristics between transplanted and discarded kidneys.

	Transplanted kidneys (*n* = 293)	Discarded kidneys (*n* = 149)	*p*-value
Macroscopic characteristics			
Perfusions quality, (good/medium/bad), %	93.5/4.4/2.0	92.2/7.1/0.7	0.310
Organ quality (good/medium/bad) %	73.4/24.9/1.7	61.0/29.8/9.2	**<0.001**
Atherosclerosis (No/Mild/Severe), %	38.2/16.8/45.0	36.4/14.5/49.1	0.864
Histopathological characteristics			
Glomerulosclerosis, %	11.3 ± 17.2	10.1 ± 12.8	0.305
FSGS, %	2.1	9.4	**0.013**
Banff Lesion Scores (0/1/2/3), %			
Interstitial inflammation (i)	82.3/15.7/2.0/0.0	86.1/9.9/2.0/2.0	0.130
Tubulitis (t)	88.9/11.1/0.0/0.0	85.1/13.9/0.0/1.0	0.288
Intimal arteritis (v)	99.0/1.0/0.0/0.0	96.0/4.0/0.0/0.0	0.085
Glomerulitis (g)	86.4/12.1/1.0/0.5	88.1/7.9/1.0/3.0	0.244
Peritubular capillaritis (ptc)	100.0/0.0/0.0/0.0	100.0/0.0/0.0/0.0	>0.999
Interstitial fibrosis (ci)	78.8/19.2/1.5/0.5	61.4/25.7/7.9/5.0	**<0.001**
Tubular atrophy (ct)	60.6/37.4/1.5/0.5	33.7/53.5/7.9/5.0	**<0.001**
Interstitial fibrosis and tubular atrophy (IFTA), % (0–10/10–25/25–50/>50)	73.4/12.3/14.0/0.3	54.7/18.9/23.0/3.4	**<0.001**
Interstitial fibrosis and tubular atrophy (IFTA), % MW (±SD)	3.10 ± 6.80	7.39 ± 13.09	**<0.001**
Vascular fibrous Intimal thickening (cv)	38.4/37.9/20.2/3.5	24.8/43.6/26.7/5.0	0.120
cv ≥ 1	61.6	75.2	**0.018**
GBM double contours (cg) (0/1/2/3)	97.0/3.0/0.0/0.0	98.0/0.0/0.0/2.0	**0.030**
Mesangial matrix expansion (mm)	82.8/12.1/2.0/3.0	86.1/5.0/1.0/7.9	0.058
Arteriolar hyalinosis (ah)	33.3/38.9/22.7/5.1	29.7/49.5/13.9/6.9	0.163
Interstitial fibrosis and tubular atrophy (IFTA)	3.10 ± 6.80	7.39 ± 13.09	**<0.001**
Thrombotic microangiopathy, %	5.6	14.9	**0.007**
Nephrocalcinosis (No/Mild Moderate/Severe), %	89.4/4.0/6.6/0.0	88.1/7.9/4.0/0.0	0.260
Tubular hypertrophy, %	18.7	27.7	0.073
Epithelial cell flattening (0/1/2/3), %	4.0/39.4/32.8/23.7	7.9/33.7/32.7/25.7	0.461
Brush border membrane defect (0/1/2/3), %	1.0/25.8/46.5/26.8	2.0/18.8/43.6/35.6	0.291
Vacuolization (0/1/2/3), %	7.6/22.2/21.2/49.0	4.0/24.8/20.8/50.5	0.660
Loss of nuclear staining (0/1/2/3), %	2.5/28.3/37.9/31.3	0.0/22.8/44.6/32.7	0.251
Cellular detritus (0/1/2/3), %	16.2/40.9/22.2/20.7	7.9/33.7/25.7/32.7	**0.036**

Continuous variables are presented as mean ± standard deviation. MW, mean value; SD, standard deviation.
Bold values represent statistically significant parameters.

Navarro et al in their study transplanting kidneys with a score 6–7 were not confirmed later by others [47].

In summary, the majority of the scores are not suitable for procurement biopsies because they include information, which is not available during procurement. Beyond that, the scores were developed after examination of paraffin embedded renal tissue, a procedure that is time consuming and not practical in the limited time setting of allocation. The only exception is the Remuzzi score, which was based on frozen sections. However, in our experience frozen sections are often difficult to evaluate due to inappropriate handling during transport [31].

Procurement may also lead to needless discards if the histopathologic evaluation is conducted by general pathologists and not by nephropathologists. The failure of pretransplant biopsies to predict graft outcomes was highlighted in an older metaanalysis of 47 studies testing 15 scores [48]. In a recent paper, more than half of kidneys discarded in US would have been suitable for transplant in France, where procurement biopsies are rarely performed [49]. Furthermore, their usefulness has been questioned due to low reproducibility and poor predictive power [50], albeit there are centers proposing punch- instead of wedge or needle- biopsies as a means to improve standardization, sample adequacy and reproducibility [51]. At all, scores based on preimplantation biopsies can be implemented to predict graft function but their applicability to decide on transplantation or discard has probably been overestimated [52].

Strengths of our study were the comprehensive evaluation exclusively of procurement biopsies by an experienced nephropathologist according to the most recent Banff criteria [29] and the validation of the most known scores for the endpoints for which they have been developed.

Limitations should also be recognized. **First**, the definition of DGF as need for dialysis within the first week after transplantation, an endpoint that may be influenced by various clinical factors (such as heart failure, hyperkalemia, etc.) is not uniformly accepted. Furthermore, we excluded PNF, because it has a different pathogenesis [40] and was not tested as outcome parameter in the scores. The extraordinarily high incidence of PNF and DGF was probably due to bias by indication; our cohort was highly selective since biopsies were performed only in those donors whose organs were supposed to be of lower quality. Another reason was the higher incidence of donors with AKI an acknowledged risk factor for both outcomes [53]. **Second**, the scores have been constructed on preimplantation biopsies, which are in terms of prognostication completely different from procurement biopsies due to the accrued damage during cold preservation and transport as well as the reperfusion injury after implantation. **Third,** the number of missing data implies that each score was tested on

TABLE 8 | Previously published scores for the quality assessment of DDKs tested in this study including the endpoints they were designed for and their performance in the original publication.

Publication/Score	Endpoints	Performance in original publication	Performance in our cohort
Delayed graft function			
[15]*Balaz et al.* (n = 171)	DGF	AUC (95% CI)$_{CIV\ Score}$: 0.659 (0.606–0.710) AUC (95% CI)$_{CIV\ Score\ +\ donor\ age\ +\ cause\ of\ death}$: 0.694 (0.642–0.743)	AUC (95% CI)$_{CIV\ Score}$: 0.506 (0.417–0.595) AUC (95% CI)$_{CIV\ Score\ +\ donor\ age\ +\ cause\ of\ death}$: 0.490 (0.401–0.579)
[6]Chapal et al. (n = 131)	DGF	AUC (95% CI): 0.73 (0.68–0.77)	AUC (95% CI): 0.709 (0.617–0.801)
[a7]Irish et al. (n = 223)	DGF	AUC 0.704	AUC (95% CI): 0.684 (0.612–0.757)
[8]Jeldres et al. (n = 223)	DGF	AUC: 0.743	AUC (95% CI): 0.503 (0.423–0.582)
[11]Schold et al. (n = 222)	DGF	Rate of DGF Donor Grade I: 16.7% Donor Grade II: 23.1% Donor Grade III: 30.3% Donor Grade IV: 39.2% Donor Grade V: 46.3% AUC (95% CI): NA	Rate of DGF Donor Grade I: 42.9% Donor Grade II: 70.0% Donor Grade III: 68.6% Donor Grade IV: 61.2% Donor Grade V: 53.2% AUC (95% CI): 0.451 (0.373–0.530)
Graft survival			
[17]*Navarro et al.* (n = 223)	5 years graft survival	HR (95% CI)$_{Full\ Score}$: NA HR (95% CI)$_{Score\ >5\ vs.\ \leq5}$: 6.95 (1.57–30) AUC (95% CI)$_{Full\ Score}$: NA AUC (95% CI)$_{Score\ >5\ vs.\ \leq5}$: NA	HR (95% CI)$_{Full\ Score}$: 1.501 (1.143–1.972) HR (95% CI)$_{Score\ >5\ vs.\ \leq5}$: 1.994 (0.975–4.079) AUC (95% CI)$_{Full\ Score}$: 0.617 (0.513–0.722) AUC (95% CI)$_{Score\ >5\ vs.\ \leq5}$: 0.567 (0.462–0.673)
[19]Port et al. (n = 223)	1 and 3 years graft survival	1 year graft survival for RR < 1.7/≥1.7: 90.6/84.5% AUC (95% CI)$_{1\ year}$: NA 3 years graft survival for RR <1.7/≥1.7: 79.4/68.0% AUC (95% CI)$_{3\ years}$: NA	1 year graft survival for RR <1.7/≥1.7: 91.0/80.7% AUC (95% CI)$_{1\ year}$: 0.662 (0.369–0.955) 3 years graft survival for RR <1.7/≥1.7: 87.5/75.8% AUC (95% CI)$_{3\ years}$: 0.603 (0.515–0.692)
[26]Rao et al. (n = 223)	1, 3, and 5 years graft survival	AUC (95% CI)$_{1\ year}$: NA AUC (95% CI)$_{3\ years}$: NA AUC (95% CI)$_{5\ years}$: NA 5 years graft survival KDRI quintile 1: 82% 5 years graft survival for KDRI quintile 2: 79% 5 years graft survival for KDRI quintile 3: NA 5 years graft survival for KDRI quintile 4: NA 5 years graft survival for KDRI quintile 5: 63%	AUC (95% CI)$_{1\ year}$: 0.699 (0.459–0.939) AUC (95% CI)$_{3\ years}$: 0.557 (0.456–0.658) AUC (95% CI)$_{5\ years}$: 0.576 (0.474–0.679) 5 years graft survival KDRI quintile 1: 80.6% 5 years graft survival for KDRI quintile 2: 73% 5 years graft survival for KDRI quintile 3: 79% 5 years graft survival for KDRI quintile 4: 76% 5 years graft survival for KDRI quintile 5: 68%
[20]*Snoeijs et al.* (n = 171)	5 years graft survival	AUC: 0.74	AUC (95% CI): 0.630 (0.513–0.746)
[16]*Vusser et al.* (n = 223)	3 years graft survival	AUC (Historic cohort): 0.65 AUC (Validation cohort): 0.70	AUC (95% CI): 0.637 (0.538–0.736)
[16]*Vusser et al.* (n = 223)	5 years graft survival	AUC (Historic cohort): 0.67 AUC (Validation cohort): 0.81	AUC (95% CI): 0.620 (0.524–0.717)
[19]*Remuzzi et al.* (n = 223)	3 years graft survival	AUC: N/A	AUC (95% CI): 0.605 (0.501–0.709)
Graft function			
[14]*Anglicheau et al.* (n = 223)	1 year graft function	AUC $_{eGFR\ <\ 25\ mL/min\ at\ 1\ year}$: 0.84	AUC (95% CI) $_{eGFR\ <\ 25\ mL/min\ at\ 1\ year}$: 0.649 (0.540–0.758)
[9]Nyberg et al. (n = 223)	1 year graft function	Mean creatinine clearance Kidney Grade A: 61.1 mL/min Kidney Grade B: 51.8 mL/min Kidney Grade C: 42.6 mL/min Kidney Grade D: 33.7 mL/min	Mean creatinine clearance Kidney Grade A: 51.5 mL/min Kidney Grade B: 42.7 mL/min Kidney Grade C: 35.7 mL/min Kidney Grade D: 34.8 mL/min
[18]*Ortiz et al.* (n = 171)	1 and 2 years graft function	Kendall's tau$_{1\ year}$: 0.277 (p = 0.0006) Kendall's tau$_{2\ years}$: 0.286 (p = 0.0005)	Kendall's tau$_{1\ year}$: 0.157 (p = 0.026) Kendall's tau$_{2\ years}$: NA
Patient survival			
[13]Foucher et al. (n = 120)	Patient Survival	AUC: 0.69	AUC (95% CI): 0.642 (0.548–0.736)

Abbreviations: aHR, adjusted hazard ratio; AUC, area under the receiver operating characteristic curve; CADI, chronic allograft damage index; CI, confidence interval; ECD, expanded criteria donor; eGFR, estimated glomerular filtration rate; HR, hazard ratio; KDRI, kidney donor risk index; NA, not available; RR, relative risk; SCR, standard criteria donor. Pathological and combined clinical and pathological scores are in italics; the numbers correspond to the references in the revised manuscript.

[a]*The Irish score was applied without considering the parameter history of transition, which was not available in the majority of recipients.*

different or partially overlapping sub-cohorts. However, this problem is unavoidable, since the data required for the calculation of all scores, are not routinely collected in the ET database nor at the DSO or the transplant centers. A registry with data of all sources (DSO, ET, transplant centers) is not available. **Fourth**, the test cohort dates back approximately 10 years. However, most of the evidence base of kidney transplantation relies on data collected before 2010 and the follow-up period of our study should

TABLE 9 | Performance of the investigated scores for the prediction of discards vs. transplantation.

Score	Both kidneys were discarded ($n_{kindeys}$ = 104)	One kidney was transplanted, one kidney was discarded ($n_{kindeys}$ = 90)	Both kidneys were transplanted ($n_{kindeys}$ = 248)	Overall *p*-value	*p*-value A vs. C	*p*-value B vs. C
[15]*CIV Score (Balaz et al.)*	1.80 ± 1.42	1.33 ± 1.07	1.11 ± 1.08	**<0.001**	**0.034**	0.233
[15]*CIV Score (Balaz et al.), (<1), %*	15.9	23.4	34.1	**0.012**	0.382	**0.030**
[15]*Composite CIV Score (Balaz et al.), %(0/1/2/3)*	4.3/31.9/49.3/14.5	9.4/25.0/46.9/18.8	4.2/30.5/52.7/12.6	0.583	0.533	0.804
[6]DGFS scoring system (Chapal et al.), Value	—	−0.1440 ± 0.7896	−0.1201 ± 0.7989	0.924	—	0.924
[6]DGFS scoring system (Chapal et al.), % (Low risk/medium risk/high risk)	—	36.4/54.5/9.1	33.3/61.7/5.0		—	0.808
[7]DGF risk calculator (Irish et al.), Points	—	210.6 ± 18.9	223.6 ± 28.6	0.092	—	0.092
[7]DGF risk calculator (Irish et al.), Probability of DGF (%)	—	19.9 ± 17.3	24.1 ± 20.0	0.303	—	0.303
[8]Jeldres scoring system (Jeldres et al.), Points	—	137.9 ± 31.2	131.3 ± 35.2	0.358	—	0.358
[8]Jeldres scoring system (Jeldres et al.), Probability of DGF (%)	—	48.5 ± 20.1	44.6 ± 21.6	0.370	—	0.370
[11]Schold Risk Index	—	1.05 ± 0.32	0.95 ± 0.35	0.190	—	0.190
[11]Schold Grade I-V	—	0.0/3.8/23.1/26.9/46.2	3.6/9.7/23.0/30.3/33.2		—	0.554
[17]*Navarro Score (≤3/4-5/6-7/>7)*	59.6/13.5/10.6/16.3	62.2/22.2/6.7/8.9	69.8/15.3/10.1/4.8	**0.011**	0.165	**0.010**
[17]*Navarro Score > 5, %*	26.9	15.6	14.9	**0.022**	0.080	0.947
[19]Port	1.96 ± 0.52	1.97 ± 0.51	1.96 ± 0.47	0.991	0.909	274
[26]Rao	—	1.33 ± 0.31	1.17 ± 0.43	0.054	—	0.106
[20]*Snoeijs*	4.55 ± 3.47	3.36 ± 2.61	3.12 ± 2.39	**0.001**	**0.028**	0.387
[16]*Vusser (3 years prediction)*	66.5 ± 16.8	64.0 ± 17.4	62.6 ± 17.7	0.158	0.315	0.185
[16]*Vusser (5 years prediction)*	63.2 ± 14.8	62.3 ± 16.0	61.2 ± 16.7	0.581	0.693	0.240
[19]*Remuzzi Score (pirani)*	2.41 ± 2.76	1.90 ± 1.94	1.55 ± 1.84	**0.002**	0.141	**0.011**
[19]*Remuzzi Grading (Score 1-3/4-6/7-12) (pirani) 1-3: for single transplantation, 4-6: for dual transplantation*	67.3/24.0/8.7	83.3/15.6/1.1	85.1/12.9/2.0	**0.001**	**0.013**	0.715
[14]*Anglicheau (GS−/CP−; GS−/CP+; GS+/CP−; GS+/CP+)*	29.8/46.2/2.9/21.2	16.7/64.4/1.1/17.8	20.6/52.8/6.0/20.6	0.060	0.058	0.500
[9]Nyberg Score	—	26.1 ± 7.1	24.1 ± 9.0	0.261	—	0.261
[9]Nyberg Grading (A/B/C/D)	—	0.0/25.0/25.0/50.0	9.1/23.4/28.9/38.6			0.318
[18]*Ortiz*	4.36 ± 2.91	3.34 ± 2.35	2.83 ± 1.98	**<0.001**	**0.029**	0.148
[13]Foucher	—	9.56 ± 2.90	8.39 ± 2.04	0.236	—	0.240

CIV, chronic interstitial and vascular score according to the Banff classification; composite CIV Score: CIV score considering also clinical parameters (donor age >51 years, anoxic donor brain injury).

A, B and C refer to the first (bilateral discard), second (unilateral discard) and third (bilateral transplantation) column of the table.

Pathological and combined clinical and pathological scores are in italics, the numbers correspond to the references of the manuscript.

Bold values represent statistically significant parameters.

not have changed considerably in the decade before and after 2010.[4] **Fifth**, the indications for procurement biopsies relied not on objective criteria since they were performed on case-by- case basis and not according to a standardized protocol. For example, the macroscopic assessment of the recovered organs was quite subjective. However, it can be of value if performed in a more structured way by experienced surgeons [54]. **Finally**, an inherent, unavoidable drawback of all similar studies is the unknown performance of the certainly non-randomly discarded DDKs. Despite all these limitations, this is the only study examining the performance of these scores on the dataset for which they are most usefully from a clinical point of view: procurement biopsies for the decision of DDK transplantation or discard. We found that, that none of the tested scores should allow a confident, evidence-based decision about acceptance or discard of a DDK based on prognosis of the different endpoints within the ET context. Probably, clinical parameters not included in that scores, such as donor's AKI or donor's creatinine metrics are more important for short term outcomes [53, 55].

Here, some conclusions can be drawn: **First**, organs from donors with AKI should not be accepted for recipients at high risk for DGF or these recipients may be preferentially treated with an immunosuppression protocol based on belatacept [56]. **Second**, the recipient should return timely to dialysis to avoid losing it waitlist points if an early graft failure is expected. **Finally**, we must always keep in mind that especially for the elderly patients, rejection of organs leads in the end to an increase in mortality due to the longer waiting list time [57].

Regarding the second aim, we could indeed show that for the endpoint death censored graft survival histological [17, 20], or clinicopathological [16] scores performed marginally better than purely clinical ones. But even if the AUCs were slightly better their overall performance was moderate to poor. While for some DDKs donor and recipient parameters might be entirely sufficient for a prognosis, for some donor/recipient matches histopathology

[4]https://www.ctstransplant.org/public/introduction.shtml

might add valuable information. We are currently investigating such an approach with a facultative histopathology component including only reproducible parameters independently from each other associated with prognosis.

As to the testing of the scores in the discarded kidneys, we found that scores with a histological component were better than the solely clinical. However, an inherent bias cannot be excluded since the histologic evaluation of an offered organ is often the principal reason of its discard. Here, we can only postulate that histological assessment is warranted in kidneys supposed to be unsuitable for transplantation. Probably, the most important finding was that many of the discarded kidneys could have been successfully transplanted.

CONCLUSION

Procurement biopsies are often used during allocation to increase the possibility of acceptance of kidneys of lower quality. However, the available prognostic scores perform at best only moderately. Though none of the scores could reach an acceptable discriminatory power, those based on histopathologic criteria performed slightly better than the more practical solely clinical ones. Our findings are based on data from the Eurotransplant region but can also be applied to other Multinational or National Transplant Organizations or -even more- be valuable for individual decisions in transplant centers.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

All organ transplants were performed according the Declaration of Istanbul; no transplants from prisoners were used. The study was conducted in accordance with the Declarations of Helsinki and approved by the local ethical review board of Hannover Medical School (No. 1519-2012).

AUTHOR CONTRIBUTIONS

JB and CC conceived the study and wrote the manuscript; JB re-evaluated the biopsies; CC was responsible for the acquisition of recipient and donor data; C-LF provided donor data. FS created the tables; FS and AE performed the statistical analysis; AS provided intellectual input, reviewed, and edited 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.2023.11399/full#supplementary-material

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48 h Normothermic Machine Perfusion With Urine Recirculation for Discarded Human Kidney Grafts

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Normothermic machine perfusion (NMP) has reshaped organ preservation in recent years. In this preclinical study, prolonged normothermic perfusions of discarded human kidney grafts were performed in order to investigate perfusion dynamics and identify potential quality and assessment indicators. Five human discarded kidney grafts were perfused normothermically (37°C) for 48 h using the Kidney Assist device with a red-blood-cell based perfusate with urine recirculation. Perfusion dynamics, perfusate and urine composition as well as injury markers were measured and analyzed. Donor age ranged from 41 to 68 years. All but one kidney were from brain dead donors. Perfusions were performed successfully for 48 h with all discarded kidneys. Median arterial flow ranged from 405 to 841 mL/min. All kidneys excreted urine until the end of perfusion (median 0.43 mL/min at the end of perfusion). While sodium levels were consistently lower in urine compared to perfusate samples, this was only seen for chloride and potassium in kidney KTX 2. Lactate, AST, LDH as well as pro-inflammatory cytokines increased over time, especially in kidneys KTX 3 and 4. *Ex vivo* normothermic perfusion is able to identify patterns of perfusion, biological function, and changes in inflammatory markers in heterogenous discarded kidney grafts.

Keywords: machine perfusion, kidney preservation, normothermic machine perfusion for the donor kidney, organ assessment, urine recirculation, *ex vivo* perfusion

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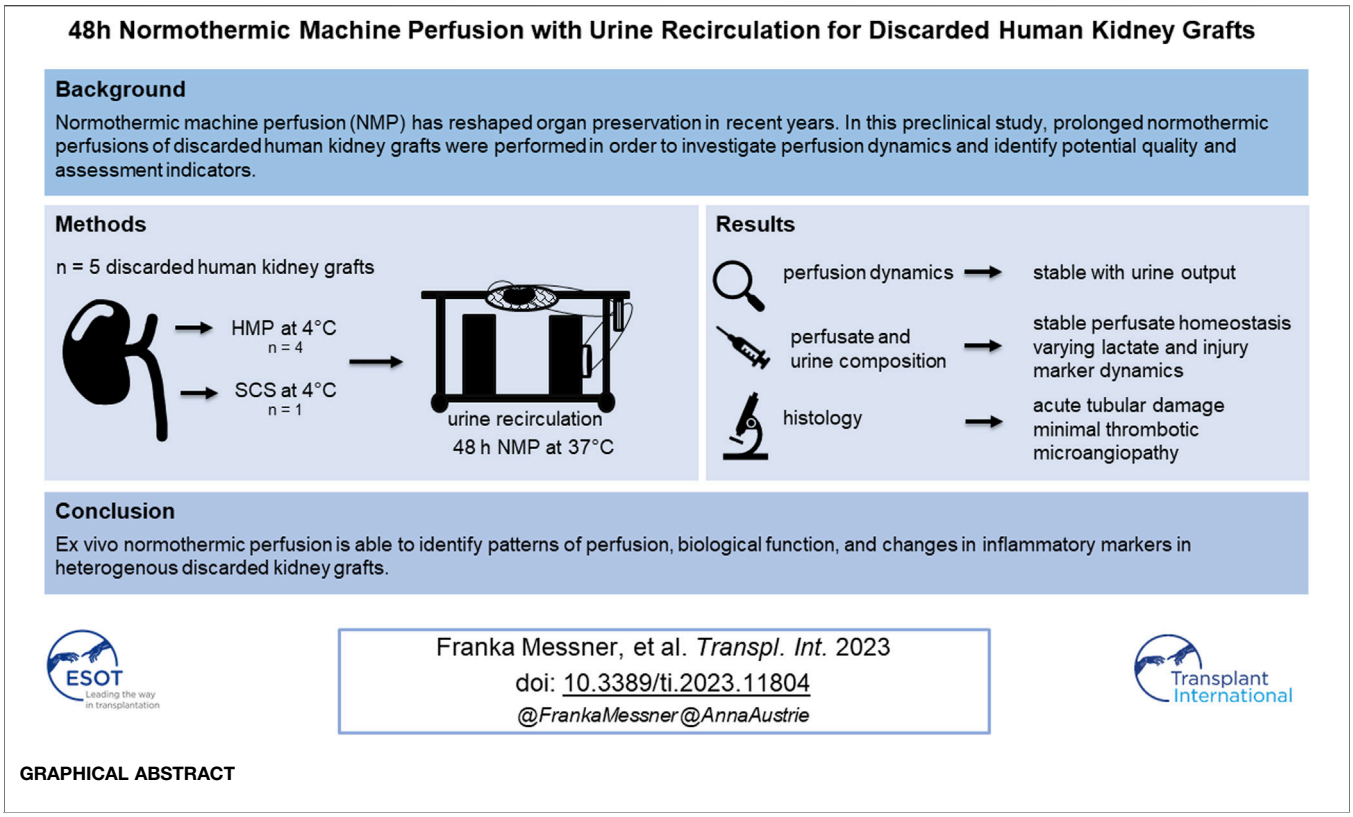
INTRODUCTION

Normothermic machine perfusion (NMP) of donor organs has seen an unprecedented interest in recent years. While liver, lung and heart NMP has become clinical routine, standard implementation of kidney NMP is still lagging behind. In contrast to other organs, kidney grafts tolerate a much longer cold ischemic time even with static storage, thus ease of logistical constrains is a less driving force in this setting. More momentum for innovative approaches is generated by a yearly growing organ shortage and a marked increase in marginal donors.

Abbreviations: AST, aspartate aminotransferase; CKD, chronic kidney disease; DCD, cardiocirculatory death donors; ECD, extended criteria donors; GM-CSF, granulocyte macrophage colony-stimulating factor; HMP, hypothermic machine perfusion; HTK, histidine-tryptophan-ketoglutarate; LDH, lactate dehydrogenase; NGAL, neutrophil gelatinase-associated lipocalin; NMP, Normothermic machine perfusion; TTF-3, trefoil factor 3; vv-ECMO, veno-venous extracorporeal membrane oxygenation.



GRAPHICAL ABSTRACT

Thus far, kidney NMP has mainly been explored in marginal, namely extended criteria donors (ECD) and donation after cardiocirculatory death donors (DCD), donors with relatively short perfusion durations [1–5]. In their latest publication, [6] reported on their randomized controlled trial that compared 170 kidneys with 1 h (hr) end-ischemic NMP to 168 statically cold stored (SCS) kidneys. With comparable rates of thrombosis, infectious complications, delayed graft function and other adverse events they demonstrated feasibility and safety for clinical application, however, NMP in this setting did not lead to superior short-term outcomes whilst adding a logistical burden.

In contrast to this end-ischemic approach, prolonged perfusion might be a tool to assess and/or condition organs prior to transplant. Longer perfusion times might also prove to be a logistic advantage by increasing summative preservation times and consequently help to increase organ utilization.

In this preclinical study, discarded human kidney grafts were perfused on the Kidney Assist device with urine recirculation in order to 1) describe perfusion dynamics, 2) investigate biological function, and 3) report on changes in inflammatory markers in a heterogenous group of kidneys over 48 h.

MATERIALS AND METHODS

The human kidney grafts included in this study were retrieved for transplant but eventually declined at the recipient center. Experimental perfusions for 48 h were performed in the laboratory of the Organ Regeneration Center of Excellence, organ-Life™, Medical University of Innsbruck after approval by the institutional ethics committee (EK Nr. 1216/2019).

Perfusion Set-Up

Custodiol® HTK (histidine-tryptophan-ketoglutarate; Dr. Franz Köhler Chemie GmbH, Bensheim, Germany) solution preserved kidneys arrived at the transplant center statically cold stored. Grafts were routinely placed on the LifePort kidney transporter (Organ Recovery Systems, Itasca, IL, USA) immediately after arrival. After deemed untransplantable, the organ was taken off the hypothermic machine perfusion (HMP) device and prepared for connection to the NMP circuit. In one case, no HMP was performed due to logistical reasons and the kidney graft was perfused normothermically after routine back table preparation and static cold storage in Custodiol® HTK.

For NMP, the Organ Assist Kidney Assist (Organ Assist BV, Groningen, Netherlands) device was used. The renal artery was cannulated with a 20-Fr (KTX 1, KTX 2) or 16-Fr (KTX 3, KTX 4, KTX 5) straight perfusion cannula (Infusion, Warsaw, Poland). The ureter was cannulated with the provided tubing. A three-way valve was included in the tubing to allow for urine collection and recirculation into the reservoir. The disposable set was adapted by implementing an in-line blood gas analyzer (CDI500, Terumo Medical Corporation, Tokyo, Japan). Perfusate temperature was set at 37°C. Oxygenation of the circuit was facilitated by manual regulation of air (21% oxygen) and CO_2. The perfusion circuit was primed with three units of packed red

blood cells (RBCs) of universal donor blood, resuspended in 1000 mL 5% human albumin solution (Albunorm®, Octapharma, Lachen, Switzerland), resulting in a total perfusate volume of approximately 1800 mL. The protocol was adapted from the protocol published by Weissenbacher et al. [7] Before connecting the kidney, the perfusate was supplemented with 750 mg cefuroxime (Sandoz, Basel, Switzerland), 10 mL calcium gluconate 10% (B.Braun, Melsungen, Germany), and 8000 IE enoxaparin (Lovenox®, Sanofi, Paris, France). For pH adjustment prior to initiation of NMP, 10 mL of sodium bicarbonate 8.4% (Fresenius Kabi, Bad Homburg, Germany) were added to the perfusate to achieve a pH level >7.0. Immediately after perfusion start, 5 mL verapamil (Mylan, Vienna, Austria) was administered directly into the arterial line. Kidneys were perfused with a median arterial pressure (MAP) of 90 mmHg. MAP was lowered to 80 mmHg in case flow reached 900 mL/min due to flow restrictions of the device (KTX 1 at 40 h and KTX 2 at 24 h into the perfusion). For glucose and electrolyte monitoring, blood gas analyzer (BGA) measurements (ABL800Flex, Drott Medizintechnik GmbH, Wiener Neudorf, Austria) were performed throughout the perfusion and total parenteral nutrition (Nutriflex® plus, containing 0.15 g/mL glucose) was administered once perfusate glucose levels dropped below 70 mg/dL. Data from the first perfused kidney have already been published as a proof of principle study [8]. To give a better overview on perfusion dynamics and explore the potential of kidney NMP in graft assessment, we included this kidney in this manuscript.

Sampling Procedure

Perfusate and urine samples were obtained throughout the duration of perfusion at hrs 1, 6, 12, 20, 24, 40, and 48. Samples were analyzed upfront by blood gas analyzer and institutional biochemistry laboratory as well as stored after centrifugation at 15,000 G for 15 min at −80°C.

In all kidney grafts, a zero-biopsy was performed and the Remuzzi score was assessed by the on-call pathologist. Follow-up biopsies were taken after 24 h of NMP and upon reaching the endpoint at 48 h. Hemodynamic perfusion parameters were recorded at corresponding timepoints.

Luminex

Kidney injury markers (Luminex Performance Human Kidney Biomarker Panel [6-Plex], #FCSTM16-06, R&D Systems, Minneapolis, United States) and cytokine (Luminex Performance Human High Sensitivity Cytokine Panel A [12-Plex], #FCSTM09-12, R&D Systems, Minneapolis, United States) levels were measured in perfusate samples stored at −80°C. Sample dilution, processing and analysis were carried out according to manufacturer's instruction.

Statistical Analysis

Results are expressed as median and interquartile range (IQR) or range. Mann-Whitney test, Kruskal Wallis, Friedman test corrected with Dunn's multiple comparison test and Wilcoxon matched-pairs signed rank test were used for non-normal distributed data. All tests were two-sided and a p-value of <0.05 was considered statistically significant. Prism GraphPad 9.0 (GraphPad Inc., San Diego, CA, USA) was used for all statistical tests.

RESULTS

Donor and Kidney Graft Demographics

Donor age ranged from 41 to 68 (median 62) years (yrs). Four out of five kidney grafts were from DBD donors, only one (KTX 3) was from a DCD donor. Causes of death were all cardiovascular events. All but one donor had a normal ranged serum creatinine level (median 1.06 mg/dL) before organ retrieval. One donor (KTX 4) was on veno-venous extracorporeal membrane oxygenation (vv-ECMO) and renal replacememt therapy (RRT) due to severe aspiration and concomitant sepsis. All donors had urine output before organ retrieval (median 85 mL/h). Two donors had a history of hypertension, none of the donors were diabetic. For details see **Table 1**.

Cold ischemic times ranged from 11.5 to 28 (median 19.5) hrs. All but one kidney were perfused hypothermically after being transported to our unit on static cold storage (SCS) using the Lifeport® device for a median of 7 h. Reasons for discard were malignancy in the contralateral kidney, poor organ quality and poor perfusion. Remuzzi scores ranged between 0 and 5 (median 3). A detailed overview can be found in **Table 2**.

Perfusion Characteristics

Arterial flow was stable throughout the perfusion of all kidney grafts (**Figure 1A**) irrespective of reason for discard. In the first three perfused kidneys, the median arterial flow exhibited higher values, measuring 841, 775, and 721 mL/min, in contrast to the second pair of kidneys, which recorded flow rates of 405 and 406 mL/min, respectively ($p < 0.001$). Correspondingly, resistance indices (**Figure 1B**) were lower in the first three

TABLE 1 | Donor demographics.

	KTX 1	KTX 2	KTX 3	KTX 4	KTX 5
Donor age (years)	62	68	51	41	65
Donor type	DBD	DBD	DCD	DBD	DBD
Cause of death	CVA	CVA	CVA	CVA	CVA
Creatinine (mg/dL)	0.9	1.06	1.17	1.98 (RRT)	0.74
Urine production (mL/h)	100	85	117	52	70
Hypertension	unknown	Yes	no	no	yes
Diabetes	no	No	no	no	no
Comment	suspected malignancy			VV-ECMO due severe to aspiration; sepsis	

DBD, donation after brain death; DCD, donation after cardiocirculatory death; CVA, cardiovascular accident; RRT, renal replacement therapy.

TABLE 2 | Kidney baseline parameters.

	KTX 1	KTX 2	KTX 3	KTX 4	KTX 5
CIT total (hours)	27	19.5	19	28	11.5
HMP duration (hours)	4.5	5	10	9.5	n.a.
Reason for discard	malignancy	poor organ quality[a]	poor perfusion	poor perfusion	poor organ quality[b]
Remuzzi score	0	4	3	2	5

CIT, cold ischemic time; HMP, hypothermic machine perfusion.
[a]*large cyst and Remuzzi score: g1, i1, t1, a1 = 4.*
[b]*Remuzzi score: g1, i1, t1, a2 = 5.*

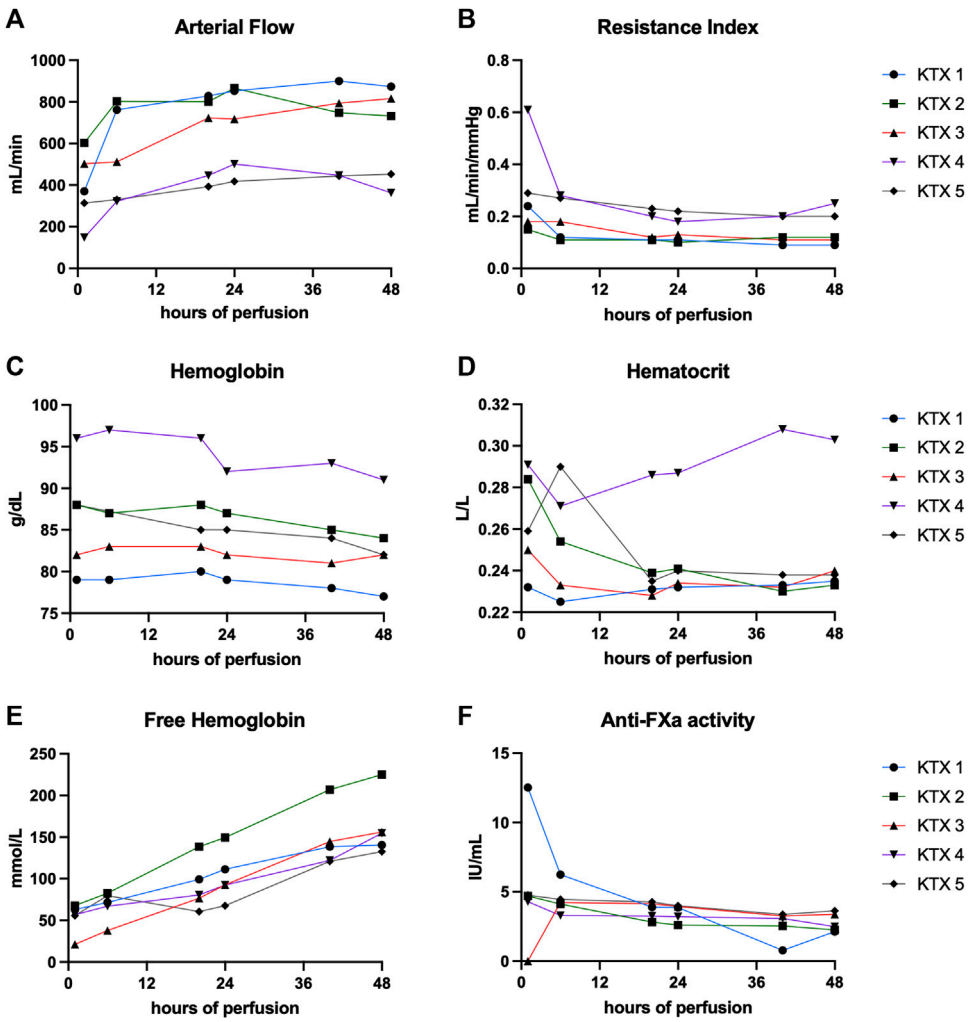


FIGURE 1 | Perfusion characteristics and composition over 48 h of perfusion. **(A)** Arterial flow, corresponding resistance indices **(B)**, hemoglobin levels **(C)** and hematocrit **(D)**, free hemoglobin **(E)** and anti-FXa activity **(F)** is shown at hours 1, 6, 20, 24, 40, and 48.

kidneys compared to the last two ($p < 0.001$). Median hemoglobin and hematocrit levels ranged between 9.5 and 7.9 g/dL, and 0.23 and 0.29 L/L (**Table 3**; **Figures 1C, D**). Free hemoglobin increased over time (**Figure 1E**). Median free hemoglobin levels ranged from 74 to 144 mg/dL (**Table 3**). Anti-FXa activity ranged between 2.7 and 4.1 UI/mL. Levels were slowly decreasing over time and only KTX2 demonstrated a clear clearance during perfusion (**Figure 1F**).

Median (IQR) venous pO_2 und pCO_2 levels were 113 (125–107) mmHg and 37.8 (39.6–36.1) mmHg for KTX 1, 79.9 (86.9–78.5) mmHg and 34.6 (37.8–31.2) mmHg for KTX 2, 81.9 (107–76.2) mmHg and 36.3 (40.7–29.1) mmHg for KTX 3, 90.3

TABLE 3 | Perfusion characteristics. Additive use (total amount), perfusion characteristics (median [IQR]) as well as biochemical markers (median [IQR]) throughout the 48 h perfusion.

	KTX 1	KTX 2	KTX 3	KTX 4	KTX 5	*p*-value
Sodium bicarbonate (mL)	15	32	32	40	20	
Nutriflex (mL)	55	45	69	65	35	
Arterial flow (mL/min)	841 (664–881)	775 (700–819)	721 (510–800)	405 (279–461)	406 (326–446)	<0.001
Resistance index (mmHg/mL/min)	0.11 (0.15–0.09)	0.12 (0.13–0.11)	0.13 (0.18–0.11)	0.23 (0.36–0.20)	0.23 (0.28–0.20)	<0.001
pH	7.15 (7.08–7.15)	7.29 (7.02–7.37)	7.16 (7.08–7.22)	7.09 (6.87–7.20)	7.34 (7.22–7.40)	0.070
Lactate (mg/dL)	89.5 (86–102)	119 (81–136)	114 (93.3–194)	179 (143–183)	98.5 (86.5–112)	0.063
Venous pO_2 (mmHg)	113 (125–107)	79.9 (86.9–78.5)	81.9 (107–76.2)	90.3 (97.4–79.7)	80.3 (89.2–73.8)	<0.001
Venous pCO_2 (mmHg)	37.8 (39.6–36.1)	34.6 (37.8–31.2)	36.3 (40.7–29.1)	13.4 (22.2–10.8)	24.8 (32.5–19.8)	<0.001
Sodium (mmol/L)	158 (159–156)	163 (163–153)	167 (169–163)	173 (177–158)	176 (177–171)	0.001
Chloride (mmol/L)	128 (129–121)	118 (122–110)	119 (123–117)	117 (117–114)	135 (136–131)	<0.001
Potassium (mmol/L)	6.85 (8.03–6.48)	9.95 (11.2–9.23)	6.00 (7.08–5.83)	8.15 (9.68–6.7)	6.35 (7.23–6.15)	<0.001
Hemoglobin (g/dL)	7.9 (7.93–7.78)	8.7 (8.8–8.48)	8.2 (8.3–8.18)	9.45 (9.63–9.18)	8.5 (9.2–8.35)	<0.001
Hematocrit (L/L)	0.23 (0.23–0.23)	0.24 (0.26–0.23)	0.23 (0.24–0.23)	0.29 (0.30–0.28)	0.24 (0.27–0.24)	0.005
Free hemoglobin (mg/dL)	10 (139–69.4)	144 (212–78.8)	84.5 (148–33.7)	86.5 (130–64.5)	73.6 (124–59.3)	0.004
Anti-FXa activity (IU/mL)	3.9 (7.8–1.8)	2.7 (4.3–2.5)	3.7 (4.2–2.4)	3.2 (3.5–2.9)	4.1 (4.5–4.6)	0.018
Urine (mL/min) at hour 48	0.43	0.56	0.37	1.14	0.40	
Urine sodium (mmol/L)	138 (151–109)	117 (137–100)	163 (163–144)	163 (174–121)	100 (171–71)	0.009
Urine choride (mmol/L)	123 (131–109)	102 (119–80)	123 (124–120)	124 (126–105)	98 (140–87)	0.063
Urine potassium (mmol/L)	7.65 (9.88–6.83)	16.2 (17.7–15.9)	6.7 (11.1–5.79)	9.45 (20.8–8.43)	15.8 (19.1–6.50)	0.009
Proteinuria, absolute (mg/dL)	3,964 (6,072–1,856)	9,310 (22,983–5,177)	30,825 (32,516–28,852)	28,251 (28,891–19,273)	6165 (34,468–521)	0.029

GOT, Glutamic-Oxaloacetic Transaminase; IQR, interquartile range; LDH, Lactate Dehydrogenase; pO_2, partial pressure of oxygen; pCO_2, partial pressure of carbon.

(97.4–79.7) mmHg and 13.4 (22.2–10.8) mmHg for KTX 4, and 80.3 (89.2–73.8) mmHg and 24.8 (32.5–19.8) mmHg for KTX 5.

With 35–69 mL of summative Nutriflex supplementation, glucose concentrations between 50 and 150 mg/dL were achieved (**Figure 2A**). Lactate levels ranged between 179 and 89.5 mg/dL. Even tough median lactate levels were similar between groups (p = 0.063), lactate dynamics differed. While lactate was cleared in KTX 1 and 5, it remained stable in KTX 2 and increased over the duration of perfusion in KTX 3 and 4 (**Figure 2B**). Supplementation of 15–40 mL sodium bicarbonate were necessary to achieve median pH levels between 7.09 and 7.34 (**Table 3**; **Figure 2C**). Despite a uniform basic perfusate formulation, kidneys exhibited different electrolyte levels in the perfusate (**Table 3**; **Figure 3**). Perfusate sodium and chloride levels increased over the first 24 h of perfusion and stabilized afterwards (**Figures 3A, C**). Perfusate potassium levels, on the contrast, decreased over the course of the first 24 h in all cases. Thereafter it further decreased, stabilized or increased depending on potassium loss via urine sampling (**Figure 3B**).

All kidney grafts had urine output throughout the perfusion. Median urine output was 0.43 mL/min at the end of perfusion. Median urine sodium content was significantly lower than in the perfusate in all perfused kidney grafts (**Figure 3D**, p = 0.031). In contrast, median chloride (**Figure 3F**, p = 0.031) and potassium (**Figure 3E**, $p < 0.001$) levels differed only in KTX 2.

Injury Markers

Perfusate samples were analyzed for generic and kidney specific injury markers (**Figure 4**) as well as for the presence of pro- and anti-inflammatory markers (**Figure 5**).

Generic injury markers aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) steadily increased over time (**Figure 4**). Significant differences in enzyme release were noticed with KTX 3 displaying the highest absolute enzyme levels ($p < 0.001$). Chemokine CXCL10 (IP-10, $p < 0.001$), trefoil factor 3 (TFF3, p = 0.010) that is found upregulated in chronic kidney disease (CKD) and the pro-inflammatory cytokine granulocyte macrophage colony-

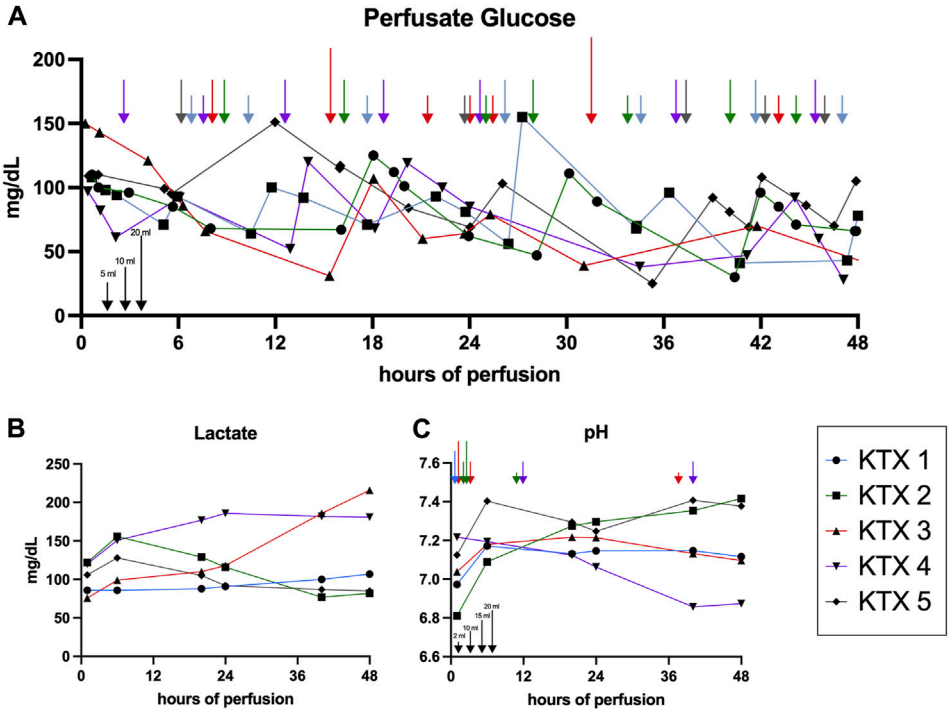


FIGURE 2 | Glucose, lactate and pH dynamics over 48 h of perfusion. **(A)** Glucose concentration assessed via perfusate blood gas analysis and resulting Nutriflex supplementation over time. Arrows indicate the Nutriflex amount administered at a given timepoint (blue, KTX 1; green KTX 2; red, KTX 3; purple, KTX 4; grey, KTX 5; for amount see indicators at the bottom left). **(B)** Lactate dynamics and **(C)** pH fluctuation and supplementation of sodium bicarbonate (blue, KTX 1; green KTX 2; red, KTX 3; purple, KTX 4; grey, KTX 5; for amount see indicators at the bottom left) for buffering over time.

stimulating factor (GM-CSF, $p < 0.001$) all displayed highest levels in KTX 3 and 5 (**Figure 4**). Neutrophil gelatinase-associated lipocalin (NGAL) is released upon tubular injury into the perfusate. Despite a numerical higher level in KTX 3, similar levels between the five perfused kidneys (**Figure 4**, $p = 0.080$) were seen. Similarly, a comparable level of Cystatin C was measured in all groups. Clusterin, a protein released from multiple cell types upon injury with cytoprotective properties, was found in abundance in all groups and displayed a similar pattern over time in all kidney grafts. Osteopontin (OPN), a widely expressed protein during inflammation and arteriosclerosis, was found to be, unlike the other injury markers, highest in KTX 1 and KTX 2.

Highest levels of proinflammatory markers TNFa, IFNy, IL-1b, IL-2, IL-4 IL-5, IL-6 and IL-12p70 were seen in KTX 3 and KTX 4, respectively (**Figure 5**). In the histological work-up, KTX 4 was found to suffer from fungal contamination. A steep increase of these cytokines especially towards the end of the 48 h perfusion duration might reflect the presence of fungal contamination (**Figure 5**). Anti-inflammatory IL-10 expression spiked after 6 and 20 h in DBD and DCD organs, respectively. In addition, IL-10 levels increased after fungal contamination in KTX 4.

Macroscopical and Histological Assessment

All kidneys had a good reperfusion. Only the two poorly perfused kidneys, KTX 3 and KTX 4, showed some purple cortical areas (**Figure 6**). These vanished over the first 6 h of perfusion and further homogenous reperfusion was achieved until the end of the 48 h experiment. Despite favorable perfusion dynamics, macroscopic appearance of KTX 2 was the worst in our series, and KTX 4, despite a favorable appearance, was overall performing poorly.

All kidneys displayed unspecific acute tubular injury that showed moderate progression over the course of the 48 h of perfusion. No glomerular and/or tubular necrosis. In biopsies of kidneys perfused for 48 h, a small fraction (2%) of glomeruli had signs of acute thrombotic microangiopathy (**Figure 6B**). No evidence of thrombotic events were found at earlier timepoints. Focal oidia of the *Candida* type were present in tubules and glomeruli of KTX 3.

DISCUSSION

This study explored normothermic machine perfusion of human discarded kidney grafts using the Kidney Assist device and a blood based perfusate for a duration of 48 h. Perfused kidney grafts varied substantially in donor characteristics. While KTX 1 would have been transplantable without presence of malignancy in the contralateral kidney, the other kidneys were considered too marginal to be transplanted. Preexisting kidney injury, as reflected by the Remuzzi score, did not correlate with behavior during NMP.

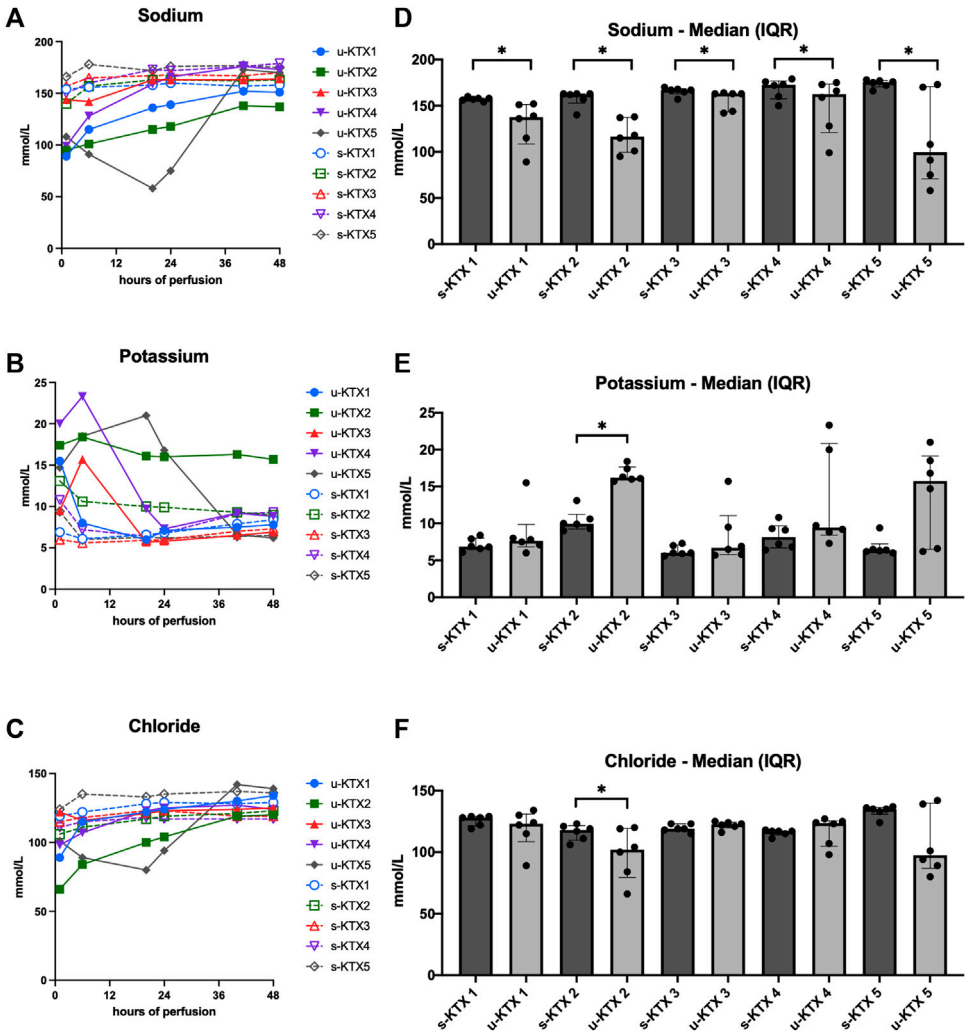


FIGURE 3 | Perfusate and urine electrolyte levels. **(A–C)** Absolute sodium, potassium and chloride concentrations in serum (s-KTX, solid line) and urine (u-KTx, interrupted line). **(D–F)** Median (IQR) perfusate (s-KTX) and urine electrolyte levels (u-KTX) over the course of the 48 h perfusion were illustrated and compared using the Wilcoxon matched-pairs signed rank test. *$p < 0.05$; IQR, interquartile range.

Irrespective of donor characteristics, reason for discard, ischemic time and cold storage method, stable normothermic *ex vivo* perfusions were achieved for the whole 48 h perfusion duration. Similarly, as demonstrated earlier with 24 h perfusion experiments [7, 9], urine recirculation led to a stable perfusate composition over the prolonged perfusion time and subsequently no perfusion had to be terminated early. In two kidneys the targeted MAP of 90 mmHg resulted in such a high flow that it had to be lowered to 80 mmHg in order to stay within the technical limits of the used perfusion device. Unlike in other studies [10], flow rates did not correlate with other surrogate markers of function like lactate clearance and levels of injury markers in our series. That perfusion parameters are not necessarily reflecting clinical outcome was also reported by Hosgood et al. [2] The group perfused the kidneys of an uncontrolled DCD donor for 1 h normothermically before transplantation. Despite stable perfusion, favorable macroscopic appearance and urine output both organs experienced primary non function. As a result, the authors questioned whether their standard perfusion duration of 1 h might be too short to properly perform a pre-transplant assessment. In our series, both kidneys with poor perfusion as contributing factor for discard demonstrated the least favorable perfusion characteristics and prolonged perfusion did not alter dynamics. In addition to pre-existing thrombi, that are residual from retrieval, DeRito et al. [11] described the formation of cold storage-induced microvascular obstructions that are building up with prolonged cold storage and that might also negatively impact perfusion. They could demonstrate that the addition of plasminogen and rt-PA was able to successfully lyse these plugs. Eventually this treatment led to a significant reduction in renal injury markers, lower intrarenal resistance as well as higher urine output. In our experiment, enoxaparin, a low-molecular-weight heparin, was used as anticoagulant. A single application led to stable anti-FXa levels throughout the perfusion. Despite being eliminated renally, a clear decrease of anti-FXa activity was only observed in KTX 1.

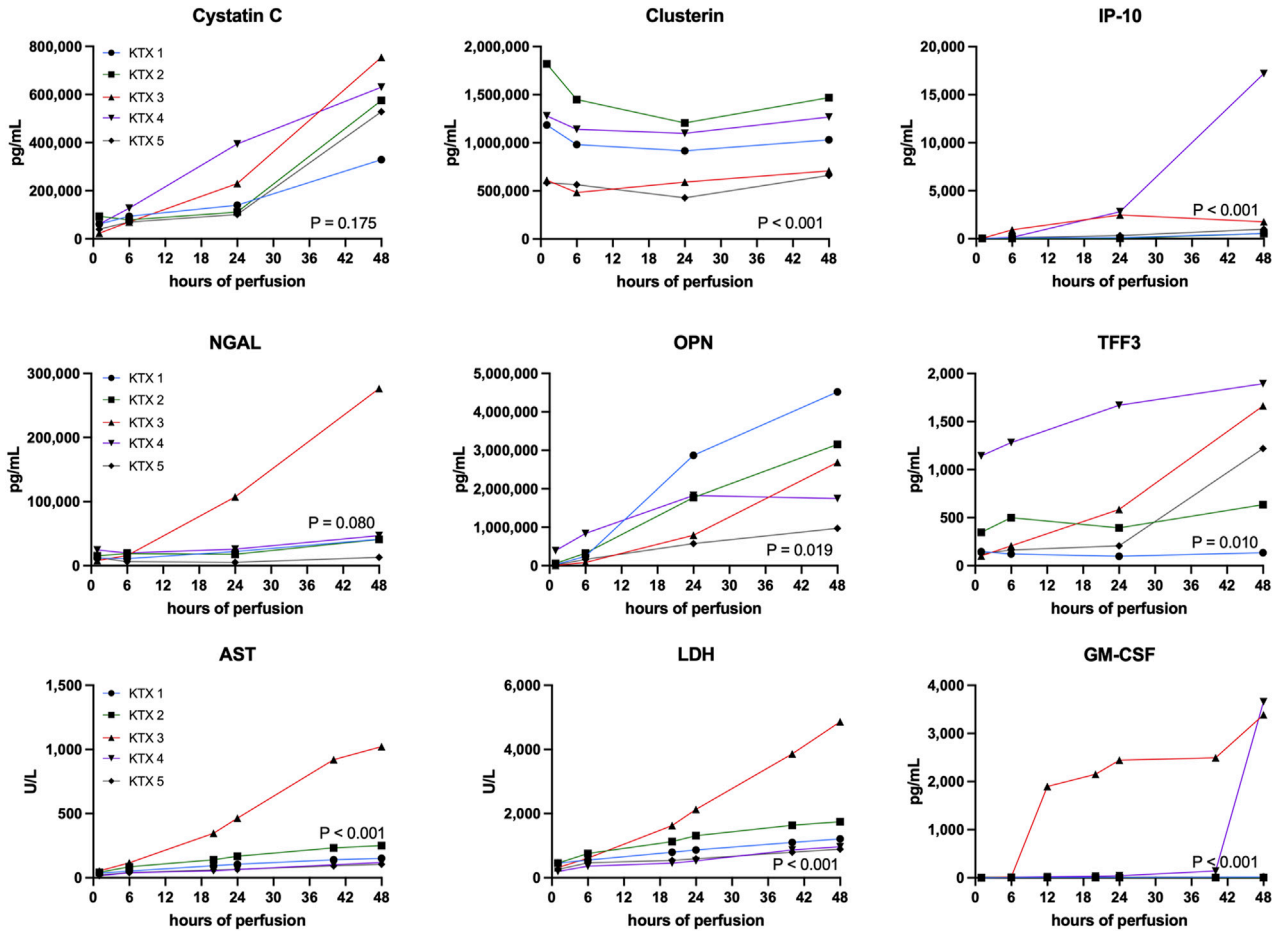


FIGURE 4 | Quantification of injury markers. Unspecific and kidney specific injury markers have been measured at corresponding timepoints throughout the 48 h perfusion. A comparison between perfused kidneys was performed using the Friedman test. AST, aspartate aminotransferase; GM-CSF, granulocyte macrophage colony-stimulating factor; LDH, lactate dehydrogenase; IP-10, CXCL10; NGAL, Neutrophil Gelatinase-associated Lipocalin; OPN, Osteopontin; TFF3, Trefoil Factor 3.

The two poorly perfused organs, KTX 3 and KTX 4, demonstrated the highest need for exogenous glucose supplementation and accumulation of lactate, while better performing grafts, like KTX 1, 2 and 5 displayed stable or decreasing perfusate lactate levels and lower glucose supplementation needs indicating that higher glucose consumption might not necessarily indicate better metabolic activity. Similar to our findings *ex vivo*, lactate clearance has previously been shown to correlate with posttransplant renal function [12].

Urine output was present in all of our kidney grafts irrespective of donor type, CIT, cause of death and reason for discard. As already reported by others [12], the presence of urine did not seem to be an indicator for kidney graft quality. In addition, all kidneys were reabsorbing sodium, at least for the first 24 h of perfusion and most even beyond this point challenging the value of tubular sodium reabsorption as quality discriminator. Three out of five kidneys had comparable potassium urine and perfusate levels 24 h after perfusion. Only KTX 2 and KTX 5 showed significant urine potassium excretion beyond this point and strikingly KTX 1, a supposedly good quality kidney, showed poor potassium excretion.

By measuring different injury markers in the perfusate during the perfusion, we could detect some interesting dynamics. Firstly, NGAL which is a commonly used kidney injury marker [13], did not show significant differences in levels in our series. More generic markers like AST and LDH have been described to correlate with outcome after transplantation [12, 13] and were exceptionally elevated in our DCD organ. CXCL10, TFF3 and GM-CSF as well as proinflammatory markers TNFa, IFNy, IL-1b, IL-2, IL-4 IL-5, IL-6 and IL-12p70 were elevated in KTX 3 and 4. While KTX 3 had elevated perfusate levels already early, most markers increased in KTX 4 at much later timepoints. This differences in dynamics might reflect on the different reasons for poor organ quality. Finally, histologic assessment revealed, besides acute tubular injury, no glomerular and/or tubular necrosis. Thrombotic events were found in a small fraction of glomeruli after 48 h of NMP, but not at earlier timepoints. The progressive increase of tubular damage together with the presence of glomerular thrombotic events after prolonged perfusion for 48 h might indicate time limits for *ex vivo* perfusion and/or are potential signs of perfusate exhaustion.

Kidney NMP has safely been translated in the clinical setting by various groups. All applications, however, are thus far limited to short (1–3 h) perfusion durations [1, 3, 5, 6, 10, 14–16]. Novel

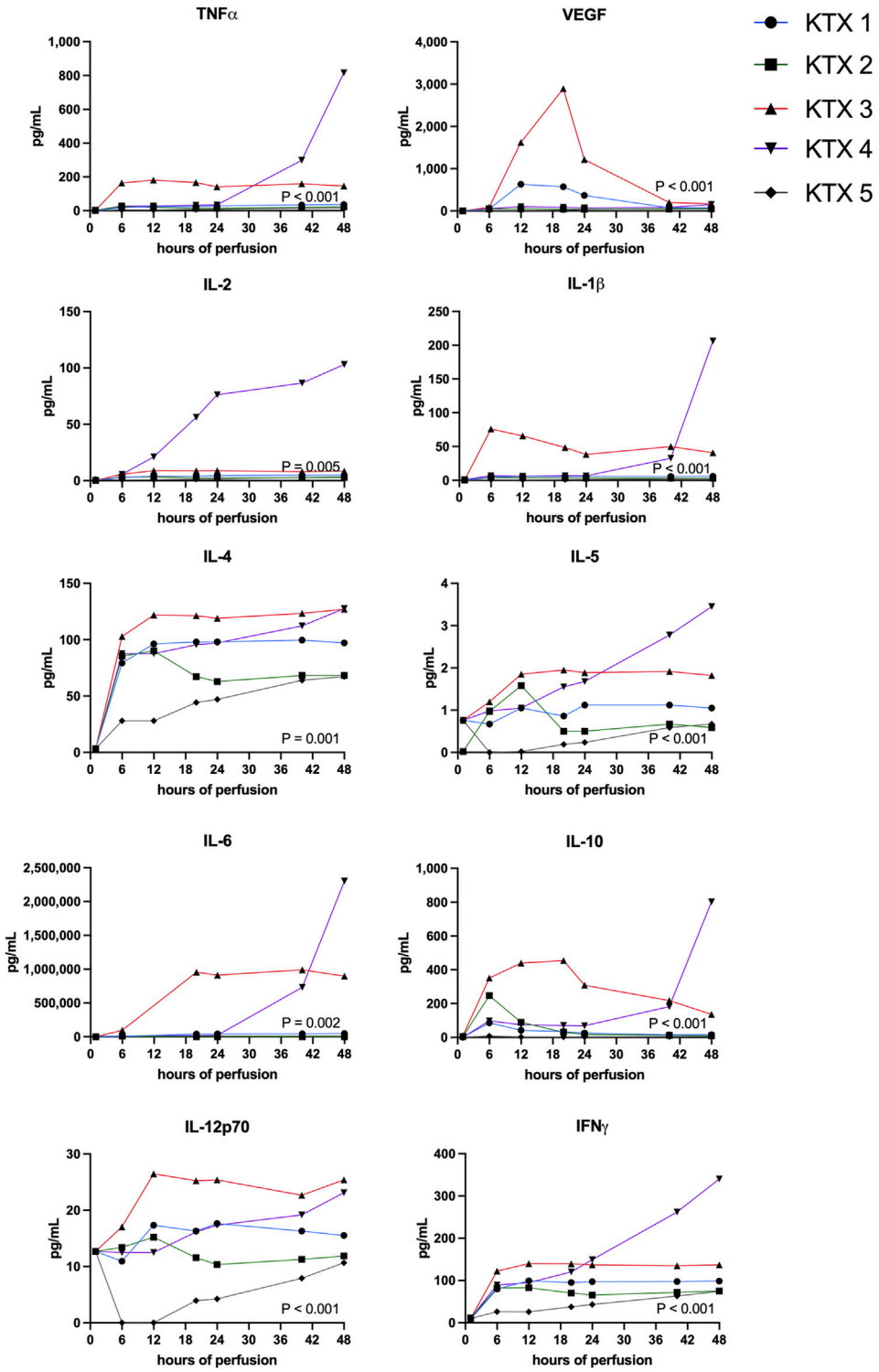


FIGURE 5 | Pro- and anti-inflammatory protein perfusate levels during 48 h kidney NMP were measured and compared between perfused kidneys using the Friedman test.

insights from a magnetic resonance imaging study found, that perfusion during NMP takes at least 1–2 h to reach the renal cortex in a range comparable to *in vivo* and authors warned from over-interpretation of quality assessment markers for NMP at early timepoint as they may not reflect actual physiology [17]. This phenomenon might also be reflected in the dynamics of

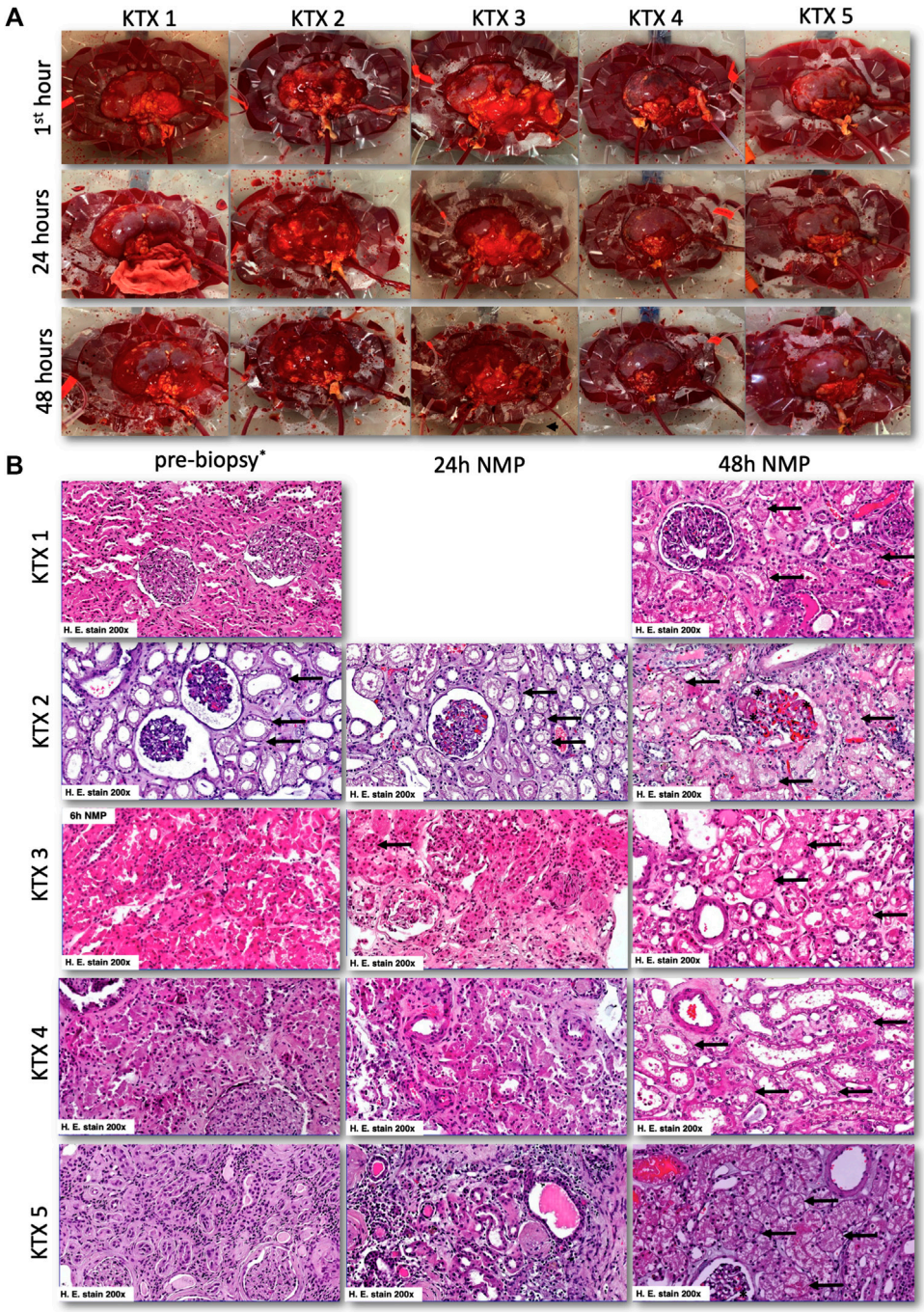


FIGURE 6 | Macroscopical and histological appearance of normothermically perfused kidney grafts. **(A)** Macroscopic aspects of normothermically perfused kidneys. The upper row shows a representative image in the first hour after reperfusion, the middle row after 24 h and the lower row after reaching the endpoint at 48 h. **(B)** Representative histologic images of discarded human kidney grafts pre-NMP (left row), after 24 h of perfusion (middle row), and at 48 h of perfusion (right row). In the pre-biopsy, acute tubular damage (black arrows) with glomerular collapse was seen. This tubular damage then slightly progressed in the first 24 h of NMP (black arrows). After 48 h of NMP, again a slightly progressive tubular damage was seen together with glomerular endothelial swelling. At 48 h, 2% of glomeruli displayed signs of thrombotic microangiopathy (black asterix). No 24 h biopsy was taken in KTX1, and no pre-biopsy was available for KTX 3. For the latter one, we showed histological damage after 6 h of NMP to demonstrate progressive tubular damage. H, hours; NMP, normothermic machine perfusion.

biochemical markers in the present study, where similarly low levels were found in early sampling points and relevant differences were only apparent after longer perfusion durations.

Limitations of this study include the low number of kidneys that have been perfused together with the huge variety in organ quality and donor characteristics. In addition, none of

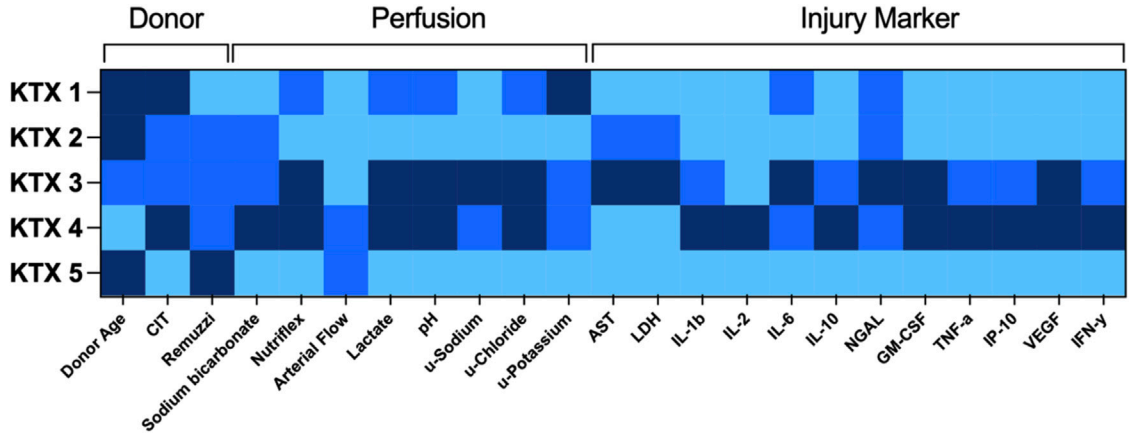


FIGURE 7 | Heat map of donor characteristics, perfusion parameters and injury markers. Single factors were graded according to a semiquantitative three-tier scale (light blue = good/short/low, medium blue = intermediate, dark blue = bad/long/high). AST, aspartate aminotransferase; CIT, cold ischemic time; u, urine; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN-y, interferon gamma; IL, interleukin; IP-10, CXCL10; LDH, lactate dehydrogenase; NGAL, Neutrophil Gelatinase-associated Lipocalin; TNF-a, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

these kidneys has been transplanted and thus, no correlation to a clinical outcome can be made. Despite using the same standard recipe for all kidney perfusions, perfusate composition varied between different runs. Contributing factors might include differences in RBCs (volume, age, potassium and lactate content), tubular reabsorption capacity, urine production and sampling, and the need for exogenous buffering (depending on initial lactate and clearance). As perfusate compositions and perfusion device in different centers vary, comparison of these data to others might be limited [18].

In this series of human kidney perfusion, lactate dynamics, pH, potassium excretion, as well as upregulation of injury markers show a comparable dynamic over the 48 h perfusion. By using a heat-map for donor and perfusion characteristics as well as injury markers (**Figure 7**), it is apparent that KTX 3 and KTX 4 are consistently performing poorer than the other three kidneys. Next in line regarding overall performance comes KTX 1, followed by KTX 2. KTX 5 had the most beneficial perfusion and injury profile.

The two kidneys with consistently undesirable profiles had the most extended donor profiles with KTX 3 being from a DCD organ with poor perfusion after retrieval, and KTX 4 from a DBD organ with poor perfusion from a septic donor on RRT and vv-ECMO. Despite lacking correlation to clinical outcome, the incorporated parameters are, as previously described by others, possible quality indicators for kidney on NMP. Prolonged perfusion might help to better identify perfusate dynamics. Long-term, longer than 24 h, *ex vivo* perfusion of the kidney, however, might be limited by accumulating tubular damage as well as *de-novo* glomerular thrombotic events when currently available devices (clinically licensed for 6 h kidney NMP only) are applied. Optimization of the perfusate might be key to improve kidney NMP outcomes further.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by Medical University of Innsbruck. 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

AW and FM carried out the experiment. FM wrote the manuscript with support from AW, AS, HN, SS, and DÖ. AS was responsible for histopathological analysis. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

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ORIGINAL RESEARCH
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Assessment of Donor Derived Cell Free DNA (dd-cfDNA) at Surveillance and at Clinical Suspicion of Acute Rejection in Renal Transplantation

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In our prospective, unicenter cohort study, we collected blood samples from 30 newly kidney transplanted patients, at month 1, 2, 3, and 5 for dd-cfDNA analysis, along with creatinine/eGFR and DSA monitoring, and from 32 patients who underwent an indication biopsy and whose dd-cfDNA levels were measured at the time of biopsy and 1 month afterwards. Fourteen of 32 (43.8%) patients in the biopsy group were diagnosed with TCMR and 5 of 32 (15.6%) with ABMR. Dd-cfDNA proved to be better than creatinine in diagnosing rejection from non-rejection in patients who were biopsied. When a dd-cfDNA threshold of 0.5% was chosen, sensitivity was 73.7% and specificity was 92.3% (AUC: 0.804, 0.646–0.961). In rejection patients, levels of dd-cfDNA prior to biopsy (0.94%, 0.3–2.0) decreased substantially after initiation of treatment with median returning to baseline already at 1 month (0.33%, 0.21–0.51, $p = 0.0036$). In the surveillance group, high levels of dd-cfDNA (>0.5%) from second month post-transplantation were correlated with non-increasing eGFR 1 year post-transplantation. The study used AlloSeq kit for kidney transplant surveillance for first time and confirmed dd-cfDNA's ability to detect rejection and monitor treatment, as well as to predict worse long-term outcomes regarding eGFR.

Keywords: dd-cfDNA, kidney allograft, transplantation, rejection, biomarker

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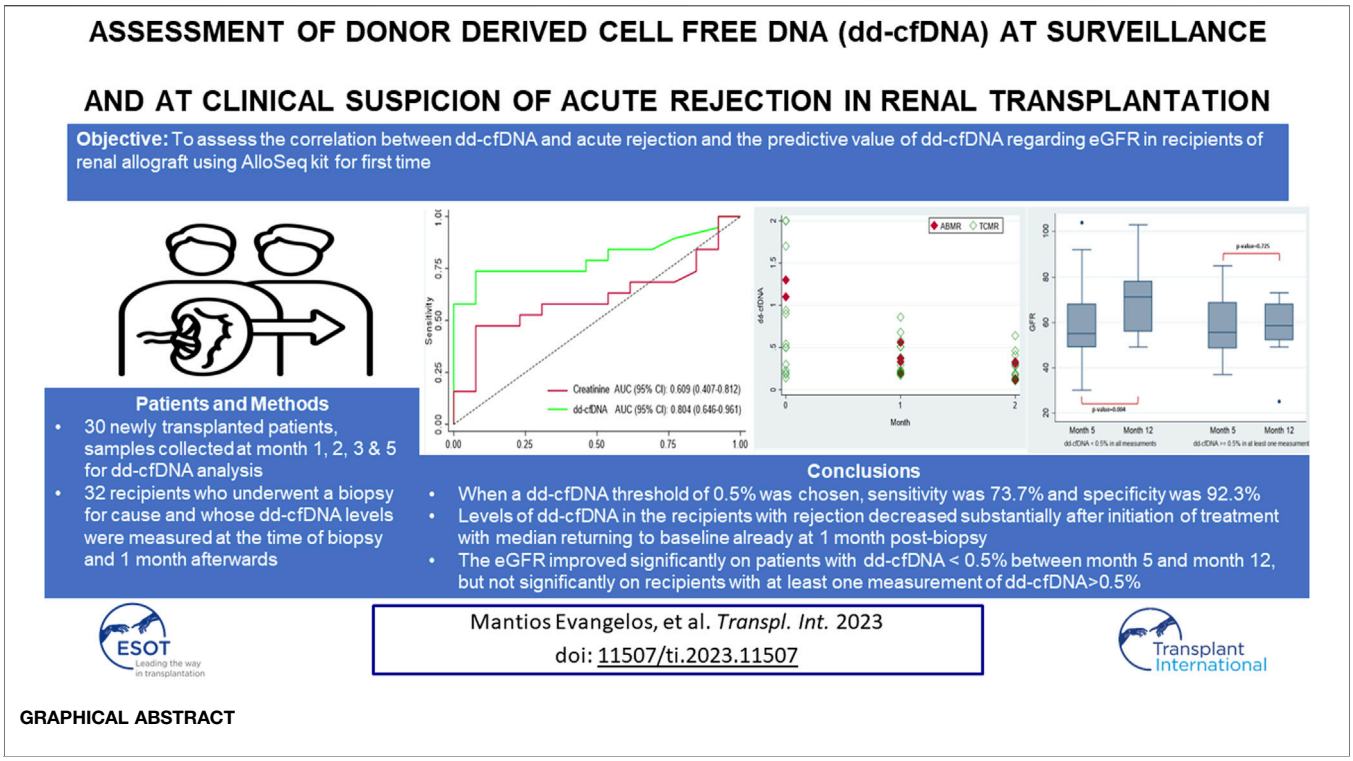
INTRODUCTION

Rejection, antibody-mediated, and T-cell mediated, remains the first cause of death-censored allograft loss in kidney recipients [1, 2]. Despite the standardization of needle biopsy for rejection diagnosis, it is rarely used for surveillance due to its cost, logistics, potential complications, and patient discomfort. Only 17% of US centers conduct surveillance biopsies, and another 21% do so on a selective basis [3]. Donor-derived cell-free DNA (dd-cfDNA) has been proposed as a non-invasive marker for transplant rejection, not only in kidney [4–9], but also in lung [10, 11] and heart transplants [12, 13], since it may itself trigger inflammation and thus add insult to injury [14, 15]. In renal transplant recipients who developed *de novo* donor specific antibodies (dnDSAs), a rise in dd-cfDNA > 0.5% occurred a median of 91 days preceding detection of dnDSAs [16]. The first large multicenter trials aiming to compare dd-cfDNA measurements with the



GRAPHICAL ABSTRACT

molecular phenotype of kidney transplant biopsies [17], as well as short patient series trying to enhance the use of dd-cfDNA information to guide clinical practice and immunomodulation decisions [18] have recently been published, while additional interventional studies are in progress [19].

We launched a prospective study for the assessment of dd-cfDNA in renal transplantation, which is an observational longitudinal cohort with 62 patients and used Alloseq kit, that was implemented locally for dd-cfDNA testing in order to provide information about the clinical performance of the biomarker in surveillance and rejection detection for first time. By using AlloSeq cfDNA assay, study aims to evaluate the correlation between dd-cfDNA values in plasma and DSA formation, as well as between the dd-cfDNA measurements and histopathology reporting, based on "for cause" renal biopsy. Additionally, we aimed to examine the long-term relationship between elevation in dd-cfDNA and estimated glomerular filtration rate (eGFR).

PATIENTS AND METHODS

Study Population

A total of 30 adult kidney transplant recipients in one transplant center were monitored with dd-cfDNA testing at month 1, 2, 3, and 5 post-transplant (surveillance group). The initial surveillance group included 39 patients, 9 of whom underwent an indication biopsy during the surveillance period and therefore were "transferred" to the biopsy group. The biopsy group was consisted of 32 renal recipients who were biopsied for cause and were monitored with dd-cfDNA prior to biopsy and 1 month afterwards. Data was collected between 1 November 2020, and 20 January 2022. The study performed in accordance with international standards, and it did not form part of a broader study. The patients were managed prospectively as standard of care without dd-cfDNA in the context of post-transplant care, with dd-cfDNA data captured being retrospectively examined. Using the center's medical records, we determined clinical events (e.g., rejection, infection) and routine laboratory tests (creatinine, DSAs). Participants had to meet the inclusion criteria of the study; male or female, aged 12 years or above, recently transplanted and willing and able to give informed consent for participation in the trial and to comply with all trial requirements. Pregnant women, recipients of multiple organs, patients with significant hepatic impairment or short life expectancy, monozygotic twins and patients who had previously received bone marrow transplants were not allowed to participate in the study. None of the recipients were excluded from participation. Polyomavirus infection did not constitute an exclusion criterion from the study.

dd-cfDNA Testing

Venous blood was collected in Cell-Free DNA BCT tubes (Streck, La Vista, NE) and plasma isolated according to manufacturer's instructions (Streck) used for analysis. An analysis sample of 240 dd-cfDNA measurements was collected from 62 patients for this study. The cell-free DNA was extracted from the isolated plasma by using QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) and then 10 ng inputed for library preparation with AlloSeq cfDNA kit following assay manual documentation

TABLE 1 | Indications for biopsy in the biopsy group.

Indications for biopsy	*N* = 32
sCr increase	15
Non satisfactory sCr decrease (early post-Tx period)	6
Extended DGF (>20 days)	1
BK viremia + sCr increase	5
Deterioration of proteinuria	1
De novo DSAs	4

IFU084 version 6.0, September 2021 provided by the manufacturer (CareDx Pty, Fremantle, WA, Australia). The resulting amplified products were sequenced on the MiSeq sequencing system (Illumina, San Diego, CA), and sequencing data was analyzed with AlloSeq cfDNA software version 1.0 (CareDx Pty). The AlloSeq cfDNA is a commercially available next-generation sequencing (NGS)-based assay that identifies the fraction of donor-specific cfDNA by analyzing 202 targeted single-nucleotide polymorphisms (SNPs), chosen to have genome-wide coverage (equally distributed), multiethnicity coverage and high uniformity. Genetic relationship between donor and recipient was entered into AlloSeq cfDNA Software, and the algorithm adjusts % the dd-cfDNA calculation accordingly. Assuming a reporting range of <50% for kidney post-transplant, no recipient or donor samples were provided, and AlloSeq cfDNA software algorithm assumed the minor represented cfDNA fraction as the donor fraction to calculate the % dd-cfDNA. In addition to % dd-cfDNA, AlloSeq cfDNA QC metrics for all loci, mean coverage, uniformity, and locus count were monitored.

Diagnosis of Graft Dysfunction and Biopsy-Defined Rejection

Results of for-cause kidney transplant biopsies were recorded. Among the indications for for-cause biopsy were changes in creatinine, worsening proteinuria, the development of dnDSA, or a combination of these factors (**Table 1**). A single pathologist blinded to dd-cfDNA results assessed biopsy reports for study analysis. Interpretations of biopsy results were made in accordance with Banff 2019 classification scheme [20]. Antibody-mediated rejection (ABMR) group included also mixed rejection cases. Borderline cases were captured and categorized in the T cell–mediated rejection (TCMR) group.

Other concomitant pathologic diagnoses, such as calcineurin inhibitor toxicity, glomerulopathy, or acute tubular injury or acute tubular necrosis (or both) were classified as no rejection. Rejection treatment decisions were made following the center's clinical protocol. As part of the surveillance group of 30 newly transplanted patients and of the group of those who had a biopsy, all dd-cfDNA levels were collected, along with eGFR changes and dnDSAs.

Statistical Analyses

Distributions of categorical variables were summarized through absolute and relative (%) frequencies. For continuous variables, mean and standard deviation (SD) were used for the normally distributed variables, while median and interquartile range (IQR) for the non-normally distributed ones. Statistical analysis was performed by either Wilcoxon rank-sum (Mann-Whitney U), Wilcoxon Signed Rank or Kruskal-Wallis H nonparametric statistical tests (non-normally distributed continuous variables). In addition, ROC analysis and a two-way repeated-measures analysis of variance (ANOVA) were performed. All statistical analyses were performed using Stata version 16.0 program. The level of statistical significance was set at 0.05.

Univariable and multivariable exact logistic regression models were used to identify factors associated with rejection for patients with biopsy. Rejection was determined as the binary dependent variable [outcomes: rejection/non-rejection; T cell-mediated rejection (TCMR)/non-rejection] and dd-cfDNA in month 0, age, gender, ABO incompatibility, DSAS preformed, DSAS *de novo*, days after transplantation and Crossmatch B flow as possible explanatory (independent) variables. The significance level was set equal to 0.10 for the univariable analyses and equal to 0.05 for the multivariable analyses. Odds ratios (ORs) and 95% confidence intervals (95% CI) are reported.

dd-cfDNA and eGFR Analysis

Kidney function was determined by eGFR calculated using the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation. Dd-cfDNA and eGFR for each month was assessed. There were two categories of patients: those with a high dd-cfDNA (any measurement above 0.5%) and those with a low dd-cfDNA (all measurements below 0.5%). A two-way repeated-measures analysis of variance (ANOVA) was performed for the analysis.

RESULTS

The demographics of the 62 patients enrolled in our study depict a population of high immunological risk (**Table 2**). An ABO incompatible transplant was performed on one patient out of five in both the surveillance and biopsy groups. It was noted that 23.3% of patients who were newly transplanted had preformed DSAs, while 43.8% of patients who were biopsied had preformed DSAs. Plasmapheresis and intravenous immunoglobulin were administered prior to surgery to one of every three recipients either due to DSAs or because of ABO incompatibility. Among the biopsy group, the rejection diagnosis was identified in 19 out of 32 patients (59.4%), with 14 of the 19 being classified as TCMR. In three patients, ABMR was diagnosed, while in two recipients, mixed rejection was detected, which was also classified as ABMR.

Association of dd-cfDNA Levels and Acute Rejection Events

Using 32 for cause biopsies from 32 patients with biopsy-paired dd-cfDNA results, the association between dd-cfDNA levels and any allograft rejection status was evaluated. Even though changes in serum creatinine make up the largest proportion of reasons for a biopsy in our study, there was no statistically significant difference in

TABLE 2 | Descriptive statistics of (i) newly transplanted patients (n = 30) and (ii) patients with biopsy (n = 32).

Variable	Newly transplanted patients (n = 30)	Patients with biopsy (n = 32)
Mean age [years, (SD)]	46.5 (10.8)	41.5 (14.3)
Primary disease [*n*, (%)]		
DN	1 (3.3)	1 (3.1)
Glomerulonephritis	12 (40.0)	13 (40.6)
Nephronophthisis	0 (0.0)	2 (6.3)
Obstructive uropathy	2 (6.7)	5 (15.6)
Other	0 (0.0)	1 (3.1)
PKD	5 (16.7)	4 (12.5)
Unknown	10 (33.3)	6 (18.8)
Median years of haemodialysis (IQR[a])	1.5 (0.0, 8.0)	1.5 (0.5, 7.5)
Transplantation [*n*, (%)]		
Deceased donor	8 (26.7)	11 (34.4)
Living donor	22 (73.3)	21 (65.6)
Donor (relation) [*n*, (%)][b, c]		
Husband	6 (27.3)	2 (9.5)
Wife	3 (13.6)	3 (14.2)
Father	2 (9.1)	1 (4.8)
Mother	9 (40.9)	13 (61.9)
Brother	0 (0.0)	1 (4.8)
Sister	0 (0.0)	1 (4.8)
Aunt	2 (9.1)	0 (0.0)
Mean age of donor [years, (SD)]	55.4 (15.0)	55.1 (15.5)
Donor history [factors; *n*, (%)]		
0	13 (43.3)	12 (37.5)
1	12 (40.0)	14 (43.8)
2 or 3	5 (16.7)	6 (18.8)
ABO incompatibility [*n*, (%)]		
No	24 (80.0)	25 (78.1)
Yes	6 (20.0)	7 (21.9)
DSAS preformed [*n*, (%)]		
No	23 (76.7)	18 (56.2)
Yes	7 (23.3)	14 (43.8)
DSAS de novo [*n*, (%)]		
No	30 (100.0)	28 (87.5)
Yes	0 (0.0)	4 (12.5)
Crossmatch B flow [*n*, (%)]		
No	24 (80.0)	26 (81.3)
Yes	6 (20.0)	6 (18.7)
Crossmatch T flow [*n*, (%)]		
No	29 (96.7)	30 (93.8)
Yes	1 (3.3)	2 (6.2)
RTX [*n*, (%)]		
No	19 (63.3)	22 (68.8)
Yes	11 (36.7)	10 (31.2)
PLEX + IVIG [*n*, (%)]		
No	20 (66.7)	20 (62.5)
Yes	10 (33.3)	12 (37.5)
ATG [*n*, (%)]		
No	27 (90.0)	25 (78.1)
Yes	3 (10.0)	7 (21.9)
Median days after transplantation (IQR)	—	106.5 (19.0, 185.0)
Rejection [*n*, (%)]		
No	—	13 (40.6)
ABMR[d]		5 (15.6)
TCMR[e]		14 (43.8)
Prednisone pulses [*n*, (%)]		
No	—	15 (46.9)
Yes		17 (53.1)
PLEX [*n*, (%)]		
No	—	28 (87.5)
Yes		4 (12.5)
ATG [*n*, (%)]		
No	—	30 (93.8)
Yes		2 (6.2)

(Continued on following page)

TABLE 2 | (*Continued*) Descriptive statistics of (i) newly transplanted patients (*n* = 30) and (ii) patients with biopsy (*n* = 32).

Variable	Newly transplanted patients (*n* = 30)	Patients with biopsy (*n* = 32)
Leflunomide [*n*, (%)]	—	
No		29 (90.6)
Yes		3 (9.4)
Eculizumab [*n*, (%)]	—	
No		31 (96.9)
Yes		1 (3.1)

[a]*IQR, interquartile range.*
[b]*Newly transplanted patients:* n = *22.*
[c]*Patients with biopsy:* n = *21.*
[d]*ABMR, antibody-mediated rejection.*
[e]*TCMR, T cell-mediated rejection.*

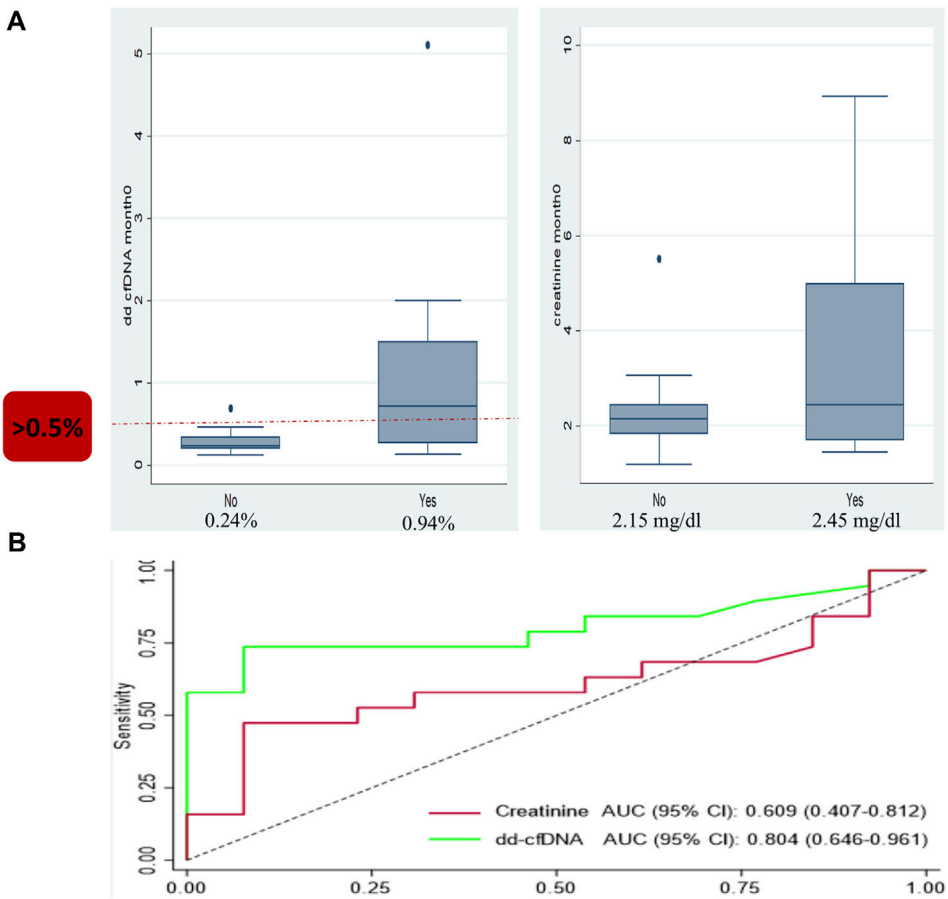


FIGURE 1 | Box and whisker plot and ROC analysis showing the median donor-derived cell-free DNA (dd-cfDNA) and creatinine levels observed in patients with and without allograft rejection. **(A)** Box and whisker plot for dd-cfDNA (left) showing a median of 0.24% seen in patients with no rejection and 0.94% in patients with allograft rejection; $p = 0.004$. Box and whisker plot for creatinine (right) with a median creatinine of 2.15 mg/dL in patients with no rejection versus 2.45 mg/dL in patients with allograft rejection; $p = 0.3$. **(B)** The ROC analysis for dd-cfDNA: area under the receiver-operating characteristic curve (AUROC) 0.804. The ROC analysis for creatinine: AUROC 0.609.

the median creatinine in patients with a no rejection biopsy (2.15 mg/dL; interquartile range [IQR]: 1.82–2.44 mg/dL) and patients with Banff-defined rejection (2.45 mg/dL; IQR: 1.70–4.98 mg/dL); $p = 0.3$ (**Figure 1**). The AUROC for creatinine was 0.609 (95% CI: 0.407–0.812). In comparison, the median dd-cfDNA level among patients with a no rejection biopsy was 0.24% (IQR: 0.20%–0.34%), which was significantly lower than the median dd-cfDNA in patients with biopsies demonstrating defined cellular or antibody-mediated rejection (0.94%; IQR: 0.30%–2.0%); $p = 0.004$. The AUROC for all rejection dd-cfDNA was 0.804 (95% CI: 0.646–0.961). The Youden's

TABLE 3 | Multivariable exact logistic regression estimates using rejection as the binary outcome variable (outcomes: rejection/non-rejection).

Explanatory variable	Adjusted odds ratio	95% Conf. Interval	*p*-value
dd-cfDNA (in month 0)			
*<0.5%	—	—	—
≥0.5%	25.57	(3.44, +Inf)	<**0.001**

**Reference category.*
Patients with dd-cfDNA higher than 0.5% had a more than 25 times higher odds of rejection compared to those with dd-cfDNA lower than 0.5% (p <0.001).

TABLE 4 | Multivariable exact logistic regression estimates using TCMR as the binary outcome variable [outcomes: T cell-mediated rejection (TCMR)/non-rejection].

Explanatory variable	Adjusted odds ratio	95% Conf. Interval	*p*-value
dd-cfDNA (in month 0)			
*<0.5%	—	—	—
≥0.5%	12.35	(1.18, 746.10)	**0.031**

Univariable and multivariable exact logistic regression models were used to identify factors associated with rejection for patients with biopsy. Rejection was determined as the binary dependent variable [outcomes: rejection/non-rejection; T cell-mediated rejection (TCMR)/non-rejection] and dd-cfDNA in month 0, age, gender, ABO incompatibility, transplantation, DSAS, DSAS de novo*, days after transplantation and Crossmatch B flow as possible explanatory (independent) variables.*
**Reference category.*
Patients with dd-cfDNA higher than 0.5% had a more than 12 times higher odds of TCMR compared to those with dd-cfDNA lower than 0.5% (p = 0.031).

index for dd-cfDNA was 0.58%. When a dd-cfDNA threshold of 0.5% was chosen, sensitivity was 73.7% and specificity was 92.3%.

ABMR was diagnosed in 5 biopsies. Among these patients, compared to non-rejection patients, the median dd-cfDNA was 13%; $p < 0.001$. TCMR was diagnosed in 14 biopsies. Patients with TCMR, compared to nonrejection patients, had a median dd-cfDNA value of 0.52%; $p = 0.038$.

In terms of discrimination, dd-cfDNA was effective for distinguishing among biopsies that show no rejection or any rejection. However, when it exceeded a specific threshold, it could rise the possibility for any type of rejection. Patients with dd-cfDNA higher than 0.5% had more than 25 times higher odds of rejection compared to those with dd-cfDNA lower than 0.5% ($p < 0.001$) and more than 12 times higher odds of TCMR compared to those with dd-cfDNA lower than 0.5% ($p = 0.031$) (**Tables 3**, **4**).

Monitoring Anti-Rejection Treatment

Dd-cfDNA kinetics were evaluated in 19 recipients diagnosed with rejection (**Figure 2**). In order to achieve a longer monitoring period, dd-cfDNA levels were also measured 2 months after biopsy in 15 out of 19 rejection recipients. Levels of dd-cfDNA before biopsy (0.94%; IQR: 0.3–2.0) decreased substantially after initiation of treatment already at first month (0.33%; IQR: 0.21–0.51); $p = 0.0036$. The difference was even more significant when comparing median dd-cfDNA levels at month 2 (0.19%; IQR: 0.12–0.33) to median levels at month 0 ($p = 0.0007$).

According to our study, the median value of dd-cfDNA for 30 surveillance patients from the first 5 months post-transplantation was 0.23% (IQR: 0.18%–0.36%). Moreover, nine transplant recipients who were initially enrolled in the surveillance group had median dd-cfDNA of 0.33% (IQR: 0.24%–0.37%) before being referred for a graft biopsy and being 'transferred' to the biopsy group. These findings suggest that median dd-cfDNA levels returned to baseline levels already at the first month after anti-rejection treatment, while dd-cfDNA levels at month 2 were similar to the median dd-cfDNA levels of the surveillance group.

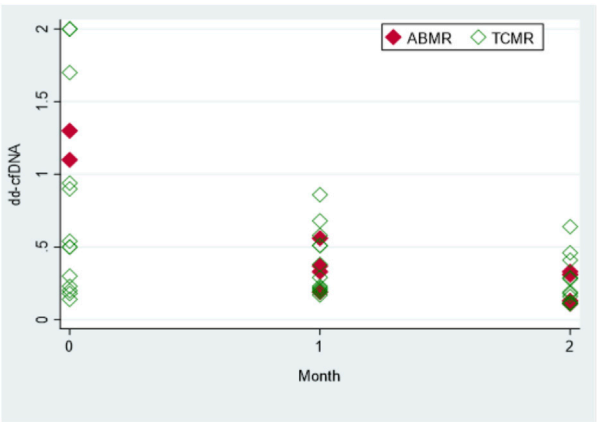


FIGURE 2 | The dd-cfDNA kinetics with anti-rejection treatment. Total of 15 patients with biopsy and rejection [antibody-mediated rejection (ABMR) or T-cell mediated rejection (TCMR)]. Values shown are at month 0 (time of biopsy and diagnosis), and at 1 and 2 months (after rejection treatment was initiated). For the sake of clarity, four patients with high levels of dd-cfDNA (>2.0%) were excluded from the graph presented. Each diamond represents a biopsy specimen.

Association of dd-cfDNA Elevation and eGFR Progression

In the surveillance group of the 30 newly transplanted recipients, an effort was made to assess how the elevation of dd-cfDNA affects changes in eGFR 1 year post-transplantation. The two-way repeated-measures ANOVA was run on the eGFR at month 5 and 12 in two groups of 22 (dd-cfDNA < 0.5% in all measurements—month 1 excluded) and 8 (dd-cfDNA ≥ 0.5% at least in one

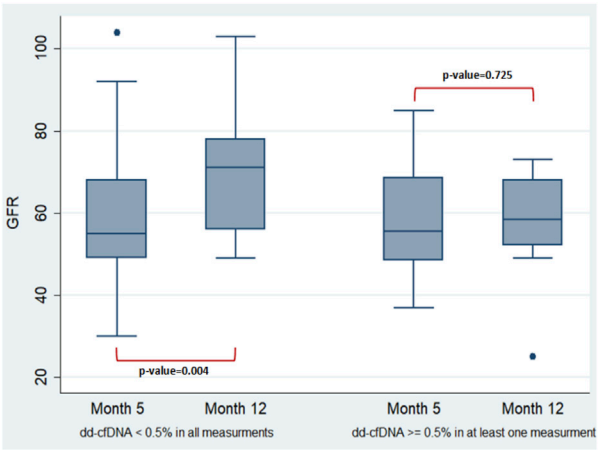


FIGURE 3 | Two-way repeated-measures ANOVA performed to examine the effect of group (newly transplanted patients were grouped according to the percentage of dd-cfDNA and the number of measurements) and time on the eGFR revealed non-significant main effect of group (p = 0.2235), non-significant main effect of time (p = 0.2008) and non-significant interaction between factors (the effects of group and time on eGFR) (p = 0.0652). In more detail, the analysis determined that the mean value of eGFR has not been significantly different between the groups and the timepoints. A difference of the mean value of eGFR between the timepoints was observed only for those with dd-cfDNA < 0.5% (p = 0.004). The two-way repeated-measures ANOVA analysis with the Greenhouse-Geisser correction (performed to check if the data do not meet the compound symmetry assumption) confirmed the previous estimates.

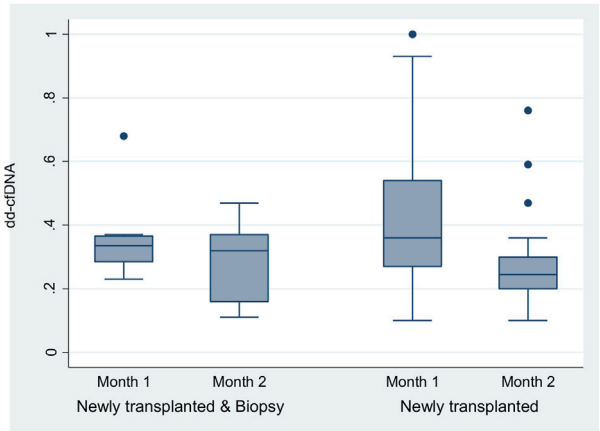


FIGURE 4 | The two-way repeated-measures ANOVA that was run to examine the effect of group (newly transplanted patients and newly transplanted patients who had experienced biopsy) and time on the dd-cfDNA revealed non-significant main effect of group (p = 0.5480), a significant main effect of time [$F(1, 36) = 5.72$, $p = 0.0221$] and non-significant interaction between factors (the effects of group and time on dd-cfDNA) (p = 0.3083). In more detail, the analysis determined that the mean value of dd-cfDNA has not been significantly different between the groups, but has been significantly different between the timepoints (month 1 and 2). The difference of the dd-cfDNA between the timepoints was observed mainly for the newly transplanted patients (p = 0.001). The two-way repeated-measures ANOVA analysis with the Greenhouse-Geisser correction confirmed the previous estimates.

measurement—month 1 excluded) newly transplanted patients. Groups were defined according to the percentage of dd-cfDNA (cut-off point = 0.5%) and the number of measurements (**Figure 3**). A difference of the mean value of eGFR between month 5 and month 12 was observed for patients with dd-cfDNA < 0.5% (p = 0.004) compared to recipients with at least one high measurement of dd-cfDNA (≥0.5%) (p = 0.725), whose eGFR did not seem to rise that efficiently 1 year post-transplantation. However, the mean value of eGFR has not been significantly different between the two groups in month 12.

Correlation of Alterations in dd-cfDNA Over Time With Indication Biopsies

As mentioned above, nine of the 32 renal recipients who underwent a biopsy had been enrolled in the surveillance group at the beginning of the study but were shifted to the biopsy group after an indication for a for cause biopsy was received. All these recipients were biopsied after the second month post-transplantation. As a result, nine patients had at the end of the study at least two monthly dd-cfDNA measurements prior to the biopsy event. We decided to compare the first two measurements of these recipients to the first two dd-cfDNA measurements of the 30 surveillance patients who managed to complete 5 months post-transplantation without the need of a for cause biopsy.

The two-way repeated-measures ANOVA performed on the dd-cfDNA at month 1 and 2 in two groups of 9 newly transplanted with biopsy and 30 newly transplanted patients showed a greater reduction of dd-cfDNA in patients who did not need a biopsy (p = 0.001) compared to those who needed one the first months post-transplantation (**Figure 4**).

Relationship Between dd-cfDNA Level and Identification of dnDSAs

None of the 30 surveillance recipients developed dnDSAs the first year post-transplantation and only 4 of the 32 patients who performed a biopsy did so. Due to these circumstances, an analysis was not possible between dd-cfDNA and DSA formation.

DISCUSSION

Our study is the first conducted in Europe and also the first one of the Greek cohort of kidney transplant patients to investigate the clinical performance of dd-cfDNA in both surveillance and for-cause biopsies, by using AlloSeq kit, a laboratory product that can be implemented and operated, without the need to send samples to a centralized service. There have been larger studies that have derived similar conclusions, but these used centralized service tests for dd-cfDNA and primarily included US cohorts [6, 16]. This commercially available *in vitro* diagnostics kit was implemented locally for dd-cfDNA testing and investigated

Greek cohort for the first time. Several studies have also examined dd-cfDNA's diagnostic potential in different areas of kidney transplantation using the Alloseq cfDNA kit. Mayer et al. assessed the diagnostic value of dd-cfDNA in the diagnosis of ABMR based on Alloseq, as an adjunct to the detection of DSA [21], as well as its ability to differentiate rejection from BK nephropathy [22]. Moreover, the researchers used AlloSeq to investigate whether dd-cfDNA levels are affected by clazakizumab, a promising anti-rejection treatment [23]. Other authors assessed AlloSeq's value as a surveillance tool after reduction of immunosuppression in order to accomplish seroresponse in transplant recipients who had not responded in previous COVID-19 vaccinations [24, 25]. AlloSeq cfDNA assay was also used in other studies to examine different analytical techniques for the quantification of donor-derived cell-free DNA in plasma and urine [26, 27].

Using the AlloSeq assay, we measured dd-cfDNA in renal transplantation as a percentage rather than an absolute measurement. It is hotly debated whether absolute quantification is superior to fractional measurement in discrimination of rejection. A cross-sectional study in Australia compared diagnostic performance of dd-cfDNA (cp/mL) and dd-cfDNA (%), and similar results were obtained for composite diagnosis of ABMR [30]. In a German prospective cohort, the comparison of % versus cp/ml dd-cfDNA results were not significantly different regarding NPV and PPV, even though the AUC for cp/ml was significantly higher [28]. A single-Center Cohort in California proposed combining cp/ml and fractional results, but the suggested superior diagnostic performance was based only on the results of a cohort of 9 rejection cases [9]. Furthermore, the multi-centric Trifecta study did not find a significant difference in dd-cfDNA performance between reporting with cp/ml and reporting with fractions. AUC increased only slightly when cp/ml and fraction were combined [29]. On the contrary, R. Gohn et al. found that absolute quantification of dd-cfDNA did not provide any additional discriminating power over dd-cfDNA fraction for detection of allograft rejection [17]. Moreover, while % cut-offs have been observed to be consistent across cohorts and sites, it is important to note that cp/mL are difficult to standardize across sites: 21 and 12 cp/mL (used by Whitlam JB et al. [30]) vs. 52 cp/mL (proposed by Oellerich M et al. [28]) and vs. 78 cp/m (used at Trifecta [29]).

It has been reported in the recent ADMIRAL study that patients with clinical ABMR had significantly higher levels of dd-cfDNA (2.2% versus 0.34%) [16], whereas in our cohort the ABMR median was even higher (13.0% versus 0.24%). Statistically significant increases in dd-cfDNA were also observed in patients with clinically evident TCMR (0.52% vs. 0.24%) compared to patients without clinical evidence of rejection. The results of a recent meta-analysis, which included six studies that used a 1.0% threshold for dd-cfDNA to diagnose rejection, indicated a diagnostic odds ratio of 8.18 for the biomarker [31]. The high median value of ABMR, as well as the high odds ratio for rejection in general when dd-cfDNA exceeded 0.5% in our cohort [25], was attributed to the small sample size of the study and some really high dd-cfDNA measurements in 3 out of 5 ABMR patients, 2 of whom had stopped their immunosuppression and ended up in allograft loss as a consequence of these devastating rejection episodes. Some high measurements in the TCMR group were also the reason for the high odds ratio for TCMR [12] despite the anticipated, compared to the literature, TCMR median value (0.52%). Despite the general perception, based on several studies [32–34], that dd-cfDNA is less effective in diagnosing TCMR than ABMR, the high odds ratio for TCMR in our study comply with the latest work of Aubert et al. [35], who included 1,210 biopsies in 992 patients and concluded that higher levels of dd-cfDNA were observed for ABMR and TCMR or both compared to other diagnoses. Sigdel et al. also reported higher dd-cfDNA fractions in TCMR patients [36].

Given the small number of participants, we decided to include the 2 patients diagnosed with borderline rejection in the TCMR group (14 patients overall), keeping in mind although the heterogeneous injury within this diagnosis, as Stites et al. [37] proved by risk-stratifying recipients with TCMR1A and borderline rejection depending on their dd-cfDNA level prior to biopsy. As a result of the small sample size, no TCMR analysis was conducted according to TCMR grade, which remains a current knowledge gap in literature: nine studies included in the meta-analysis of Wijtvliet V. et al [34], who did not find higher dd-cfDNA levels in TCMR patients compared to recipients without rejection at indication biopsy, reported no dd-cfDNA fractions for different grades of TCMR, making a distinction between dd-cfDNA fractions in low versus high grades of TCMR impossible. Due to the high heterogeneity of TCMR and the lack of differentiation between low grade and high grade TCMR in the published dd-cfDNA studies, further research is required on dd-cfDNA values for different grades of TCMR.

It was calculated that our optimal threshold for dd-cfDNA using AlloSeq cfDNA assay in order to discriminate rejection from non-rejection was 0.58%, which complies with the ADMIRAL study, whose Youden Index for dd-cfDNA was 0.69%, while the AUROCs in the two studies when using the same threshold were similar [16].Former studies have considered 1.0% as the appropriate threshold [5, 15, 36]. In order to increase its sensitivity, more studies which will combine dd-cfDNA with other biomarkers such as urinary chemokines, may be of great interest in the future.

The delta between serial dd-cfDNA was also associated with increased possibility for an indication biopsy, suggesting that dd-cfDNA alterations can be an alarming sign for the allograft quiescence. Using the dd-cfDNA as an indicator, Anand et al. [38]showed that a 141% increase in dd-cfDNA is associated with abnormal pathology. Our AlloSeq study showed that not only the increase, but also the non-satisfactory decrease in dd-cfDNA in the early post-transplant period can indicate that a patient may require closer monitoring or even invasive procedures in the future.

Wolf-Doty et al. [39] have monitored dd-cfDNA in 35 patients from the DART study who received anti-rejection treatment and concluded that 1 month post-rejection dd-cfDNA levels returned from 0.62% to 0.35%, which was almost the baseline for the

non-rejection recipients of the DART study (0.30%). Our AlloSeq study confirms these findings, with median rejection value before biopsy (0.94%; IQR: 0.3–2.0) returning to baseline already at first post-rejection month (0.33%; IQR: 0.21–0.51); $p = 0.0036$.

It was concluded that recipients with low dd-cfDNA levels showed a clear increase in eGFR after month 12 ($p = 0.004$), while those with at least one high dd-cfDNA value excluding first month did not show the same increase. It should be noted, however, that due to the short follow-up period, the mean value of eGFR has not been significantly different between the two groups after month 12. Bu et al. [16] demonstrated a correlation between higher levels of dd-cfDNA and a subsequent decline in eGFR, while Huang et al. [40] stated that as compared to assessing graft survival using only biopsy characteristics alone, the addition of dd-cfDNA to Banff biopsy scores provides a superior prognostic assessment.

It has been demonstrated by Aubert et al. [41] that dnDSAs have a detrimental effect on the graft survival in comparison with preexisting DSAs, while a study by Lionaki et al. [42] reported a link between dnDSAS and reduced allograft survival, even in the absence of clinically evident ABMR. 87 patients from the DART study were identified by Jordan et al. [43]as evidence that the PPV of dd-cfDNA increases when used in combination with dnDSAs. After a 1 year follow-up period, none of our surveillance recipients with serial dd-cfDNA monitoring developed dnDSAs, and only four of the patients who were biopsied had developed dnDSAS. It should be noted that three of the four patients who had dnDSA had ABMR and had levels of dd-cfDNA >1%, while the fourth patient had recurrence of primary FSGS without any rejection and had a low level of dd-cfDNA at diagnosis (0.31%). A small sample size made it impossible to perform any analysis.

Previously mentioned, the major limitation of our study was the small sample size, which prevented the correlation of dd-cfDNA with DSA formation, and in combination with some very high values in ABMR patients, led to large confidence intervals and high diagnostic odds ratios regarding rejection. Nevertheless, these limitations did not affect the AUROC performance or usefulness of the biomarker as a monitoring tool. A longer follow up period could also strengthen the correlation between high dd-cfDNA and worse outcome regarding eGFR over time. Moreover, in view of the evident multifactoral value of considering dd-cfDNA as part of the clinical assessment of the patient, further research is required to determine the optimal monitoring interval. The novelty of this study is that Alloseq dd-cfDNA kit was used locally for dd-cfDNA testing and useful clinical data was provided about how this kit performed in the real-world.

In summary, this report using AlloSeq cfDNA kit for local testing confirms large multicenter service-based trials regarding dd-cfDNA's validity as a tool to surveil the allograft quiescence, to detect rejection and monitor treatment, as well as to predict outcomes regarding graft survival. However, since dd-cfDNA is positioned to be added within the existing panel of current routine testing rather than be used as single information for taking clinical decision, it is undisputed that further research combining dd-cfDNA with other biomarkers is required to improve our diagnostic tools in relation to allograft rejection.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by Ethics Committee of General Hospital Laiko Athens. 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

Conceptualization, SC and JNB; methodology, VF, PC, SC, SM, and JNB; software, EM; validation, EM, VF, and GL; formal analysis, EM, VF, and AV; investigation, EM and VF; resources, PC and SC; data curation, EM; writing—original draft preparation, EM; writing—review and editing, VF, SC, SM, and JNB; visualization, EM and VF; supervision, SM and JNB; project administration, JNB. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

The study received a grant from CareDx and Genotypos-Bioanalytica. SC has an affiliation with CareDx. PC has an affiliation with Genotypos-Bioanalytica.

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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ORIGINAL RESEARCH
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Excess Mortality in Kidney and Kidney-Pancreas Transplant Recipients in the COVID-19 Pandemic in Portugal—A Cohort Study

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The COVID-19 pandemic increased morbidity and mortality worldwide, particularly in the Kidney and Kidney-Pancreas Transplant Recipient (KTR/KPTR) population. Aiming at assessing the absolute and relative excess mortality (EM) in a Portuguese KTR/KPTR cohort, we conducted a retrospective observational study of two KTR/KPTRs cohorts: cohort 1 (*P1*; n = 2,179) between September/2012 and March/2020; cohort 2 (*P2*; n = 2067) between March/2020, and August/2022. A correlation between relative and absolute EM and age, sex, time from transplantation and cause of death was explored. A total of 145 and 84 deaths by all causes were observed in *P1* and *P2*, respectively. The absolute EM in *P2* versus *P1* was 19.2 deaths (observed/expected mortality ratio 1.30, p = 0.006), and the relative EM was 1.47/1,000 person-months (95% CI 1.11–1.93, p = 0.006). Compared to the same period in the general population, the standardized mortality rate by age in *P2* was 3.86 (95% CI 2.40–5.31), with a peak at 9.00 (95% CI 4.84–13.16) in *P2*C. The higher EM identified in this population was associated, mainly, with COVID-19 infection, with much higher values during the second seasonal COVID-19 peak when compared to the general population, despite generalized vaccination. These highlight the need for further preventive measures and improved therapies in these patients.

Keywords: excess mortality, kidney transplant, COVID-19, mortality, pancreas allograft

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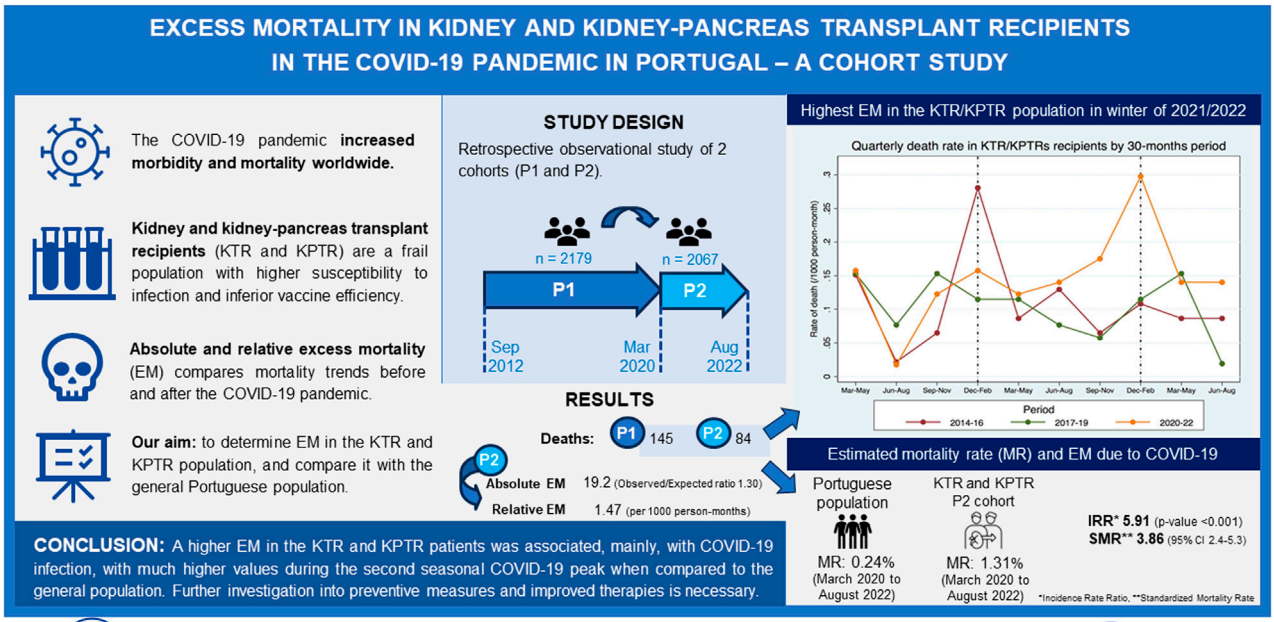
Abbreviations: BMI, Body Mass Index; CHUSA, Centro Hospitalar Universitário de Santo António (Porto, Portugal); CI, Confidence Interval; CKD, Chronic Kidney Disease; DGS, Directorate-General of Health in Portugal (*Direção Geral de Saúde*); EM, Excess Mortality; ESKD, End-Stage Kidney Disease; INE, Statistics Portugal (*Instituto Nacional de Estatística*); IR, Incidence Rate; IRR, Incidence Rate Ratio; KT, Kidney Transplant; KTR, Kidney Transplant Recipient; KPTR, Kidney and Pancreas Transplant Recipient; NA, Not Attributed; O/E, Observed/Expected; RPT, Portuguese Transplantation Registry (*Registo Português de Transplantação*); SER, Portuguese Electronic Health Registry (*Registo de Saúde Eletrónico*); SMR, Standardized Mortality Rate; SOT, Solid Organ Transplant; UK, United Kingdom.



GRAPHICAL ABSTRACT |

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the highly contagious etiological agent of the novel coronavirus disease 2019 (COVID-19), which was responsible for the unprecedented pandemic that started at the beginning of 2020.

In Portugal, epidemiologic studies in the general population reported a total of 5,417,101 confirmed cases and 24,855 deaths due to COVID-19 between March 2020, and August 2022 [1]. In 2021, the number of deaths attributed to COVID-19 noticeably increased to 12,004 (9.6% of all deaths), when compared to a total of 6,972 deaths reported due to COVID-19 in 2020, and January 2021 was the month with the highest number of deaths due to COVID-19 (n = 5,804) [1, 2]. We noticed that, in the second winter, January 2022 registered the highest monthly record of infections (1,277,754 confirmed cases) but only 1,002 deaths, representing an estimated 82.7% reduction in COVID-19-related deaths compared to the homologous month of the previous year. The emergence of vaccination strategies against SARS-CoV-2 and less lethal SARS-COV-2 variants were believed to have significantly reduced the morbidity and mortality associated with COVID-19. As countries worldwide started waiving the tight sanitary measures and progressively lifting the protective measures in place in 2022, it has led to increased exposure of SOT recipients, who are immunosuppressed patients prone to infection in general and to SARS-CoV-2 in particular. The morbidity and mortality associated with COVID-19 have been the subject of great concern in kidney transplant and kidney-pancreas transplant recipients (KTR/KPTRs), and we believe it is crucial to assess the impact of COVID-19 infection in this frail patient population, whether mortality and EM during the COVID-19 pandemic in these patients differed from the general public, and to discuss the role of preventive measures such as vaccination in KTRs.

This study aims to investigate the mortality rate and EM in the KTR/KPTR patient population during the COVID-19 pandemic and compare them with those of the general Portuguese population.

MATERIALS AND METHODS

This was a retrospective cohort study of patients who received a kidney or kidney and pancreas transplant before August 2022, at Centro Hospitalar Universitário de Santo António (CHUSA) and remained active transplant recipients between September 2012, and August 2022. Patients with time since last transplant less than 6 months were excluded. Data were retrieved from the Portuguese Transplantation Registry (*Registo Português de Transplantação*, RPT), Portuguese Electronic Health Registry (*Registo de Saúde Eletrónico,* RSE), and CHUSA database.

Two patient cohorts were considered pertaining to two time intervals: one comprising 90 months before the COVID-19 pandemic (i.e., between September 2012, and March 2020; pre-pandemic cohort [P1]), and another comprising

30 months of COVID-19 pandemic (i.e., between March 2020, and August 2022; pandemic cohort [P2]).

A time-dependent prospective model was used to calculate expected deaths in the kidney transplant population between cohorts. We first determined the likelihood of death in person-months among a database of observed deaths and mortality rate in a given time-frame (between September 2012 and March 2020, among kidney transplant recipients with time of transplant prior to March 2020), adjusted by exposure (months since time of transplant) during the pre-pandemic cohort (P1). Our survival model allowed us to estimate the expected absolute mortality and the absolute observed/expected mortality (O/E) ratio in the COVID-19 era, using a second dataset of observed deaths (both COVID-19 and non-COVID-19 related deaths). By establishing the mortality rate among KTR included in the second cohort (P2), absolute EM was estimated by subtracting the expected absolute mortality from the observed absolute mortality. We were able to determine the relative excess mortality (or incidence rate ratio, *IRR)* in the KTR population during the COVID-19 pandemic (P2), after adjusting observed deaths (between March 2020 and August 2022) to exposure (months since time of transplant), and comparing both mortality rates in person-months (incidence rates, *IR*) between our two different time-frames (P1 and P2). The mortality rate per 1,000 person-months and incidence rate ratio (IRR) in P1 and P2 were estimated with a 95% confidence interval. We used a univariate analysis to adjust our EM findings to several variables, including age (<20 years, 20–40 years, 40–50 years, 50–60 years, 60–70 years, >70 years), sex, time from transplantation (0–6 months, 6–12 months, 12–24 months, 24–48 months, 48–120 months, >120 months), and cause of death (cardiovascular disease, malignancy, COVID-19 infection, non-COVID-19 infection, all infections, other causes).

EM in the KTR population during the pandemic (P2) was further assessed according to three consecutive 10-month intervals: P2A (from March to December 2020), P2B (from January to October 2021), and P2C (from November 2021 to August 2022).

The significance as a function of the considered variables in EM was estimated using the Chi-square test (x^2).

Statistical analysis was performed using Stata® for Windows.

Publicly available sources (Statistics Portugal [*Instituto Nacional de Estatística*, INE], the Directorate-General of Health [*Direção Geral de Saúde*, DGS], and other readily available online sources) were used to retrieve epidemiological data regarding incidence peaks of COVID-19 infection and COVID-19 vaccination rates and coverage in the general Portuguese population. The EM identified in the KTR study cohort between March 2020, and August 2022, was compared to EM of the general Portuguese population.

RESULTS

A total of 2,179 KTR/KPTRs (corresponding to an exposure of 144.641 person-months) were included in P1, and 2,067 KTR/KPTRs (corresponding to an exposure of 57.080 person-months) were included in P2.

Global Excess Mortality in the KTR/KPTR Population Before (P1) and During (P2) COVID-19 Pandemic

The absolute and relative EM found in the KTR/KPTR population *in P1 and P2 is* depicted in **Tables 1**, **2**, respectively.

Overall, 145 and 84 deaths by all causes were observed in this patient population in P1 and P2, respectively (**Tables 1** and **2**). The absolute EM in P2 compared to P1 was 19.2 deaths (O/E ratio 1.30, $p = 0.006$), and the relative EM was 1.47 per 1,000 person-months (95% CI 1.11–1.93, $p = 0.006$).

Stratifying the analysis according to the three pandemic time intervals considered, 21, 26, and 37 deaths by all causes were identified in P2A, P2B, and P2C, respectively (**Tables 1**, **2**). P2C had the highest absolute and relative EM of the three time periods. Compared to P1, the absolute EM was 2.1 deaths in P2A (O/E ratio, 1.11, $p = 0.621$), 6.2 deaths in P2B (ratio O/E, 1.32, $p = 0.154$), and 15.4 deaths in P2C (O/E ratio, 1.71, $p = 0.001$). This translates into a relative EM of 1.12 in the pandemic period P2A ($p = 0.602$), 1.37 in P2B ($p = 0.149$), and 1.90 in P2C ($p = 0.001$) compared to before the pandemic (P1).

The highest EM in the KTR/KPTR population during the COVID-19 pandemic was seen in 2021/2022 winter, with a mortality rate approximately 3 times higher than that of the two previous pre-pandemic periods (**Figure 1**). Conversely, in 2020/2021 winter, only a moderate mortality increase was observed in this patient population [3]. An opposite trend was observed in the general Portuguese population during the pandemic, with the highest EM recorded in 2020/2021 winter (20–60% EM compared to the previous winter), and only a marginal increase in EM (−4 to +6%) seen in 2021/2022 winter compared to the last pre-COVID-19 winter.

Excess Mortality by Age of Transplant Recipients

An absolute EM (i.e., >0 deaths and/or O/E ratio >1.0) was identified in most age subgroups assessed. The exception was the 20–40 year-old subgroup, where the O/E ratio was 0.93. However, statistical significance was only achieved in the subgroup of patients aged between 60–70 years, where an observed absolute mortality of 29 deaths, an expected absolute mortality of 20 deaths, and an O/E ratio of 1.45 ($p < 0.020$) were reported, together with an increase in relative EM (IRR 1.792, 95% CI, 1.066–2.984, $p = 0.021$) (**Tables 1**, **2**).

Excess Mortality by Sex of Transplant Recipients

An absolute EM was observed in both female and male transplant recipients, with a higher number of observed and expected deaths in men (50 and +37.9, respectively) compared to women (34 and +26.9, respectively). IRR was also higher in men (1.508, 95% CI 1.041–2.166 vs. 1.413; 95% CI 0.900–2.183 in women), but the mortality rate per 1,000 person-months was lower (IR 1.436 vs. 1.531 in women). However, this difference was not statistically significant ($p = 0.112$) (**Tables 1**, **2**).

TABLE 1 | Absolute excess mortality in the KTR/KPTR population in the P2 period.

Subgroup	*P1*	*P2*				
	Observed (deaths)	Observed (deaths)	Expected (deaths)	Excess	O/E death ratio	*p*-value
Total	145	84	64.8	19.2	1.30	0.006
P2A	—	21	18.9	2.1	1.11	0.621
P2B	—	26	19.8	6.2	1.31	0.154
P2C	—	37	21.6	15.4	1.71	0.001
Age						
0–20	0	1	0.3	0.7	3.33	0.271
20–40	12	4	4.3	−0.3	0.93	0.558
40–50	17	9	6.7	2.3	1.34	0.311
50–60	29	13	12.2	0.8	1.07	0.789
60–70	38	29	20	9.0	1.45	0.020
70+	49	28	22.8	5.2	1.23	0.204
Sex						
Male	84	50	37.9	12.1	1.32	0.024
Female	61	34	26.9	7.1	1.26	0.113
Time since last KT						
0–6 months	11	8	4.3	3.7	1.86	0.048
6–12 months	4	1	1.1	−0.1	0.91	0.694
12–24 months	5	1	1.3	−0.3	0.77	0.626
24–48 months	12	7	4.0	3.0	1.75	0.084
48–120 months	44	21	14.7	6.3	1.43	0.047
+120 months	69	46	31.1	14.9	1.48	0.003
Cause of death						
Global	145	84	64.8	19.2	1.30	0.006
Cardiovascular	45	18	17.8	0.2	1.01	0.961
Malignancy	34	12	13	−1.0	0.92	0.737
COVID-19 infection	36	27	17.8	9.2	1.52	0.014
No COVID-19 infection	36	13	13.9	−0.9	0.94	0.782
All infections	36	40	21.5	18.5	1.86	<0.001
Other causes	30	14	12.5	1.5	1.12	0.608

KPTR, Kidney and pancreas transplant recipient; KT, Kidney transplant; KTR/KPTRs, Kidney/kidney-pancreas transplant recipients; O/E, observed/expected; P1, Pre-COVID-19 pandemic period; P2, COVID-19 pandemic period; P2A, March to December 2020 pandemic period; P2B, January to October 2021 pandemic period; P2C, November 2021 to August 2022 pandemic period.

Excess Mortality by Time Since the Last Active Kidney Transplant

In this study, KTRs who received an active transplant less than 6 months before August 2022 had a significant increase in absolute EM (O/E ratio 1.86, $p = 0.048$). KTRs with an active transplant for 48–120 months and >120 months also showed a significant rise in the number of deaths and absolute EM (O/E ratio 1.43, $p = 0.047$ and O/E ratio 1.48, $p = 0.003$, respectively). The mortality rate in KTRs with >120 months of active kidney transplant was 1.429 per 1,000 person-months, and the relative EM was highest in this patient subgroup (IRR 1.800, 95% CI 1.212–2.652, $p = 0.003$). Interestingly, there was no absolute or relative EM in the periods between 6 and 12 months of active kidney transplant (O/E ratio 0.91, $p = 0.694$; IRR 0.881, $p = 0.981$) or between 12 and 24 months of active transplant (O/E ratio 0.77, $p = 0.626$; IRR 0.743, $p = 0.865$) (**Tables 1**, **2**).

Excess Mortality by Non-COVID-19 Causes

The absolute and relative EM due to cardiovascular causes, malignancy, or other non-COVID-19 causes was not statistically significant ($p > 0.5$ for all subgroups). In the subgroup of death by all causes of infection, the absolute EM was +18.5 (O/E ratio 1.86, $p < 0.001$), and IRR was 2.82 (95% CI 1.750–4.546, $p < 0.001$). However, when excluding deaths attributed to COVID-19 infection in *P2*, the absolute and relative EM due to all causes of infection (excluding COVID-19) was not statistically significant ($p > 0.5$) (**Tables 1**, **3**)

Excess Mortality by COVID-19 Infection

Of the total 84 deaths observed in P2, 32% ($n = 27$) were due to COVID-19 infection. The analysis by 10 months periods showed that 5 out of 21 deaths (24%) in P2A, 4 out of 26 deaths (15%) in P2B, and 18 out of 37 deaths (49%) in P2C were attributed to COVID-19. Among deaths due to infection ($n = 40$), nearly 68% ($n = 27$) were attributed to COVID-19 infection (**Tables 1**, **3**, **4**).

In a stratified correlation analysis between EM and cause of death, the expected deaths by COVID-19 infection in transplant recipients reached 17.8 (vs. 27 observed deaths), with an O/E ratio of 1.52 ($p = 0.014$). The mortality rate due to COVID-19 in transplant recipients was 0.473 per 1,000 person-months, and the IRR was 1.901 (95% CI 1.110–3.219).

According to data from INE, DGS, and other online sources [4], 24,855 deaths due to COVID-19 infection were recorded in the general Portuguese population (10,206,016 inhabitants)

TABLE 2 | Mortality rates in the KTR/KPTR population in P1 and P2 periods, and relative excess mortality in P2.

Subgroup	*P1*			*P2*			IRR (95% CI)	*p*-value
	Observed (deaths)	Exposure (person-months)	IR (per 1,000 person-months)	Observed (deaths)	Exposure (person-months)	IR (per 1,000 person-months)		
Total	145	144,641	1.003	84	57,080	1.472	1.468 (1.108–1.934)	0.006
P2A	—	—	—	21	18,631	1.127	1.124 (0.675–1.784)	0.602
P2B	—	—	—	26	18,929	1.374	1.370 (0.866–2.090)	0.149
P2C	—	—	—	37	19,459	1.901	1.897 (1.285–2.738)	0.001
Age								
0–20	0	2,190	0	1	814	1.229	NA	0.271
20–40	12	18,357	0.654	4	6,781	0.590	0.902 (0.212–2.977)	0.895
40–50	17	32,816	0.518	9	11,319	0.795	1.535 (0.603–3.640)	0.307
50–60	29	36,478	0.795	13	14,944	0.870	1.094 (0.522–2.172)	0.774
60–70	38	32,616	1.165	29	13,887	2.088	1.792 (1.066–2.984)	0.021
70+	49	2,285	2.209	28	9,335	3.000	1.358 (0.822–2.204)	0.201
Sex								
Male	84	88,262	0.952	50	34,829	1.436	1.508 (1.041–2.166)	0.024
Female	61	56,288	1.084	34	22,209	1.531	1.413 (0.900–2.183)	0.112
Time since last KT								
0–6 months	11	4,721	2.330	8	1,394	5.739	2.463 (0.860–6.723)	0.064
6–12 months	4	9,128	0.438	1	2,589	0.386	0.881 (0.018–8.907)	0.981
12–24 months	5	17,871	0.280	1	4,808	0.208	0.743 (0.016–6.643)	0.865
24–48 months	12	34,235	0.351	7	9,086	0.770	2.198 (0.733–6.055)	0.114
48–120 months	44	71,204	0.618	21	20,799	1.010	1.634 (0.923–2.807)	0.072
+120 months	69	86,893	0.794	46	32,182	1.429	1.800 (1.212–2.652)	0.003

CI, Confidence interval; IR, Incidence rate; IRR, Incidence rate ratio; KTR/KPTRs, Kidney/kidney-pancreas transplant recipients; KT, Kidney transplant; KTR, Kidney transplant recipient; NA, Not attributed; P1, Pre-COVID-19 pandemic period; P2, COVID-19 pandemic period; P2A, March to December 2020 pandemic period; P2B, January to October 2021 pandemic period; P2C, November 2021 to August 2022 pandemic period.

between March 2020 and August 2022, accounting for a mortality rate of approximately 0.24% (**Table 4**). In a subanalysis, the observed deaths and mortality rate due to COVID-19 infection in the KTR population in P2 were directly compared with those of the general Portuguese population, considering the same 10-month stratification periods: period A, between March 2020, and December 2020; period B, between January 2021, and October 2021; and period C, between November 2021, and August 2022; **Table 4**), and the pre-COVID cohort (P1) was not included in this comparison. Overall, relative EM was estimated with an IRR of 5.91 due to COVID-19 infection between March 2020 and August 2022 and a standardized mortality rate (SMR) by age of 3.86 (95% CI 2.40–5.31, $p < 0.001$). A much higher EM was observed during period C (+16.7) compared to periods A and B (+2.8 and +3.0, respectively) in the KTR/KPTR population, and a relatively high EM due to COVID-19 was also observed during period C in transplant recipients compared to the general population (IRR 14.19, SMR 9.00, 95% CI 4.84–13.16, $p < 0.001$).

DISCUSSION

The emergence of vaccination strategies against SARS-CoV-2 significantly reduced the morbidity and mortality associated with COVID-19 in the general public. A large-scale observational US study from 2021 reported a mortality reduction of over 80% in SARS-CoV-2-vaccinated individuals (i.e., with two doses of the mRNA Pfizer-BioNTech® vaccine,

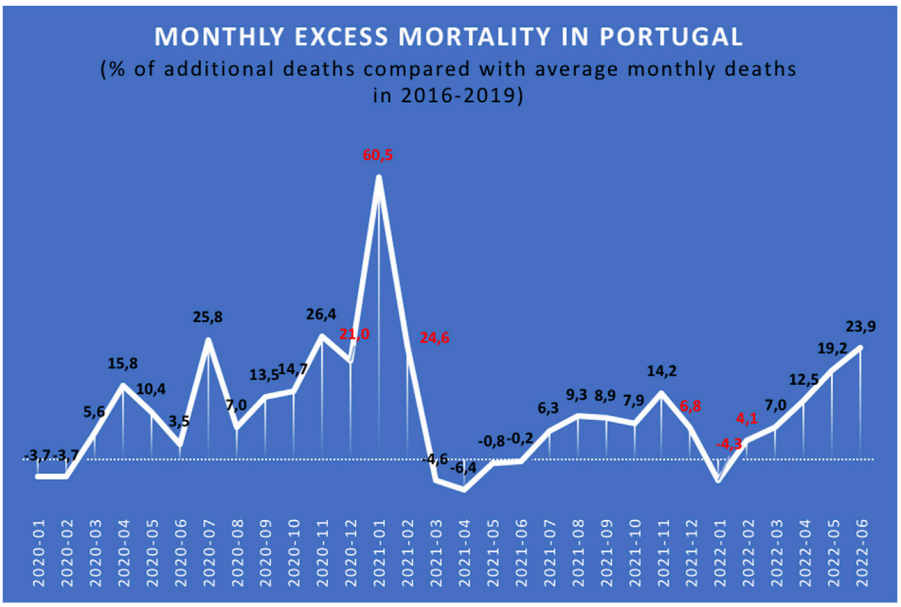


FIGURE 1 | Relative excess mortality (%) in the Portuguese population during the COVID-19 pandemic [3].

TABLE 3 | Relative excess mortality in the KTR/KPTR population in P1 and P2 periods according to cause of death.

Subgroup	*P1*		*P2*		IRR (95% CI)	*p*-value
	Observed (deaths)	IR (per 1,000 person-months)	Observed (deaths)	IR (per 1,000 person-months)		
Cause of death						
Global	145	1.003	84	1.472	1.468 (1.108–1.934)	0.006
Cardiovascular	45	0.311	18	0.315	1.014 (0.552–1.786)	0.946
Malignancy	34	0.235	12	0.210	0.894 (0.422–1.771)	0.759
COVID-19 infection	NA	NA	27	0.473	1.901 (1.110–3.219)	0.014
No COVID-19 infection	36	0.249	13	0.228	0.915 (0.445–1.767)	0.802
All infections	36	0.249	40	0.701	2.816 (1.750–4.546)	<0.001
Other causes	30	0.207	14	0.245	1.183 (0.579–2.300)	0.597

CI, Confidence interval; IR, Incidence rate; IRR, Incidence rate ratio; KTR/KPTRs, Kidney/kidney-pancreas transplant recipients; NA, Not attributed; P1, Pre-COVID-19 pandemic period; P2, COVID-19 pandemic period.

two doses of the mRNA Moderna® vaccine, or one dose of the Johnson & Johnson® adenovirus vaccine) [5]. In Portugal, the vaccination campaign started on December 2020 [6]. In our experience, transplant recipients were among the first citizens eligible to receive their first dose of vaccination, as they are a priority group, and started receiving the boost dose by October 2021. By December 2021, nearly all patients had at least full dose vaccination for COVID-19.

According to several sources [3, 7], the monthly excess mortality (EM) associated with the COVID-19 pandemic in Portugal was much higher in January 2021 (between 21.0% and 60.5%) than in January 2022 (between −4.3% and 6.8%) (**Figure 1**) [3]. Possible explanations for this EM reduction are vaccine effectiveness in the general Portuguese population, on the one hand, and the emergence and implementation of the SARS-CoV-2 Omicron variant, on the other, as a fast-spreading variant that several authors argue is less lethal than its Delta variant predecessors [8, 9].

In our KTR cohort in the pandemic period and until August 2022, we found an absolute EM of 19, with an O/E death ratio of 1.30, and an increased death IRR of 1.47. These results are in

TABLE 4 | Mortality due to COVID-19 infection in KTR/KPTRs and in the general Portuguese population between March 2020 and August 2022.

Subgroup	General Portuguese population		Subgroup	KTR/KPTR population				IRR	*p*-value	SMR by age (95% CI)
	Observed (deaths)	Mortality rate (%)		Observed (deaths)	Mortality rate (%)	Expected (deaths)	Excess			
T	24,855	0.24	T	27	1.31	4.6	22.4	5.91	<0.001	3.86 (2.40–5.31)
A	6,906	0.07	*P2A*	4	0.21	1.2	2.8	3.22	0.050	2.50 (0.31–4.69)
B	11,250	0.11	*P2B*	5	0.26	2.0	3.0	1.96	0.074	1.33 (0.03–2.64)
C	6,699	0.07	*P2C*	18	0.91	1.3	16.7	14.19	<0.001	9.00 (4.84–13.16)

A, COVID-19 period between March 2020 and December 2020; B, COVID-19 period between January 2021 and October 2021; C, COVID-19 period between November 2021 and August 2022; IRR, Incidence rate ratio; KTR/KPTRs, Kidney/kidney-pancreas transplant recipients; P2A, March to December 2020 pandemic period; P2B, January to October 2021 pandemic period; P2C, November 2021 to August 2022 pandemic period; SMR, Standardized mortality rate; T, COVID-19 period between March 2020 and August 2022.

agreement with those reported in other studies. For example, Massie et al. found a 41.2% increase in expected deaths and a 1.42 O/E death ratio in KTRs between March 2020 and March 2021 [10].

When investigating probable EM causes, an increased risk of EM was found to be associated with some parameters. COVID-19 introduced a new morbidity and mortality risk globally, and more so in the transplant recipient population. The results of the present study confirm an absolute and relative EM due to COVID-19 infection but not due to other infection etiologies. Furthermore, when looking at deaths in the KTR population during the 10 months (P2C) period that included the second seasonal peak of the COVID-19 pandemic (January 2022), COVID-19 infection accounted for nearly half of deaths from all causes (49%), with an IRR of 1.9, which surpassed expectations.

In line with previous publications, an age-related effect on EM was identified in transplant recipients during the pandemic. Requião-Moura et al. showed that age and time after transplantation were both related to an increased probability of death in KTRs [11]. A systematic review by Roxanne Opsomer et al. demonstrated a consistent correlation between mortality due to COVID-19 and age in SOT recipients, although data supporting transplantation time as an independent risk factor for mortality in severe COVID-19 disease was more disputed [12]. Our analysis showed significantly higher absolute and relative EM by age (between 60 and 70 years-old), and by time since last transplant (over 120 months), and interestingly, considering the infectious risk in the early post-transplant period, we did not find a significant correlation between EM and early time of transplant or younger age. One major explanation is higher mortality due to more age-related comorbidities (cardiovascular and others) in kidney recipient patients with a prolonged active transplant status, considering the long time of exposure of the majority of our transplant cohort samples, which include active grafts that date back as early as 1983, and newly transplanted patients during the study time period were relatively very few.

As expected, we faced limitations. A true case-control mortality model was not possible, since our cohort model had significant overlap between patients from the P1 and P2 transplant recipient cohorts. Also, we should clarify that new kidney transplants during both pre-COVID-19 and the COVID-19 era were included in our sample, as long as the time since the last transplant was at least 6 months. Besides, analysis of excess mortality in a kidney and pancreas transplant recipient subgroup was scarce and inconclusive, and the data was not published. We also lacked data which could allow to stratify the risk of death in the transplant recipient populations for other variables, such as diabetes, smoking, BMI and cardiovascular disease. Conceptually, our statistical approach would not allow for a multivariate analysis.

The comparison of mortality between the cohort of KTRs with the general Portuguese population during the same pandemic time-frame provided interesting results, moreso when searching for different mortality trends during different COVID-19 pandemic waves. To reduce selection bias, we calculated SMR adjusted by age. EM was high in both groups during the first COVID-19 seasonal peak, but the second seasonal peak brought a reduction in mortality rate and EM in the general Portuguese population, together with a much higher rate of primary full-dose SARS-COV-2 vaccination compared to the first peak. Among transplant recipients, the second seasonal peak was associated not only with higher mortality, but also with a several-fold increase in EM (estimated SMR of 9.00), especially in the last 10 months of the study when most (if not all) active KTRs already had full-dose SARS-COV-2 vaccination. The higher mortality is also illustrated in **Figure 2**, and was partially explained by an approximately 3-fold higher incidence of COVID-19 infection in the general population, mainly during the second seasonal peak. Additional factors may have contributed to the increase in EM observed in the present study, including the shift in social restriction policies that took place in Portugal in 2022 [13, 14]. **Figure 2** also shows a seasonal peak in the 2014/2015 winter, probably related to an unusually high EM "from all causes" among the general Portuguese population when compared to the previous winter (global EM of 17%, maximum weekly EM of 36%), largely explained by a high-intensity flu epidemic with unusually low temperatures [15].

CKD is a well-established risk factor for COVID-19-associated mortality [16–21], and questions have arisen about the effectiveness of the COVID-19 vaccine in patients with chronic kidney disease (CKD), end-stage kidney disease (ESKD) on chronic dialysis, and KTR/KPTRs undergoing chronic immunosuppression [22–24].

The seroprevalence of SARS-CoV-2 antibodies has been suggested elsewhere as a measure of vaccine effectiveness [25, 26]. The seroprevalence and decay of SARS-COV-2 antibodies

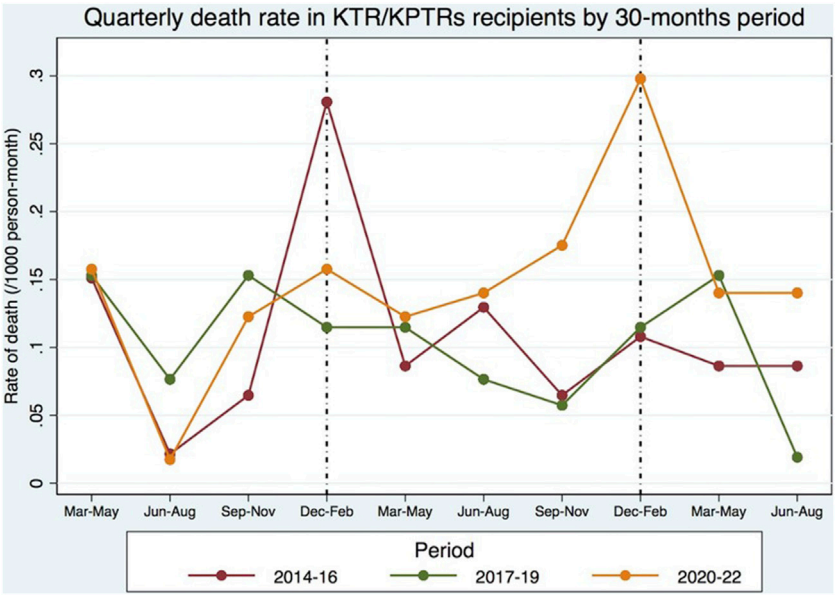


FIGURE 2 | Global mortality in the KTR/KPTR population by 30 month-periods, including in P1 (between 2014 and 2019) and P2 (between March and August 2022) [3].

after full-dose vaccination of patients with CKD stages 4 and 5, can be very similar to non-CKD controls [27, 28]. In dialysis patients, the COVID-FRIAT study and other studies reported that the prevalence of antibodies in hemodialysis patients was slightly lower (80–95%), and had an earlier and faster decline compared to the general population [28–31]. Studies on antibody seroprevalence in the KTR/KPTR population showed exceedingly lower values compared to non-transplant counterparts. Sanders *et al.* reported a SARS-CoV-2 seroprevalence after complete vaccination of only 57.7% at day 28% and 49% at 6 months [28]. A Scottish cohort study reported a vaccine effectiveness rate against COVID-19 of only 39% in KTRs who received a full-dose vaccine regimen (i.e., 2 doses of ChAdOx1 nCoV-19 (Vaxzevria) vaccine or 2 doses of mRNA BNT162b2 vaccine) compared to 67–80% in the general UK population [23]. Vaccine effectiveness against hospitalization is also relatively low in this patient population (40%), and the mortality rate due to COVID-19 is 10% versus <0.1% in the general population. Bell et al. compared survival rates in ESKD and KTR patients in two consecutive COVID-19 pandemic waves and showed that survival rates at 28 days after positive SARS-CoV-2 testing in ESKD patients were higher during the second pandemic wave, while in KTRs were nearly overlapping between the first and second waves [23]. It can be hypothesized that this difference is due to the lack of COVID-19 vaccine effectiveness in KTRs, and EM may be an indirect marker of failure of current vaccination strategies among transplant recipients. Interestingly, in other countries, some authors have reported a reduced overall COVID-19 mortality rate among KTRs until 2021 [32, 33], possibly explained by high accessibility to SARS-CoV-2 testing, new treatments, and vaccination, also referring to different timelines and unaccounted confounding factors, such as younger age and less comorbidities, in the study population of the second pandemic wave.

More recently, studies on the serological response in kidney transplant recipients after 3 and 4 doses of a SARS-COV-2 vaccine found higher seroconversion among this population (up to 75%), but Thomson et al. highlight that a significant proportion of transplant recipients still remain seronegative after 3 and 4 doses of SARS-CoV-2 vaccines [34].

Results retrieved from this study emphasize the need for vigilance and a continuous search for alternative therapies to prevent COVID-19 infection and mortality in the solid organ transplant recipient population. Therapies with SARS-COV-2 neutralizing antibodies targeting viral surface proteins in immunocompromised patients seemed promising for SOT recipients, but the rate of appearance of new SARS-CoV-2 strains and the cost and availability of immunoprophylaxis with monoclonal antibodies pose considerable restraints [35]. Recently, the PANAMO study in critically ill patients with COVID-19 infection showed promise using monoclonal antibodies targeting the complement system (C5a), and another study with a neutralizing antibody targeting the receptor binding site for the virus also showed interesting results [36, 37]. Further therapies are also expected to emerge and significantly improve the prognosis of SOT recipients with COVID-19 infection.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by Comissão de Ética CHUdSA/ICBAS. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

MC and JAF participated in the performance of the research, data analysis and writing of the paper. JCF, RC, and SV participated in the writing of the paper. CR, JS, SP, and MA participated in the performance of the research. LM and JM participated in the research design, the performance of the research and data analysis. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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Telehealth Experience Among Liver and Kidney Transplant Recipients: A Mixed Methods Study

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Telehealth has become widely available to solid organ transplant (SOT) recipients during the COVID-19 pandemic. While evidence suggests that telehealth serves as an acceptable alternative for most SOT recipients, their satisfaction and its context remain unclear. This study used a mixed methods approach to investigate the perspectives of SOT recipients (i.e., liver, kidney, and simultaneous liver-kidney) on the benefits and disadvantages of telehealth. A total of 252 adult SOT recipients completed an online survey that quantitatively assessed telehealth experience and satisfaction. Fifteen of them further shared their perspectives by participating in either a focus group or individual interview. Approximately 70% of online survey participants had previously used telehealth for their transplant care. The quantitative data documented that, while recipients were mostly satisfied with telehealth, especially with its effectiveness and convenience, they were less satisfied with the reliability of navigating the telehealth system. The qualitative data further showed that telehealth could be less effective for SOT recipients who perceived themselves as clinically and/or socially vulnerable, needed urgent care, and were concerned about privacy. These findings suggest that the plan for using telehealth to provide transplant care should prioritize personalization, considering unique needs and preferences of each SOT recipient.

Keywords: telehealth, solid organ transplant, healthcare delivery, patient-centered care, mixed methods design

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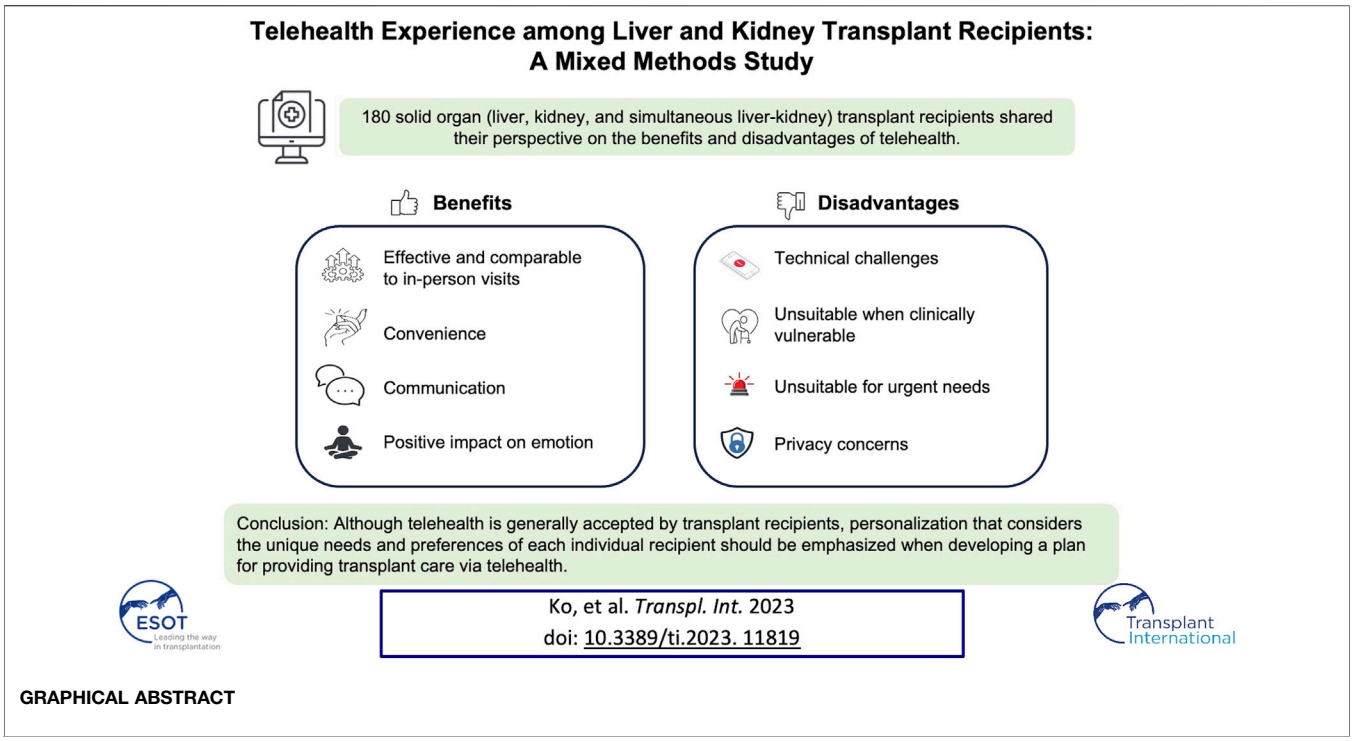
INTRODUCTION

Telehealth refers to the delivery of healthcare, education, and support using telecommunications technologies, such as live videoconferencing [1]. Telehealth has been primarily used to support chronic disease care management that requires regular clinic visits, particularly for individuals living in rural areas [2]. Indeed, telehealth has shown to improve access by reducing travel time and costs [3] while advancing patient outcomes, such as better quality of life and decreased rehospitalization [4]. Despite such benefits, telehealth was not widely available before the COVID-19 pandemic due to multiple barriers, including interstate licensing restrictions,

Abbreviations: IQR, Interquartile Range; REDCap, Research Electronic Data Capture; SOT, Solid Organ Transplant; TUQ, Telehealth Usability Questionnaire.



GRAPHICAL ABSTRACT

insurance coverage, and lack of infrastructure [5, 6]. The COVID-19 pandemic, however, encouraged many providers and insurers to embrace telehealth.

Solid organ transplant (SOT) recipients, such as liver, kidney, and simultaneous liver-kidney, are required to engage in lifelong care that involves taking immunosuppressants as prescribed and regular follow ups to maintain long-term transplant function. Telehealth has played a critical role in providing essential care for SOT recipients during the COVID-19 pandemic [7]. Telehealth appears to be an acceptable alternative for most SOT recipients. They reported comparable satisfaction to in-person visits with minimized burden of travel [8, 9]. While beneficial in many ways, however, disadvantages of telehealth may exist. Some types of care that require physical contact may not be feasible via telehealth [10]. Lack of technological literacy or reduced access to telecommunications infrastructure among SOT recipients may hinder the effective use of telehealth [9].

Understanding benefits and disadvantages of telehealth from SOT recipients' perspectives could suggest ways to improve telehealth for them. An approach of continuous improvement is particularly critical because SOT recipients have reported their willingness to use telehealth for certain care services, including synchronous and asynchronous communication with their care providers [11]. While the existing literature has examined the experiences of SOT recipients in relation to transplant care delivered through telecommunications technologies [8, 12–15], evidence is insufficient to fully understand their experiences. Many studies assessed satisfaction of SOT recipients using questionnaires that have not been psychometrically tested [16, 17]. Further, there is a lack of studies employing a mixed method design, which holds the potential to provide a comprehensive understanding of the underlying context influencing their satisfaction. Addressing these gaps in knowledge may inform strategies to advance the telehealth experience among SOT recipients. Thus, this study aimed to understand perspectives of SOT recipients (i.e., liver, kidney, and simultaneous liver-kidney) on the benefits and disadvantages of telehealth.

METHODS

A mixed method design was used to obtain holistic understanding of the perspectives of SOT recipients on telehealth. Quantitative data was collected through an online self-report survey and qualitative data was collected through focus groups and individual interviews.

Survey Design and Recruitment

Participants were recruited using paid Facebook advertisements between May and August 2021. A series of images and descriptions were used over time to improve the efficacy of the advertising. When a potential participant clicked on the advertisements, they were directed to the study page at Research Electronic Data Capture (REDCap) [18, 19]. Individuals were eligible to participate in the survey if they met the following criteria: (1) were aged 18 years old or greater; (2) had received a liver, kidney, or simultaneous liver-kidney transplant; and (3) were currently receiving care for their transplants at a transplant center located in the United States. Individuals who confirmed that they met all

three of these criteria provided online informed consent and completed the survey at REDCap. A total of 876 individuals clicked on the advertisements, 653 were eligible to participate in the study, and 252 individuals completed the survey (response rate 38.6%).

Measures

All participants were asked if they had used telehealth for transplant care after the United States declared a national emergency due to the COVID-19 pandemic in March 2020. Participants who had not used telehealth were asked to provide reasons for not using telehealth. Those who had used telehealth (n = 180) completed a series of questions asking about the use of telehealth. These include types of telehealth, confidence in using telehealth, level of assistance needed to complete telehealth visits, and telehealth satisfaction. Telehealth satisfaction was assessed using the 21-item Telehealth Usability Questionnaire (TUQ), which has strong content validity in assessing the usability of telehealth service [20]. The TUQ has five subscales: usefulness, ease of use, effectiveness, reliability, and satisfaction. Usefulness measures how effective telehealth was at completing desired function. Ease of use determines how easy it was for a patient to complete their appointments and care using telehealth. Effectiveness measures the quality of interaction with clinicians compared to in-person appointments. Reliability measures how well the telehealth system's online help and feedback was in guiding a patient to navigate the system or correct an error. Satisfaction measures how pleased a patient was with their experience overall. Participants were asked to rate their satisfaction with telehealth they received from their transplant center from 1 (strongly disagree) to 7 (strongly agree). With the TUQ developer's consent, several items were adjusted to limit the scope of telehealth services provided for transplant care (**Supplementary Table S1**). Mean total and subscale scores were calculated if at least 75% of the items were answered. Higher scores indicate a greater sense of satisfaction with telehealth care provided. Cronbach's α coefficients of the scores in this study ranged between 0.75 and 0.97. Demographic and clinical characteristics, such as types of organ transplant and time since transplant, were self-reported by all participants.

Focus Group and Interview Design and Recruitment

The survey included a question asking if the respondent would be interested in participating in a focus group discussion or interview. A total of 107 survey respondents indicated their willingness to participate and provided an email address. A study team member contacted all 107 respondents to schedule a call. Among them, 92 could not be contacted or withdrew from participation. A total of 15 participants provided online informed consent via REDCap that explained the purpose and procedure of the focus group.

We conducted two focus group discussions between November 2021 and February 2022, each involving four participants. The focus group was moderated by a trained study team member. The focus group moderator's guide discussed the following topics: quality and connectedness in telehealth compared to in-person visits; participants' ability to manage their medication and self-monitoring; benefits and challenges of using telehealth; confidentiality; suggestions to improve telehealth; and the pandemic's influence on utilizing telehealth. The focus group moderator left time for probing, following up, and cross-talk between participants. Focus groups were held via a HIPAA compliant Zoom meeting (Zoom Video Communications, Inc. San Jose, CA) and lasted approximately 60 min. The focus group transcripts were transcribed verbatim and corrected by a study team member who had observed the proceedings.

We also conducted seven individual interviews with survey respondents who could not attend one of the scheduled focus group meetings. Consent and interview procedures matched what was done for the focus groups. Interviews lasted 10–30 min and were recorded and professionally transcribed. The study team member who conducted the interviews corrected the transcripts.

Pseudonyms were used during focus group discussions and individual interviews and in transcripts to protect participant confidentiality. A $30 gift card was given to participants who participated in a focus group or individual interview.

Data Analysis

We performed quantitative data analysis using IBM SPSS Statistics version 27 (IBM, Armonk, NY). Descriptive statistics were used to describe participant characteristics and scores for the study measures. Sociodemographic characteristics of telehealth users were compared to those of non-users using the Mann-Whitney U test and chi-square test. Associations between characteristics and TUQ scores were assessed using Spearman's rho correlation and Kruskal-Wallis test. Post-hoc comparisons were conducted using Mann Whitney U test for statistically significant Kruskal-Wallis test results. The level of statistical significance was set at $p < 0.05$. While p values were not corrected for multiple tests given the exploratory nature of the study, Bonferroni corrected p values were used on *post hoc* pairwise comparisons.

We analyzed the qualitative transcripts following a hybrid inductive-deductive approach [21]. The moderator's guide and the preliminary codebook we used to analyze transcripts reflected our understanding of the benefits and drawbacks of telehealth for this specific group of patients. We allowed new codes to emerge from the transcripts in response to concepts and themes introduced spontaneously by the focus group respondents, such as perception of risks.

We used NVivo version 12 to code all transcripts. The third author created a codebook that included 29 codes (organized into broad themes), a definition of each code, and representative quotations drawn from the transcripts. The team met to review the codebook and clarify the guidelines for applying codes. The third author then coded one focus group transcript. The team met again to review the coding, to

TABLE 1 | Participant characteristics.

Characteristics	Frequency (%) or median (IQR)		*p*-value[a]
	Telehealth users[b] *n* = 180	Telehealth non-users *n* = 72	
Age	*n* = 166 62.0 (55.0, 68.0)	*n* = 61 64.0 (55.0, 69.5)	0.357
Race	*n* = 177	*n* = 71	0.137
White	155 (87.6%)	65 (91.5%)	
Black or African American	8 (4.5%)	0 (0.0%)	
Asian	4 (2.3%)	0 (0.0%)	
Other	10 (5.6%)	6 (8.5%)	
Gender	*n* = 178	*n* = 71	0.355
Female	126 (70.8%)	46 (64.8%)	
Male	52 (29.2%)	25 (35.2%)	
Ethnicity	*n* = 175	*n* = 72	0.223
Not Hispanic	169 (96.6%)	67 (93.1%)	
Hispanic	6 (3.4%)	5 (6.9%)	
Education	*n* = 177	*n* = 70	**0.017**
Some high school or high school graduate, a diploma or equivalent (e.g., GED)	18 (10.2%)	26 (22.9%)	
Some college credit, no degree or vocational training	54 (30.5%)	26 (37.1%)	
Associate degree (e.g., AA, AS)	23 (13.0%)	3 (4.3%)	
Bachelor's degree (e.g., BA, BS)	50 (28.2%)	13 (18.6%)	
Graduate degree	32 (18.1%)	12 (17.1%)	
Marital Status	*n* = 175	*n* = 71	0.098
Single	43 (24.6%)	25 (35.2%)	
Single, living with a partner	7 (4.0%)	4 (5.6%)	
Married	101 (57.5%)	29 (40.8%)	
Widowed	21 (12.0%)	9 (12.7%)	
Separated	3 (1.7%)	4 (5.6%)	
Employment Status	*n* = 176	*n* = 71	0.125
Employed full or part-time	49 (27.8%)	23 (32.4%)	
Retired	60 (34.1%)	27 (38.0%)	
Unemployed	4 (2.3%)	4 (5.6%)	
On Disability	58 (33.0%)	13 (18.3%)	
Other	5 (2.8%)	4 (5.6%)	
Area of Residence	n = 178	n = 71	0.052
City/Urban	47 (26.4%)	28 (38.9%)	
Suburb	68 (37.8%)	17 (23.9%)	
Country/Rural/Small Town	63 (35.0%)	26 (36.6%)	
Miles traveled to visit the transplant center (roundtrip)	*n* = 178	*n* = 72	0.282
0–10 miles	23 (12.9%)	12 (16.7%)	
11–25 miles	34 (19.1%)	18 (25.0%)	
26–50 miles	20 (11.2%)	10 (13.9%)	
51–100 miles	36 (20.2%)	10 (13.9%)	
101–200 miles	35 (19.7%)	7 (9.7%)	
200+ miles	30 (16.9%)	15 (20.8%)	
Income	*n* = 157	*n* = 60	0.174
Less than $20,000	23 (14.6%)	13 (21.7%)	
$20,000 to $34,999	26 (16.6%)	17 (28.3%)	
$35,000 to $49,999	19 (12.1%)	4 (6.7%)	
$50,000 to $74,999	32 (20.4%)	9 (15.0%)	
$75,000 to $99,999	27 (17.2%)	6 (10.0%)	
Over $100,000	30 (19.1%)	11 (18.3%)	
Organ type	*n* = 178	*n* = 71	0.061
Liver	32 (18.0%)	7 (9.9%)	
Kidney	135 (75.8%)	63 (88.7%)	
Simultaneous liver-kidney	11 (6.2%)	1 (1.4%)	
Time since transplant (months)	*n* = 177 55 (22.5, 127.5)	*n* = 70 62.5 (30.5, 118.0)	0.555

(Continued on following page)

TABLE 1 | (*Continued*) Participant characteristics.

Characteristics	Frequency (%) or median (IQR)		*p*-value[a]
	Telehealth users[b] *n* = 180	Telehealth non-users *n* = 72	
Insurance coverage	*n* = 175	*n* = 72	0.340
Public	77 (44.0%)	36 (50.7%)	
Non-government insurance/private	42 (24.0%)	14 (19.7%)	
Both	55 (31.4%)	19 (26.8%)	
None	1 (0.6%)	2 (2.8%)	

[a]*Mann-Whitney or Chi-square test.*
[b]*Have used telehealth for transplant care.*
Note: Bold values denote statistical significance at the p < *0.05 level.*
Missing values were excluded for calculation of percentages.

determine if any new codes needed to be added to the codebook, and to review preliminary findings. Once the team reached agreement about codes, themes, and coding guidelines, the third author completed coding all transcripts.

RESULTS

Quantitative Results

Participant Characteristics

Our full sample of 252 respondents included liver (*n* = 39, 15.7%), kidney (*n* = 198, 79.5%), and simultaneous liver-kidney (*n* = 12, 4.8%) transplant recipients (excluding three missing values, **Table 1**). Among them, 180 had used telehealth for transplant care. Their median age was 62.0 years (interquartile range [IQR], 55.0–68.0). Most telehealth users were White (87.6%), female (70.8%), and married (57.5%). The majority were either retired (34.1%) or on disability (33.0%) and most had public insurance (44.0%). The median time since receiving their transplant was 55 months (IQR, 22.5–127.5). The characteristics of telehealth non-users were not significantly different than telehealth users, except that telehealth users had higher levels of educational attainment compared to telehealth non-users (**Table 1**). Among the 72 nonusers, the primary reasons for not using telehealth included: a) telehealth not available at their transplant centers (23.1%), b) not comfortable with technology (16.9%), c) no interest in telehealth (15.4%), and d) no access to telehealth equipment or adequate internet or bandwidth (10.8%).

Telehealth Use

More than half of telehealth users had used multiple types of telehealth (55.3%), with real-time video visits being most common (79.4%; **Supplementary Table S2**). Over a third of users reported using telehealth for 81%–100% of their visits to their transplant centers over the past 12 months (36.9%). The majority of users rated themselves as very confident in communicating with their provider via telehealth (58.1%) and no assistance needed (87.8%), whereas some users were concerned about the effectiveness of telehealth (34.8%) or reported lack of familiarity or comfortability with the technology (21.2%). Only 13% of telehealth users reported being not likely to use telehealth for transplant care in the future (**Supplementary Table S2**).

TABLE 2 | Summary of Telehealth Usability Questionnaire (*n* = 180 telehealth users).

Telehealth usability questionnaire	Median (IQR)
Usefulness, *n* = 180	5.8 (5.0–6.7)
Ease of Use, *n* = 179	5.8 (4.8–6.7)
Effectiveness, *n* = 177	5.6 (4.4–6.6)
Reliability, *n* = 178	4.3 (3.5–5.3)
Satisfaction, *n* = 178	6.0 (4.3–6.8)
Total, *n* = 179	5.6 (4.5–6.3)

Telehealth Satisfaction

The total TUQ scores (**Table 2**) indicated that transplant recipients were mostly satisfied with telehealth they received for transplant care (Median = 5.6, IQR = 4.5–6.3). The median score of the reliability subscale was the lowest, whereas the median score of the satisfaction subscale was the highest. **Table 3** and **Figure 1** summarize the statistically significant associations between participants' characteristics and their telehealth satisfaction. Age and time since receiving transplants were inversely correlated with TUQ total and every subscale scores (r_s = −0.20–−0.29 and r_s = −0.16–−0.21; **Table 3**). Male recipients had significantly lower median scores in TUQ total and every subscale than female recipients (**Figure 1**). Recipients who were employed full- or part-time had a significantly higher median score in the TUQ usefulness subscale than those who were retired (Bonferroni-corrected p = 0.038; **Figure 1**). Finally, recipients who obtained some college credit or vocational training had significantly lower median scores in TUQ usefulness, ease of use, and satisfaction subscales than those who had a Bachelor's degree (Bonferroni-corrected p = 0.034, 0.049, and 0.017, respectively; **Figure 1**).

Qualitative Results

Benefits of Telehealth

As illustrated in **Table 4**, focus group participants and interviewees praised the quality and benefits of telehealth. The most frequently mentioned benefit was efficiency, followed by convenience, communication, and affectability. Participants frequently spoke about their satisfaction with telehealth and its benefits not only for managing their transplant recovery but also for other medical care.

TABLE 3 | Telehealth satisfaction by telehealth user characteristics.

Variables	TUQ					
	Usefulness	Ease of use	Effectiveness	Reliability	Satisfaction	Total
	[a]r_s (*p-value*)	r_s (*p-value*)	r_s (*p-value*)	r_s (*p-value*)	r_s (*p-value*)	r_s (*p-value*)
Age (*n* = 166)	−0.236 (0.002)	−0.285 (<0.001)	−0.195 (0.012)	−0.230 (0.003)	−0.236 (0.002)	−0.255 (<0.001)
Time since transplant (*n* = 177)	−0.202 (0.007)	−0.173 (0.021)	−0.211 (0.005)	−0.162 (0.032)	−0.213 (0.005)	−0.214 (0.004)

[a]*Spearman ρ correlation coefficients.*

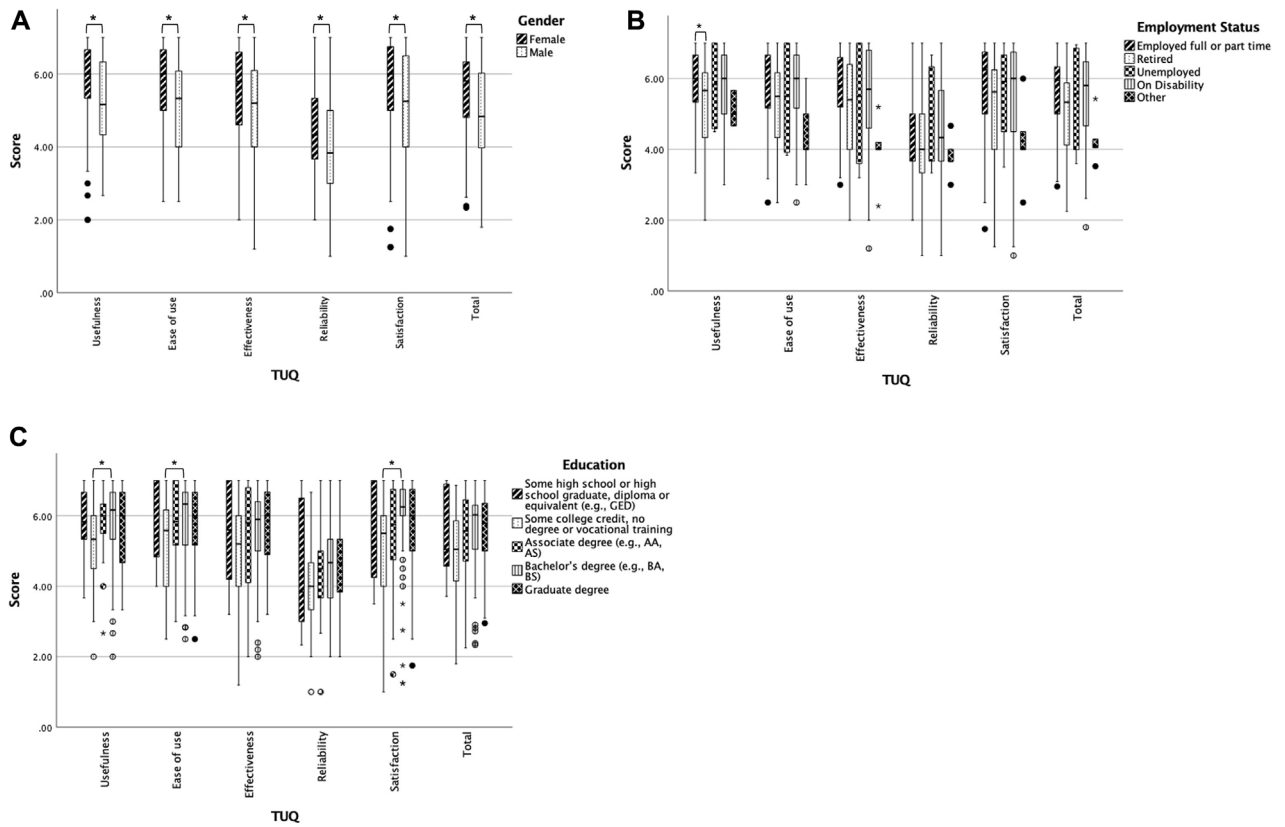


FIGURE 1 | Box plots of telehealth satisfaction by characteristics **(A)** TUQ scores by sex, **(B)** employment status, and **(C)** education. $p < 0.05$ or Bonferroni-corrected $p < 0.05$. The circles are values between 1.5 and 3 IQRs from the end of a box. The stars are extreme outliers indicating values more than 3 IQRs from the end of a box.

Efficiency

Participants repeatedly described their telehealth experience as "seamless." Many of them noted that it was easier to schedule telehealth appointments with their providers, how smoothly the appointments went, and that the appointments were faster or quicker than an office visit (in part because they and their providers experienced fewer distractions or interruptions during the call).

Convenience

Participants frequently listed convenience when we asked them to name the benefits of telehealth. Participants described the convenience of taking their telehealth appointment at any location of their choosing. In addition to talking about joining the appointments at home, participants talked about joining an appointment at work, in their car, or even outside in a field. Participants saw telehealth as particularly beneficial for individuals who might be homebound or are unlikely to seek care. They repeatedly mentioned how they benefitted from telehealth by saving travel time. For example, one respondent reported that a trip to their provider was at least 45 min one way, while another participant was traveling across states for their in-person healthcare, which required days. Another participant, who is blind, said that they found telehealth more convenient because they would not need to arrange travel assistance.

TABLE 4 | Benefits and selected quotes.

Theme–Perceptions of quality		
Sub-themes	**Definitions and further subthemes**	**Quotes**
Efficiency	The code was applied to participants' comments when they spoke about how the appointments were without challenges or problems and utilized time effectively	
	Seamless	"It's really seamless, because, like, you sign in through the portal, then they route you to, like, a check-in person, and then they route you to the provider."
	Quick	"I, I think they're faster."
Convenience	The code was applied to participants' comments when they spoke about how well telehealth fit with their needs and required little effort from them	
	Location	"But I could do the, I can do telehealth visits when I'm at work, so it does not really matter. I've had more than one doctor's appointment where I've been sitting in the field or in my car or something, so. . ."
	Travel	"Pretty good. Um, you know, it, it, it, for in my case it saves me as 45 min one-way drive. . . And um, again 45 min drive in. Um, you know not too long in the, in the waiting room. The visit was less than 15 min. And uh, you know, I'm driving home again. So a 3 hour day for a, for a 15 min visit. And I, we could have done it over the phone."
Communication	The code was applied to participants' comments when they spoke about exchanging and understanding information about their transplant care	
	Comprehensive	"And I would have to say that my visits telehealth are more comprehensive than what I was experiencing, uh, prior to being able to have that."
	Timely	"So, uh, this, this allows a, a doctor and patient or uh, a professional, a medical professional- and patients to communicate very quickly. And with, with very little or, uh, with a great deal of benefit and very little to, um, or very little lost."
Affectability	The code was applied to participants' comments as they spoke about how emotionally reactive or negatively affected, they felt before, during or after their transplant appointments	
	Stress	"just to also to add on to what someone said before, not only do you save the stress of not having it drive in and worry about the traffic, not to deal with finding parking. 'Cause some, you know, if I- I'm going into the city. . .to, to [general hospital] and some, you know, some of those parking lots, it's like, you feel like you're in a, um, you're a car accident waiting to happen as you go. So that's the benefit of not going in person."
	Mood	"And so, (laughs) this is gonna make me sound like a jerk, but, like, going, like, ha- having to, like, rearrange work schedule, like, stressing out about that and, like, having to go there, it just puts me in a really bad headspace. And I'm, like, annoyed all the time. And, like, I hate sitting in the waiting room and, like, people are there. This whole. . . It just, it's, it ruins your day, right?"
	Anxiety	"Um, so for me, it's kind of, it's nice to have telehealth. But as I said, you know, since I have to have labs every time, it's not all that helpful to me. But, um, in the in between when he's wants to give me lab results or whatever, you know, to do it over the phone is a lot easier for me, um, because I have a lot of anxiety when I'm actually, you know, in clinic just because I have such a, I guess a bit of PTSD with things going wrong and such. So it's easier to, you know, deal with it over the phone and, and ask questions where I'm not so anxious."

Communication

Similar to previous elements, participants spoke positively about the effectiveness and comprehensiveness of their telehealth appointments. Their experiences commonly included mentions of their providers answering all of their questions and communicating in a timely fashion. In most cases, the patients stated that telehealth is especially well suited for visits that are a simple review of lab results or for regular check-in appointments.

Affectability

The emotional effects that participants described were mostly positive when talking about the benefits of telehealth. For example, they reported lower levels of stress and anxiety and better moods. Some of this is closely related to the findings described above about convenience; participants said that needing to re-arrange work schedules was troublesome. Finally, some participants said that reviewing lab results could be a stressful part of an appointment, and that being able to reduce some of the stressors (e.g., travel time) allowed them to focus on the content of the conversation.

Drawbacks of Telehealth

As illustrated in **Table 5**, participants identified several drawbacks of telehealth and instances where they perceived it to be riskier than in-person appointments. The most common risk mentioned was perceived clinical vulnerability, followed by urgency, social vulnerability, and privacy.

Perceived Clinical Vulnerability

Participants' comments about the risks associated with the drawbacks of telehealth most frequently touched on their perceived clinical vulnerability as an SOT recipient. In general, participants pointed out that the utilization of telehealth depended on how well they were doing or what issues were present. Their perception of their vulnerability was constantly present due to the second chance that they were given through the transplant. They were aware that their status could change and that their preference for telehealth versus in-person appointments might change in concert with their status. As one participant pointed out, "there is not a manual" for living with an SOT, and some other participants said that in-person appointments helped them to

TABLE 5 | Drawbacks and selected quotes.

Theme – Perceptions of risks		
Sub-themes	**Definitions and further subthemes**	**Quotes**
Perceived Clinical Vulnerability	The code was applied to participants' comments when they referred to conditions or severity of illness when talking about their perceived risk during telehealth versus in-person appointments	
	Want to be confident during recovery	"uh, but yeah, but for the first 3 months looking back, I mean, at that time I definitely would say, yeah, I wanted to go in because, you know, I did not know yet. I was still, there's no book, there's no manual, I mean, they gave me some things to look for- but there is no user manual for a kidney. So, you know, go in and I, I really wanted to, you know, be check and make sure everything was working frequent, you know, working as they expected." "I do not think I would like it, um, at the beginning, because there's a lot of worry, um, and you kind of need that comforting in-person that everything's okay, and then, you know, the checking of the labs to prove that everything's okay. Um, and, you know, the beginning of my transplant was great. But you, I still, you know, I still had constant worry that, you know, things are gonna go wrong. I still feel that way. Um, you know, it's, it's kind of a really anxious, kind of, thing to have a transplant. So in those beginning days, I kind of preferred to, you know, actually get to know the doctor in person and communicate in person and have them know me and know my issues and, you know, not just be a person on a screen."
	Concern for clinical errors or important information is missed	"If I go to the portal, I think it, one of the problems with the portal is the fact that, uh, we have not really established a, uh, common denominator for, uh, uh, navigating portals. Uh, for the example, yeah, uh, the other day I was signing up for, doing a pre-visit, uh, online. And uh, there were some medications in there that I, that were not correct. Um, they had me on three mg where I'm on 1.75. But I could not change it. Or even, or even write in about a change or anything. And then there's no one to talk to say, "Hey, this is. You know, uh, I can't make a change here that needs really seriously needs to be made because it was one of my immunosuppressant drugs." So if I was hospitalized or something I do not want them giving me an overdose on, on the immunosuppressants. So um, by, in person I could, I could do that and they can update the chart. Um, where it goes from there, I do not know. But at least I'm more in control of that situation- in person."
	Changes in health status	"Um, so I think it really kind of depends on other health issues. And for me, I would not have wanted, uh, telehealth right after having a transplant." "So I would've been fine after probably, like, the second visit to say, you know, "I'll send my labs in and, and stay home." But that's, nothing went wrong, so it might be different. But I think my provider would insist on an in-person, you know, if I were in a situation where they did need to monitor for stuff."
Urgency	The code was applied to participants' comments when they described a situation that needed immediate or timely attention	
	Readmitted for urgent care in person	"Most of my problems had, I had to have be readmitted. My lab work was up, um, you know, this test came back abnormal. They would just call me and say, we're gonna admit you, you're gonna be a direct admit. Um, so I do not think it would've been terribly advantageous for me because I had those problems that needed intervention."
Social Vulnerability	The code was applied to participants' comments that spoke about populations that they perceived or they identify with that have challenges with telehealth	
	Age and tech savviness	"I do not think it's for everybody. I think that certainly, um, like I have a father-in-law that's 84. He could never use telehealth. He just, even if you were sitting next to him, he, he still would not get the concept of the doctor being there and, and talking to him and that type of thing. So I think it really kind of depends on, um, the patient population and, and how technical savvy they are."
	Impairments	"Um, I'm legally blind, so the, kind of the service that my doctor wanted to go through was not accessible, um, so we e- we end up just doing talking, like, um, you know, just on the phone, because there are some things, um, some programs that are not accessible to, you know, blind individuals or people who use screen readers, things like that."
	Information Processing	". . .somebody Zoom meetings me and starts telling me blah, blah, blah. I'm not sure I'm as effective- at listening as when I can see, uh, you know, s- see them face-to-face. So th- there must be some chemical thing going on that you, you know, is in- a little intangible on, on the internet- that, uh, that I'm missing out on."

(Continued on following page)

TABLE 5 | (*Continued*) Drawbacks and selected quotes.

Theme – Perceptions of risks		
Sub-themes	**Definitions and further subthemes**	**Quotes**
Privacy	The code was applied when participants commented that they perceived a risk of privacy or confidentiality when using telehealth	
	Acknowledge some general risk but not too concerned	"They can hack whole hospitals now though (laughs), so- and hold them hostage. So I do not know. They can, they can take the entire Blue Cross's records and not blink an eye, so I'm not really sure there's a expectation of privacy anywhere anyway anymore"
		"I guess, for me, I'm willing to make the trade-off for the benefits that I get from telehealth."
	Has some concern after being asked	"Wow, I never thought of that 'til you actually just said that Oh my god, maybe I should. But, you know, like, I never thought of that. But you're right, that's a good interesting thing. I do not know. Like. . .'Cause you do hear of stuff like that getting out. And were you ever. . . Like, have you ever seen a little, like, note on your portal about anything, or has it ever been mentioned. . . It makes me curious. Like, 'cause, you know, you hear of those things happening with other things. Where you least expect it. And I'm like, well. . . But then again, who the heck's gonna be trying to look into my. . . Well, you never know. My health. You know. But, you never know. There's some crazy people out there, so. . .But. . . Yeah. I might have to go look on their site and see what they have to say about that now, yeah. Thanks for bringing that up."

TABLE 6 | Connectedness and selected quotes.

Theme – Connectedness	
Definition	The code was applied to descriptions of the feeling of connection and trust with their providers or lack of connection
Sub-themes	**Quotes**
Elimination of distractions	". . . I feel like I have more one-on-one attention. There's not a distraction 'cause it's just the two of us in the meeting. . ..I just feel like I get more att- undivided attention, without interruptions."
Disruption of established connection during ups and downs of recovery	". . .I have had a lot of setbacks over the last 18 years. Lots and lots. Um, so yeah, I do like seeing them face, you know, face to face too. . . you know, I, I've made friends with these people, you know, over the last 20 years. And it is nice to go in and, and see the group." "I think sometimes just knowing, it's hard to come, sometimes catch the mood or the personality of the person. . .And, you know, when a doctor knows you for a length of time, then, you know, a lot of times they say, you know, there's something that you look different, or-. . .this just does not seem right according to your labs. By your appearance, or whatever. I think, you know, that's a big factor."
Impediment to establishing trust	"For me, if I know who you are, I can trust you. . .Your credentials are very nice, but they do not mean anything to me. . .So if I can not somehow look at you and size you up in person, um, you're already at a disadvantage in my head. . . I go, 'Okay, I'm not sure who you are, so I'm gonna keep you at the proverbial arm's length.' ". . .that look on their face, that look in their eye, on the screen, there's something that's not there. . .I do not. . . Like, uh, you know, I, I do not know how to describe it, but it's definitely a, a, a less personal experience."

be confident of their recovery progress. Additionally, a few participants shared concerns about the possibility of clinical errors or that something important would be missed.

Urgency

Participants noted that telehealth may not be optimal if a problem surfaces in their lab results and they need to be readmitted urgently. If such a visit happened over telehealth, it would still require travel to the hospital or transplant center, thus negating the benefit of telehealth.

Social Vulnerability

A participant shared their personal condition of blindness and lamented that their provider's telehealth videoconferencing platform was not accessible and they had to instead utilize a phone call. Another participant shared a personal need to be in person to adequately process the information from their providers. That experience was not as largely shared by the other participants, but it illuminated a potential subset of people who might need in-person care instead of telehealth to manage transplants. A few participants surmised that elderly patients might be less likely to be "tech savvy," although technological literacy was not a barrier reported among our participants.

Privacy

Perhaps surprisingly, when we asked participants if they had concerns about confidentiality, most said they had not

considered this as a potential risk. Even when asked directly about it, they were not concerned about breaches of confidentiality, either on the provider's side or on their end (i.e., being overhead by a family member or co-worker). One participant acknowledged the potential risk of losing privacy but believed that the benefits of telehealth outweighed it. Finally, one participant, although not concerned before considering the question, became more concerned and stated they needed to find out the privacy protections for their telehealth.

Connectedness

Finally, there is some evidence in the transcripts that feelings about telehealth varied among participants, depending on the length of time that had passed since their transplant (**Table 6**). Most interviewees and focus group participants had received their transplant less than 8 years before the pandemic. But there were a couple of participants who had lived with their SOT for decades. Their reports suggest that telehealth might disrupt their relationship with the provider, which they had cultivated over many years. These people mentioned that they had "good" or "easy" rapport with their providers, which in some ways made the shift to telehealth less awkward.

Despite these generally positive remarks about telehealth, some of these long-term SOT recipients gave several reasons why they nevertheless prefer in-person care. First, they commented that they had received many years of support not only from interdisciplinary transplant team members but from everyone in the transplant center (e.g., receptionists, nursing assistants), and that using telehealth disrupted those connections, which they had established throughout their long recovery. One participant, whose travel to appointments is about 4 hours, said that they viewed these staff members as part of their care team, and that they missed those interactions over telehealth.

Among participants who had had their transplant more recently, some expressed a preference for in-person care, as a means of getting to know their providers and establishing trust. They also spoke about the "chemistry" that can be established when you sit in front of someone and its absence when the appointment is through telehealth. Conversely, the minimization of interruptions and distractions in the telehealth environment could facilitate better connection.

DISCUSSION

Telehealth has been beneficial for SOT recipients during the pandemic, by minimizing the risk of contracting COVID-19. However, there is a lack of knowledge regarding how satisfied SOT recipients are with their transplant care delivered by telehealth. SOT recipients in this study were mostly satisfied with telehealth, particularly appreciating its efficiency and convenience. Yet, they also expressed concerns that telehealth may not work for everyone.

SOT recipients rated telehealth very highly in its effectiveness to provide comparable quality of care to in-person visits, consistent with previous literature [22, 23]. Particularly, telehealth seems to be a great option for employed recipients compared to retirees due to its convenience and ability to save time and money by reducing the number of trips to their transplant centers. SOT recipients also found telehealth provides a seamless appointment experience while enhancing communication with transplant care providers. They appreciated that they could receive care anywhere that is convenient for them.

This study also, however, suggests that telehealth may not be suitable for all SOT recipients, illustrating the importance of adopting a personalized approach for post-transplant care, based on the patient's needs and preferences. As demonstrated in previous literature [9, 23], this study identified limited technical capability, lack of online communication skills, and physical disabilities, such as blindness, were possible barriers preventing SOT recipients from the successful use of telehealth. Furthermore, telehealth may not be an ideal care delivery method if recipients perceive their health status as poor. In our study, a small minority of interviewees expressed concern about the limitations of telehealth, including inability to perform physical examination or provide proper care when urgently needed.

Perhaps paradoxically, our study found that patients who had lived with an SOT for decades were most skeptical about the benefits of telehealth, largely because they felt it disrupted a sense of connectedness with transplant care providers, which is crucial to managing a SOT successfully [24, 25]. Established relationships between a patient and healthcare provider facilitates the use of telehealth. Its long-term use, however, may decrease the level of connectedness between patients and providers [9, 26]. Our quantitative and qualitative findings suggest lower satisfaction with telehealth among SOT recipients who undergone SOT a longer time ago; this may indicate that telehealth erodes the sense of connectedness that SOT patients have, not only with their provider, but with all the members of the care team. SOT recipients who have long-lasting, trusted relationships with their transplant care providers may prefer in-person visits over telehealth to maintain such relationships [9, 26]. Further research is needed to identify potential strategies to help establish or maintain the sense of connectedness while providing transplant care via telehealth.

Privacy is one of the common concern individuals may raise when using telehealth [9, 11]. Interestingly, SOT recipients in this study seemed less concerned about privacy. While this finding may indicate their acceptance of telehealth, it may suggest a lack of awareness among recipients about the potential risks of confidentiality breach. For example, our interviewees were relatively unconcerned about the likelihood of a breach on the provider's side, and none of them seemed concerned that someone in their home or workplace may overhear their conversations. Efforts to broaden use of telehealth should take equity into consideration, since it may not be possible for all recipients to find private space to complete telehealth visits at home or workplace [9, 13]. Moreover, these interviews asked patients about their experience of telehealth during a pandemic and quarantine, and some patients may have considered questions about confidentiality or privacy moot in that circumstance. As

healthcare operations return to normal, further research is needed to better understand how SOT recipients perceive the relative risks and benefits of confidentiality and privacy.

A few potential limitations of this study should be noted. While online recruitment offers advantages such as higher participant numbers at a reduced cost compared to traditional methods [27, 28], the small sample recruited online may not represent the broader SOT population. Consequently, the generalizability of the study findings might be limited. Moreover, it should be acknowledged that participants independently assessed their eligibility for participation, but we did not use any validation procedures to evaluate the accuracy of their self-assessment. Finally, the observed correlations do not indicate cause and effect due to the cross-sectional nature of the study.

In conclusion, while SOT recipients readily accepted telehealth during the pandemic, telehealth may not be suitable for all recipients. Clinicians should prioritize assessing a SOT recipient's needs and preferences when developing a patient-centered transplant care plan. Further research is needed to develop strategies to address potential drawbacks of telehealth.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by Northeastern University Institutional Review Board. 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

DK and JD contributed to the study design and acquisition of subjects and data. All authors were involved in the analysis and interpretation of the data and preparation of the manuscript. All authors read and approved the final manuscript.

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.2023.11819/full#supplementary-material

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Importance and Potential of European Cross-Border Deceased Donor Organ Allocation Through FOEDUS-EOEO Platform

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The FOEDUS-EOEO platform was relaunched in 2015 to allocate deceased donor organs across European borders when there are no suitable recipients in the donor's country. We analyzed organ offers from 01.06.2015–31.12.2021 and present the number of offers and transplants, and utilization as percentage of transplanted organs. 1,483 organs were offered, 287 were transplanted (19.4% utilization). Yearly number of offers and transplants increased from 2017 to 2021, while utilization stabilized after 2018. Utilization was highest for organs offered by Slovakia (47.2%), followed for organs offered by Lithuania, France, Greece, and Czechia (19.3%–22.9%). The most frequently offered organ was the heart (n = 405; 27.3%), followed by the lungs (n = 369; 24.9%) and the liver (n = 345; 23.3%). Utilization differed significantly by organ type (highest for liver, 35.7%; followed by heart, 18.8%; and kidney, 18.3%) and by donor age (highest for 1 to 5 year-old donors (25.0%)). FOEDUS-EOEO allowed for many European patients receiving a long-awaited transplant, especially for very young pediatric patients waiting for a liver, a heart, or a kidney. The increasing number of participating countries has increased both the number of offered organs and, to a lesser extent, the number of transplanted organs.

Keywords: organ donation, organ allocation, organ utilization, solid organ transplantation, deceased donor organ transplantation, international organ exchange

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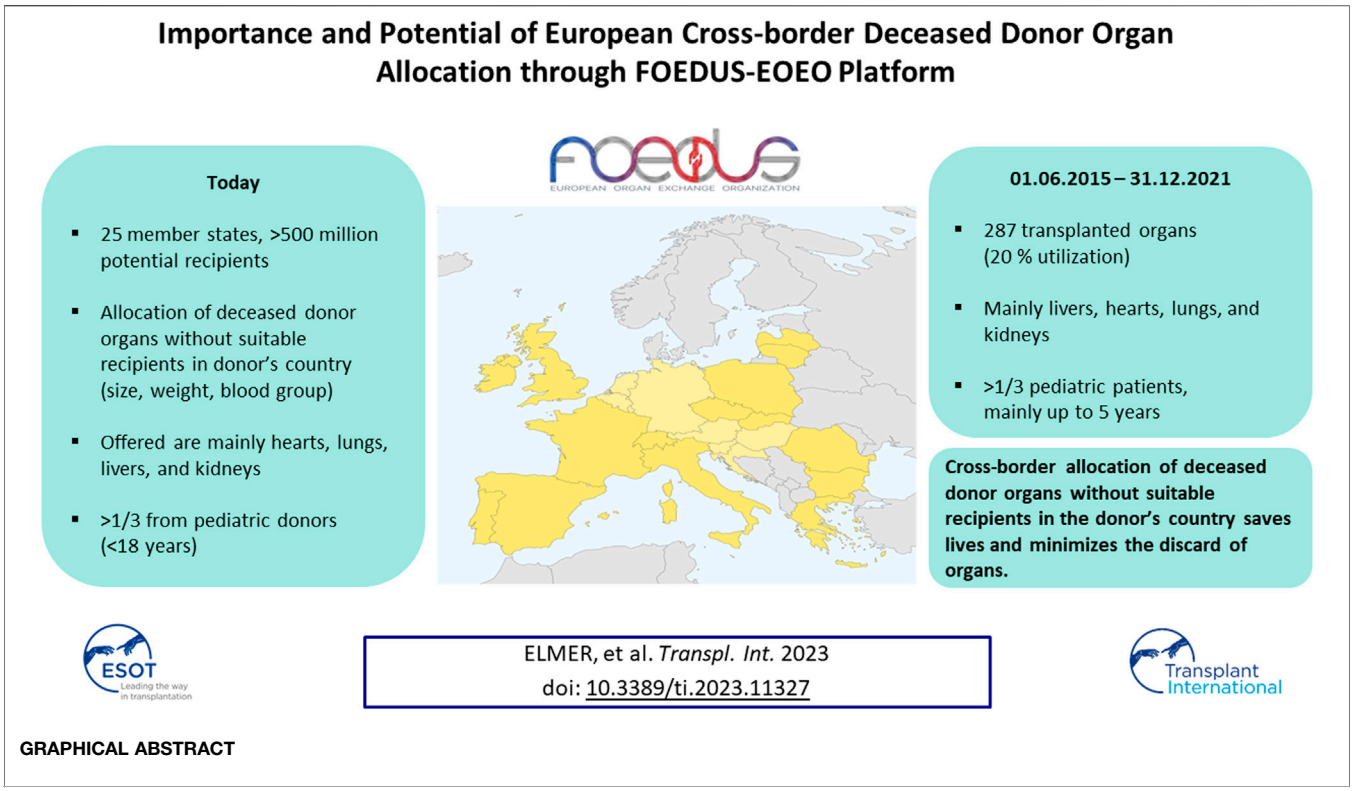
INTRODUCTION

For organs from pediatric deceased donors (size and weight mismatch) and for organs from deceased donors with the rare blood groups AB or B, there is often no compatible recipient in the donor's country. At the same time, pediatric or highly immunized patients often face long waiting times. Critically ill patients in urgent need of a transplant may even die on national wait lists due to the limited number of suitable organs. International collaboration allows deceased donor organ allocation to matching recipients on foreign wait lists, thus, minimizing the discard of valuable organs and increasing availability of organs for patients on national wait lists [1–4].

With the aim of optimizing HLA-matching in kidney allocation, Eurotransplant were the first to start cross-border organ allocation in Europe in the late 1960s. Since the early 1990s various bilateral agreements enabled the organ exchange between particular countries. A high mortality on the Swiss children heart wait list led Swisstransplant, the National Transplant Organization (NTO) in Switzerland, to introduce a "European Children Heart Waiting List" in 2009. Following



GRAPHICAL ABSTRACT

directives 2010/53/EU [5] and 2012/25/EU [6] of the European Parliament and within the EU Action Plan on Organ Donation and Transplantation (2009–2015) [7, 8], a similar project was carried out from 2013–2016 by the European Commission and the EU member states as a so-called Joint Action [9]. An important work package of this Joint Action called "Facilitating Exchange of Organs Donated in EU Members States" (FOEDUS) was the upgrading of an existing IT platform for international allocation of national organs for which no suitable recipient can be found within the donor's own country [10]. The platform had been formerly launched in 2012, by the EU-funded project called "Coordinating a European Initiative Among National Organizations for Organ Transplantation (COORENOR) [11, 12]. Following the end of the European Commission's financial support for the FOEDUS Joint Action, an agreement was signed in 2016 on the initiative of 9 countries (i.e., Czechia, France, Italy, Lithuania, Poland, Romania, Slovakia, Spain and Switzerland). This agreement established a framework for cooperation, ensured the funding and maintenance of the platform and aimed to involve other European countries. The further development and continued use of the IT platform, since then called FOEDUS-EOEO platform, has accelerated communication between responsible national authorities and increased transparency and traceability of European cross-border organ allocation [13]. In consequence, the mortality on the Swiss children heart wait list decreased from over 70 percent in 2009 to below 20 percent in 2017 [14].

We analyzed all organ offers placed on the FOEDUS-EOEO platform since the operative relaunch under the responsibility of the signatory states of the cooperation agreement, on 1 June 2015, until 31 December 2021. We show how the number of offered organs and utilization developed over time and by country and we analyze how the number of offered organs and utilization vary with respect to basic donor characteristics and organ type. Based on the results, we discuss the future potential of European deceased donor organ allocation through the FOEDUS-EOEO platform after Eurotransplant joins the FOEDUS network on 1 February 2022.

MATERIALS AND METHODS

Data

Deceased donor organ offers placed on the FOEDUS-EOEO platform from 1 June 2015 (date of the fully operational state of platform under the responsibility of the signatory states of the cooperation agreement) until 31 December 2021 were retrospectively analyzed (n = 1,519). A minimal electronically available dataset (including organ type, offer entry date, offer final status, donor gender, donor age, donor weight, donor height, donor blood group, donor rhesus factor, country of origin of the offer, countries which accepted the offer, and country where organ was transplanted), was made available by the Czechia based registered association responsible for managing the FOEDUS-EOEO platform. After a preliminary analysis, 36 offers were excluded because they were identified as duplicates, tests or mistaken database entries, leading to a total of n = 1,483 analyzed organ offers.

TABLE 1 | FOEDUS member states, respective transplant organizations and since when they participate.

State	Transplant organization	Member since	Actively using platform since	Population	Bi-/multilateral agreement implemented partly by means of FOEDUS
Belarus	RSPC, Organ and Tissue Transplantation	2017	never used	9,340,314	-
Bulgaria	Executive Agency Medical Supervision (IAMN)	2019	2020	6,949,549	-
Czechia	Czech Transplant Coordinating Centre (KST)	2016	2015	10,693,861	Slovakia, SAT
France	Agence de la biomédecine (ABM)	2016	2015	67,197,367	Switzerland, SAT
Greece	Hellenic Transplant Organization (HTO)	2019	2017	10,696,535	Italy
Ireland	Organ Donation and Transplant Ireland	2019	never used	4,966,879	-
Italy	Italian National Transplant Centre (CNT)	2016	2015	60,286,529	Greece, SAT
Latvia	National Transplant Coordination Department - Stradini Clinical University Hospital	2019	2018	1,907,094	-
Lithuania	National Transplant Bureau under the Ministry Of Health (NTB)	2016	2015	2,793,592	-
Moldova	Transplant Agency of Moldova	2019	never used	2,573,928	-
Poland	Poltransplant	2016	2015	37,941,122	-
Portugal	Instituto Português do Sangue e da Transplantação (IPST)	2017	never used	10,291,457	SAT
Romania	National Transplant Acency (NTA)	2016	2021	19,281,118	-
Slovakia	National Transplant Organization (NTO)	2016	2015	5,457,679	Czechia
Spain	Organización Nacional de Trasplantes (ONT) Catalan Transplant Organization (OCATT)	2016	2015	47,321,434	SAT
Switzerland	Swisstransplant	2016	2015	8,580,270	France, SAT
United Kingdom	NHS Blood and Transplant (NHSBT)	2017	2022	67,326,569	-
Austria, Belgium, Croatia, Germany, Hungary, Luxembourg, Netherlands, Slovenia	Eurotransplant International Foundation	2021	2022	137,501,179	-
TOTAL				511,106,476	

Note: Belarus, Ireland, Moldova, Portugal, and the United Kingdom are official member states but have never used the platform during the study period (2015–2021). If an organ offered via FOEDUS during the study period eventually had been transplanted in one of these states, the offer was counted as "not transplanted". Population figures as provided online by The World Bank (for Belarus and Moldova; 2021 projections) and Eurostat (for all other countries; 2020 projections). SAT: South Alliance of Transplantation (Czechia, France, Italy, Portugal, Spain, Switzerland).

Further donor and organ-specific data, including some data on extended criteria, was available only on handwritten, standardized forms attached to the respective electronical records as PDFs. A digital dataset with data from these PDF forms was compiled manually by final year medical student and co-author of this article within the scope of her PhD thesis, including all offers from 1 June 2015–30 September 2020. 3.8% of those offers were from donors after cardiocirculatory death. After preliminary analysis of this additional dataset we refrained from a quantitative analysis of those variables due to data incompleteness (e.g., donation type DBD/DCD was missing in 18.9% of the cases).

FOEDUS-EOEO Platform

The access-protected online platform is 24/7 available to all participating European NTOs and is maintained and operated by a Czech software company. It allows participating NTOs to quickly upload and simultaneously share organ offers for cross-border organ allocation, ensuring transparency and traceability in accordance with EU legislation [5, 6]. The main purpose of FOEDUS is to allocate organs for which no suitable recipient can be found within the donor's own country. End of 2021, 17 European states were members of FOEDUS-EOEO, of which 13 actively used the platform during the study period. Since February 2022, the Eurotransplant network has also been actively using the platform after signing the cooperation agreement in 2021. Including the eight Eurotransplant member states the FOEDUS network now covers 25 states with more than 500 million inhabitants. An overview of participating states is shown in **Table 1**.

Only organs for which no matching recipient could be found in the donor's country or under bilateral agreements are offered on the platform. Organ offers can either be sent simultaneously to all participating NTOs, or first to only a selection of NTOs, according to existing bi-/multilateral agreements between countries. Organs are allocated on a first-come, first-served basis. When a matching recipient is found, the bilateral organ

allocation begins. It is important to note that the acceptance of a foreign organ is without any obligation on the part of the accepting country. The accepting country is explicitly under no obligation to "pay back" a received organ in return. However, the receiving country needs to organize and pay for the costs of procurement and transport.

FOEDUS-EOEO also enables urgent requests for organs when no suitable organ is available in the recipient's country. In the present study, however, we analyzed only organ offers, not organ requests.

Outcomes

Each offer has a final status, which can be set as "closed," "not transplanted," or "transplanted" by the NTO which effected the offer. Status "closed" means, an offer was not accepted by any of the NTOs which received the offer. Status "not transplanted" is meant to be chosen for organ offers initially accepted by at least one NTO, but eventually the organ was not transplanted. As there is no uniform procedure or guideline when to set the status "closed," or "not transplanted," we compared only offers that led to a transplanted organ (status transplanted) versus offers that did not lead to a transplanted organ (status closed and not transplanted). As the information about the receiving country in many cases is missing, we focused in our study on the organ offering and the utilization. Presented country figures always refer to the offering country, never to the receiving country.

The primary objective was to calculate utilization as the percentage of transplanted organs among all offers and to analyze whether utilization differed by offer/donor characteristics and over time. We did not investigate refusal reasons, thus utilization does only partly reflect acceptance practices of individual transplant centers.

Statistical Analysis

As presented in **Table 2**, offers were divided into two groups as described above, transplanted offers (n = 287) vs. not transplanted offers (n = 1,196). Among these two groups, donor characteristics and organ types were compared for quantitative variables by using the t-test, or if the assumption of normality was not met, by the non-parametric Wilcoxon rank sum test. For qualitative variables Pearson's chi-square test was used, or Fisher's exact test in case of a small sample size. "Year" was treated as a numerical variable in the significance test.

For the analysis, kidneys (n = 113) and lungs (n = 345) offered together were counted as one offer. The variable organ type was regrouped as follows: "kidney left" (n = 32), "kidney right" (n = 57), and "kidneys" (n = 113) became "Kidney"; "left lung" (n = 12), "right lung" (n = 12), and "lungs" (n = 345) became "Lung"; "liver" (n = 341), "liver left" (n = 2), "liver right" (n = 2) became "Liver". BMI was calculated as the weight [in kilogram] divided by the square of the height [in meters], but only for donors over 20 years.

For all statistical analyses the freely available software R (version 4.2.2) was used [15].

RESULTS

Organ Offers and Utilization Over Time and by Country

Since the relaunch of the FOEDUS-EOEO organ allocation on 01 June 2015, 1,483 deceased donor organs were offered on the platform of which 287 were transplanted (19.4% utilization). After a sharp decrease in 2017, the yearly total number of effected offers steadily increased from 186 offers in 2017 to 269 offers in 2021 (**Table 2**; **Figure 1A**). Overall utilization per year similarly decreased until 2017, and after a maximum of 24.3% in 2018, has stabilized at just under 20% in the last 2 years of the study period (**Table 2**; **Figure 1B**).

A group of eight states together were responsible for over 95% of organ offers placed on the FOEDUS-EOEO platform during the study period, as shown in **Table 3**. Most organ offers were effected by France (n = 344 or 23.2% of total offers), followed by Switzerland (n = 330 or 22.3% of total offers). As shown in **Figure 1B**, state-specific organ utilization over the entire study period was highest for organs offered by Slovakia (47.2% transplanted offers). Organs offered by Lithuania, France, Greece, and Czechia had an average utilization over the entire study period (19.3%–22.9% transplanted offers). Organs offered by Switzerland, Spain, and Italy had an utilization below the average (9.7%–13.8% transplanted offers).

Organ Offers and Utilization by Organ Type and Donor Characteristics

The most frequently offered organ on the FOEDUS-EOEO platform was the heart (n = 405; 27.3%), followed by the lungs (n = 369; 24.9%), the liver (n = 345; 23.3%), the kidneys (n = 202; 13.6%), the small bowel (n = 86; 5.8%), and the pancreas/islets (n = 76; 5.1%). Utilization of offered livers (35.7%) was the highest and almost twice the overall average. Offered hearts (18.8%) and kidneys (18.3%) both had an average utilization, while offered lungs (11.7%), pancreas/islets (6.6%), and small intestine (3.5%) were utilized less often than the overall average. Utilization, thus, varied significantly between organ types (p < .001) (**Table 2**).

53.3 percent of effected organ offers were from male donors and the percentage of male donors was similar in transplanted (55.1%) as compared to declined offers (52.8%), respectively (p = .500). The median donor age of offered organs was 34 years (IQR = 7–55 years). Donors whose organs were transplanted were significantly younger than donors whose organs were declined (median age 28 vs. 35 years; p = .030). Thirty-five percent of organ offers (n = 516) were from pediatric donors under 18 years and among these pediatric offers, those from 1 to 5 year-old donors were most frequent (n = 212 or 14.3% of total offers). For 1 to 5 year-old donors also utilization was the highest (25%). When comparing the total pediatric donor group to the adult donor group, pediatric organs tended to be utilized more, although this was not statistically significant (21.5% vs. 18.2%; p = .124) (**Table 2**).

TABLE 2 | Organ offers placed on the FOEDUS platform from 1.6.2015 until 31.12.2021.

	Organ offers	Transplanted	Not transplanted	*p*
Total, n (%)	1,483 (100)	287 (19.4)	1,196 (80.6)	
Year of offer				
2021, n (%)	269 (18.1)	53 (19.7)	216 (80.3)	.114
2020, n (%)	256 (17.3)	50 (19.5)	206 (80.5)	
2019, n (%)	244 (16.5)	52 (21.3)	192 (78.7)	
2018, n (%)	202 (13.6)	49 (24.3)	153 (75.7)	
2017, n (%)	186 (12.5)	23 (12.4)	163 (87.6)	
2016, n (%)	230 (15.5)	40 (17.4)	190 (82.6)	
2015, n (%) only from 1.6.2015	96 (6.5)	20 (20.8)	76 (79.2)	
Organ type				
Heart, n (%)	405 (27.3)	76 (18.8)	329 (81.2)	<.001
Lungs, n (%)	369 (24.9)	43 (11.7)	326 (88.3)	
Liver, n (%)	345 (23.3)	123 (35.7)	222 (64.3)	
Kidneys, n (%)	202 (13.6)	37 (18.3)	165 (81.7)	
Small Bowel, n (%)	86 (5.8)	3 (3.5)	83 (96.5)	
Pancreas/Islets, n (%)	76 (5.1)	5 (6.6)	71 (93.4)	
Donor Characteristics				
Gender (male), n (%)	790 (53.3)	158 (55.1)	632 (52.8)	.500
Age (years), median (IQR)	34.0 (7.0–5.0)	28.0 (5.0–51.0)	35.0 (8.0–55.0)	.030
Adult group (≥18 years)	967 (65.2)	176 (18.2)	791 (81.8)	.124
Pediatric group (<18 years)	516 (34.8)	111 (21.5)	405 (78.5)	
<1 year, n (%)	128 (8.6)	21 (16.4)	107 (83.6)	.080
1–5 years, n (%)	212 (14.3)	53 (25.0)	159 (75.0)	
6–11 years, n (%)	121 (8.2)	29 (24.0)	92 (76.0)	
12–17 years, n (%)	55 (3.7)	8 (14.5)	47 (85.5)	
BMI (>20 years), median (IQR)	25.2 (22.2–28.4)	24.7 (22.0–28.0)	25.4 (22.3–28.6)	.207
Blood group				
A, n (%)	566 (38.2)	109 (19.3)	457 (80.7)	.385
0, n (%)	375 (25.3)	82 (21.9)	293 (78.1)	
AB, n (%)	274 (18.5)	45 (16.4)	229 (83.6)	
B, n (%)	268 (18.1)	51 (19.0)	217 (81.0)	

Displayed are means (±SD) and medians (IQR) for normally, and non-normally distributed numerical variables, respectively. For all categorical variables, column percentages are given in brackets in the column "Organ offers". Except for the variable "gender", where column percentages are given in brackets in the "Transplanted" and "Not transplanted" columns, row percentages are given in brackets, corresponding to utilization and refusal.

We looked at the age distribution of donors by organ type for all offers and for those which resulted in transplantation, and evaluated age-specific utilization (**Figures 2A–D**). For all organ types it applies, most offers on the FOEDUS-EOEO platform were effected in the youngest donor age group (0y–5y, first bar in histograms). In the case of the heart, the liver, and the kidney, this donor age group yielded also the most transplanted organs. In contrast, only two of 53 (4%) lung offers in the youngest donor age group were utilized. Heart and kidney utilization in the youngest donor age group was above the organ-specific average. In the case of the liver, utilization in the youngest donor age group was below average. As only three small bowels and five pancreas were transplanted in the entire study period we did not evaluate age-specific utilization for those organ types.

The median donor BMI (only donors >20 years) of all organ offers was 25.2 (IQR = 22.2–28.4) and was similar (p = .207) for transplanted offers (24.7; IQR = 22.0–28.0) as compared to declined offers (25.4; IQR = 22.3–28.6). Utilization was also similar across donor blood groups (p = .385) (**Table 2**).

DISCUSSION

Retrospects

On the initiative of the 9 European countries Czechia, France, Italy, Lithuania, Poland, Romania, Slovakia, Spain and Switzerland, the FOEDUS-EOEO platform for European cross-border allocation of deceased donor organs was relaunched in June 2015. In 6.5 years following the relaunch, the IT platform has allowed for 287 European patients receiving a long-awaited transplant. Most of these 287 transplanted organs allocated via the FOEDUS-EOEO platform otherwise would have been discarded because of no available matching recipients on national wait lists.

There are basically two ways in an organ allocation system to allow for more patients receiving a transplant. First, by simply offering more organs. Second, by accepting more of the offered organs, thereby increasing utilization. Thanks to more countries joining FOEDUS-EOEO and actively using its IT platform, the yearly number of offered organs has increased since 2017, each year by 5–21 percent, and reached 269 offers per year in 2021. Utilization slightly decreased after 2018 and seems to have

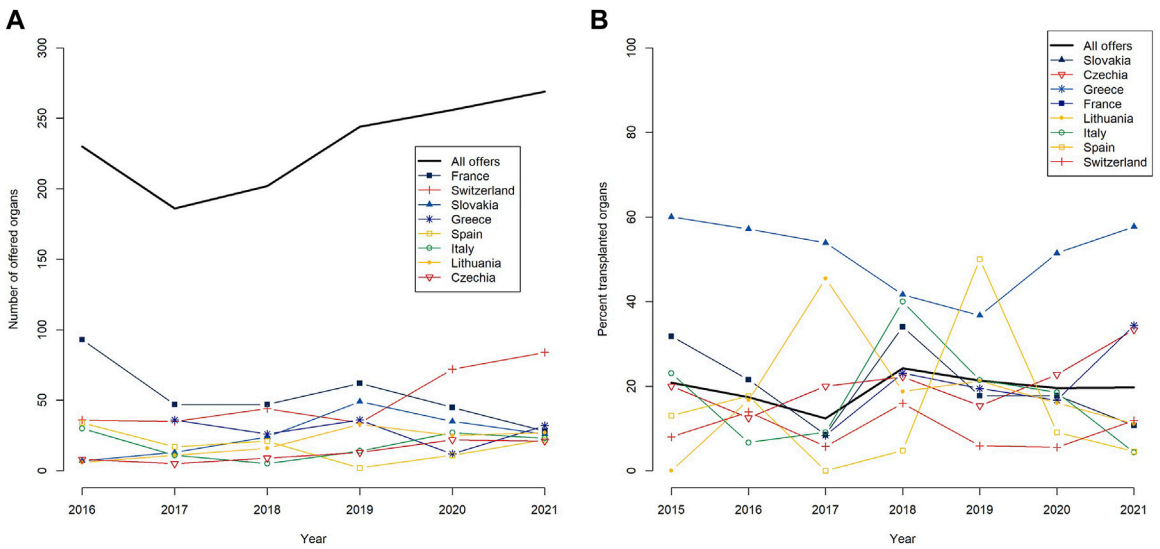


FIGURE 1 | Yearly numbers of offered organs on the FOEDUS-EOEO platform from 2016–2021 **(A)**, and yearly utilization (as percent transplanted organs) since the relaunch of the platform on 1 June 2015 **(B)**, overall and for the eight countries that together effected more than 95 percent of all offers during the study period. The legends list countries in descending order of the respective metric for the entire study period.

TABLE 3 | Organ offers placed on the FOEDUS-EOEO platform from 1.6.2015 until 31.12.2021 according to origin state in descending order of number of offers.

State (entire study period)	Organ offers	Transplanted	Not transplanted
	n (%)	n (%)	n (%)
Total	1,483 (100)	287 (19.4)	1,196 (80.6)
France	344 (23.2)	69 (20.1)	275 (79.9)
Switzerland	330 (22.3)	32 (9.7)	298 (90.3)
Slovakia	159 (10.7	75 (47.2)	84 (52.8)
Greece	142 (9.6)	29 (20.4)	113 (79.6)
Spain	130 (8.8)	13 (10.0)	117 (90.0)
Italy	123 (8.3)	17 (13.8)	106 (86.2)
Lithuania	119 (8.0)	23 (19.3)	96 (80.7)
Czechia	83 (5.6)	19 (22.9)	64 (77.1)
Poland	*26 (1.8)*	*2 (7.7)*	*24 (92.3)*
Bulgaria	*9 (0.6)*	*3 (33.3)*	*6 (66.7)*
Latvia	*9 (0.6)*	*3 (33.3)*	*6 (66.7)*
Malta	*5 (0.3)*	*0 (0.0)*	*5 (100.0)*
Romania	*4 (0.3)*	*2 (50.0)*	*2 (50.0)*

The column "Organ offers" shows numbers and column percentages in brackets. The "Transplanted" and "Not transplanted" columns show numbers and row percentages in brackets, corresponding to utilization and refusal. States in italics together account for <5% of all organ offers effected in the study period and are not shown in ***Figure 1****. Malta is not shown in* ***Table 1*** *as it is not an official FOEDUS member. However, Malta has effected 5 organ offers, all from the same donor, in 2017.*

stabilized at just under 20% in 2020 and 2021. However, as long as utilization does not decline, more organs offered obviously means more transplants.

On the overall average, almost every fifth FOEDUS-EOEO organ offer is accepted and the organ utilized. When compared to other multinational organ allocation programs this is relatively low. For example, overall average utilization of organs offered by Eurotransplant is 65% [16]. To explain this discrepancy, it is worth noting that FOEDUS is intended to allocate organs when a suitable recipient cannot be found within the donor's own country. In contrast, other European multinational organ allocation programs, such as Eurotransplant or Scandiatransplant, generally allocate organs to international recipients on a common wait list. Within FOEDUS, the exceptional high utilization of organs from Slovakia (47.2%) can partly be explained by a bilateral agreement between Czechia and Slovakia. The impact of the bilateral agreement between France and Switzerland on the allocation of livers for so-called super urgent recipients we consider negligible as with very few exceptions these allocations are processed outside the FOEDUS-EOEO platform.

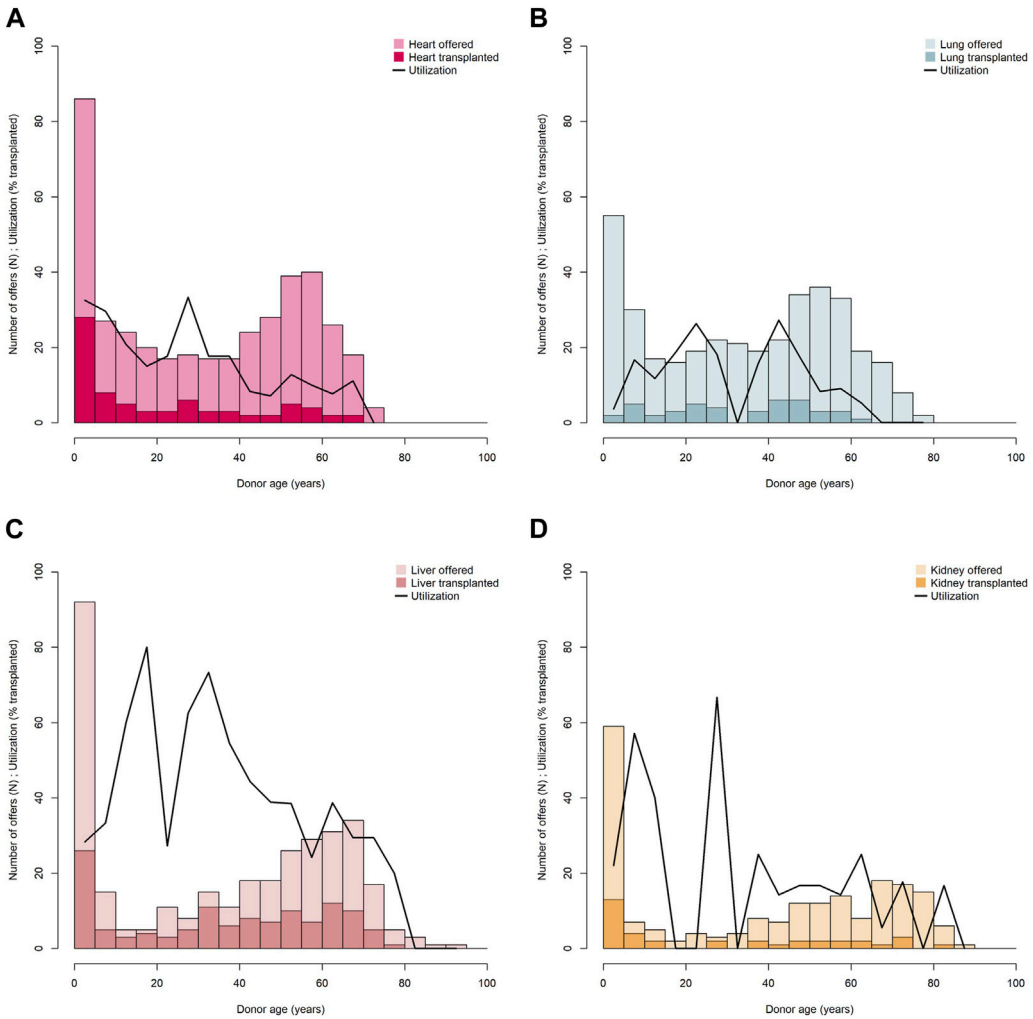


FIGURE 2 | Donor age distribution and age-specific utilization of offered hearts **(A)**, lungs **(B)**, livers **(C)**, and kidneys **(D)**. Transplanted organs are shown in dark shading, utilization is shown as curved line. Age-specific utilization is calculated for each donor age category according to histogram bars (0 ≤ 5 years, >5 ≤ 10 years, >10 ≤ 15 etc.).

The liver, however, is by far the most offered and transplanted FOEDUS organ, 123 liver transplants (or 43% of all transplants) have been facilitated through the platform, which is probably due to other bilateral agreements which are in place for this life-saving organ, in particular for pediatric liver allocation. Liver utilization is then also twice the overall average, while heart and kidney utilization are about the overall average. Lung, small bowel and pancreas offers are poorly utilized and transplanted less often. The effect of bi-/multilateral agreements (refer to **Table 1**) cannot be accurately determined due to differences in content (concerned organs and specific allocation rules) and varying portions of allocations managed through the FOEDUS-EOEO platform. Cross-border organ utilization, however, not only varies across organ types, but depends also on donor age, although this seems to be true only for certain organ types like the heart or the kidney. We can show that in particular very young pediatric patients (0–5 years) waiting for a liver, a heart, or a kidney transplant benefit most from cross-border organ allocation through FOEDUS-EOEO as this donor age group provides most liver, heart, and kidney offers on the platform and also most transplants.

If one compares the distribution of the blood groups among the FOEDUS-EOEO offers with average frequencies of a Caucasian population (data not shown), it is obvious that AB and B organs are overrepresented, most likely because there are fewer recipients on national wait lists for organs of these rare blood groups. It is noteworthy, however, that AB or B organs are not significantly less likely to be utilized than A or 0 organs when offered through an international platform. It appears that expanding the pool of potential recipients successfully facilitates donor-recipient matching for AB and B organs. Some may argue that organs from blood group A and

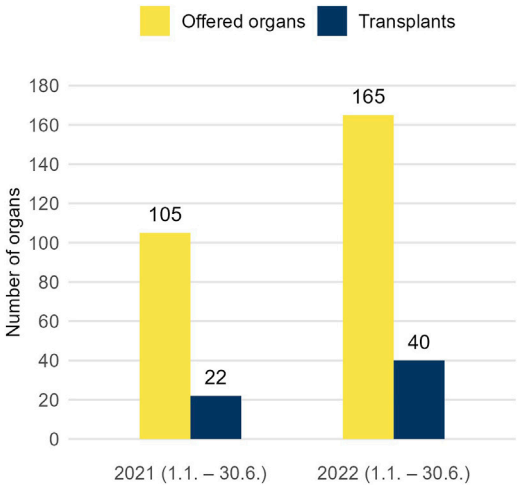


FIGURE 3 | To depict the most recent development of the FOEDUS-EOEO platform after the study period, number of offered organs (left bar) and transplanted organs (right bar) in the first semester 2021 and 2022 are shown. Eurotransplant has been actively using the platform since 1 February 2022.

0 donors offered through an international platform are of inferior quality because national recipient pools are large and, in the case of type 0 donors, unrestricted. We were not able to thoroughly analyze organ quality in the present study, but looking at utilization, A and 0 organs were not utilized less often than average. This means that the quality of these organs was considered sufficiently good for a particular international recipient, or that the lack of a suitable national recipient in the donor's country was not related to the overall quality of the organ.

Prospects

Most recent figures from 2022 (**Figure 3**) are even more encouraging. In the first semester 2022, 165 organs were offered on the FOEDUS-EOEO platform, which is a plus of 57% compared to the first semester 2021, and 40 organs were transplanted (plus 82%). Eurotransplant has been actively using the platform since February 1, 2022, making it the largest platform for cross-border organ allocation in Europe. Until end of August 2022, Eurotransplant member states in total placed alone 42 offers, which is a fifth of all offers placed in this period. Although only two Eurotransplant offers resulted in a transplant (4.8% utilization), Eurotransplant member state the Netherlands transplanted in the same period 20 organs (over one third of all transplants) offered by former FOEDUS-EOEO member states. Thus, the overall FOEDUS utilization from February to August 2022 was 25.2%, the highest since the relaunch in 2015 (**Table 2**).

Looking at these recent numbers it seems reasonable to forecast that participation of Eurotransplant in FOEDUS-EOEO may increase both, the number of offered organs, as well as utilization of the offered organs. Better utilization appears to be achieved primarily by Eurotransplant member states accepting more of the organs offered by former FOEDUS-EOEO member states, rather than more organs offered by Eurotransplant being transplanted in former FOEDUS-EOEO member states. If more states or EOEOs followed the Eurotransplant's lead and joined FOEDUS-EOEO, activity could be further increased, with positive impact on pediatric patients and patients with rare blood groups who have a hard time finding a suitable donor organ.

There are, of course, other measures which could improve the platform's utilization. For example, the fast availability of all relevant information needed for assessing organ quality is crucial. Although the use of standardized organ-specific offer forms containing key medical donor and organ information has facilitated organ evaluation for national transplant centers, our thorough analysis of these data revealed that relevant information for assessing organ quality is still missing in some offers. The forms are currently being revised and completed such that relevant information will be provided more uniformly to foreign transplant centers. Medical imaging results would be important to provide in lung offers. In the case of bilateral agreements, offering/accepting procedures must be as quick as possible to minimize loss of time when an offer subsequently is offered to all members. Other lessons learned over the years, which could serve as recommendations for regions around the world considering starting a similar collaboration, include involving users strongly during development and providing clear guidelines on how to use the system.

A major limiting factor in international organ allocation is the cold ischemia time when the organ is cold stored outside the body during transportation. Organs prone to ischemic damage are less suitable for long-distance transports. However, we believe that cooperation in legal aspects of cross-border organ allocation, together with enhanced management of organ procurement organizations, has made logistics more efficient and enabled long-distance transport also for vulnerable organs. Today, distance is practically a deciding factor only in heart allocation, but we expect, as for example, write Qin et al. in their 2022 systematic review of "Machine Perfusion for Human Heart Preservation" [17], that improvements in machine perfusion techniques may allow longer transport distances also for heart allocation in the near future. For example Swisstransplant imported two hearts in 2022 using the OCS™ Heart warm perfusion system from Rumania. As of the writing of this article Swisstransplant has imported five hearts from Czechia, France, Lithuania, and Romania in 2023.

Strengths and Limitations of the Study

This study is the first comprehensive and long-term analysis of European cross-border organ allocation with the FOEDUS-EOEO platform–a platform which has been used for more than 10 years. To the best of our knowledge, until today only preliminary results [13] or results from a single-country perspective [11] have been published in the scientific literature. We analyzed not only activity (effected organ offers on the platform), but also utility of the platform (how many of the offered organs eventually were utilized). It could encourage

European countries to participate or motivate countries in other regions of the world to set up similar programs.

Our study has also limitations. Each NTO may offer an organ either simultaneously to all participating NTOs, or, based on existing bi/multilateral agreements, first to only a selection of NTOs. This could have a significant impact on organ utilization, but we could not account for the effect size. For some NTOs it is also difficult to tell if they were actively receiving and thoroughly evaluating organ offers during the study period. Further, hearts with a maximal tolerable cold ischemia time of 4 h may not be evaluated when the donor hospital is too far away. Since the analyzed dataset does not obtain information on how many or which NTOs received and evaluated an offer, the number of potential recipients per offer remains unknown. This information, however, would help interpreting varying utilization and it would be crucial for drawing conclusions, such as if FOEDUS utilization could be increased by sending more offers to more NTOs simultaneously. Comprehensive data on donor/organ quality and refusal reasons are also important when comparing organ utilization, but in the available dataset such data were too incomplete for thorough analysis. For clarity, we treated four split livers and twelve individually offered lungs the same as whole livers and whole lungs, respectively, in our analysis. It can be argued, however, that these offers were more likely to be declined as whole liver and lung offers. Another limitation, of course, is the lack of recipient outcome data and incomplete information regarding the country of the organ's transplantation. For the latter, FOEDUS-EOEO should improve the filing of such fundamental information in the database in the future.

CONCLUSION

Over the years the FOEDUS-EOEO platform has demonstrated to be lifesaving for many European patients in need of a transplant, in particular for very young pediatric patients waiting for a liver, a heart, or a kidney transplant, or for patients waiting for a lung transplant. The increasing number of participating countries has increased both the number of offered organs and, to a lesser extent, the number of transplanted organs in Europe. In accordance with EU directives, the FOEDUS-EOEO platform ensures a high level of traceability and can be considered a best practice in European cross-border allocation of deceased donor organs for which no suitable recipient could be found under national allocation rules. To better understand and hopefully increase utilization of FOEDUS organs, more complete data on the quality of offered organs and the refusal reasons need to be analyzed. We hope that in the future the platform will be able to not only allocate those "national surplus organs", but also allocate organs on a supranational level from the beginning for specific patient categories, such as hyperimmunized patients.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

Conceptualization: FI; Methodology: AE and VL; Formal Analysis: AE and VL; Investigation: AE and VL; Data Curation: LS and CC; Visualization: AE; Writing–Original Draft: AE and VL; Writing–Review and Editing: AE, FB, CC, NK, LS, MC, and FI; Supervision: FI.

CONFLICT OF INTEREST

Coauthor LS is the CEO of the company DERS group, which is the operator of FOEDUS-EOEO platform.

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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ORIGINAL RESEARCH
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Donors With a Prior History of Cancer: Factors of Non-Utilization of Kidneys for Transplantation

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Cancer transmission from deceased donors is an exceedingly rare but potentially fatal complication in transplant recipients. We aimed to quantify the likelihood of non-utilization of kidneys for transplantation from donors with a prior cancer history. We included all intended and actual deceased donors in Australia and New Zealand between 1989 and 2017. Association between prior cancer history and non-utilization of donor kidneys was examined using adjusted logistic regression. Of 9,485 deceased donors, 345 (4%) had a prior cancer history. Of 345 donors with a prior cancer history, 197 (57%) were utilized for transplantation. Donor characteristics of age, sex and comorbidities were similar between utilized and non-utilized donors with prior cancer. The time from cancer to organ donation was similar between utilized and non-utilized donors, irrespective of cancer subtypes. Donors with a prior cancer history were less likely to be utilized [adjusted OR (95% CI) 2.29 (1.68–3.13)] than donors without prior cancer. Of all actual donors, the adjusted OR for non-utilization among those with prior cancer was 2.36 (1.58–3.53). Non-melanoma skin cancer was the most frequent prior cancer type for utilized and non-utilized potential donors. Donors with prior cancers were less likely to be utilized for transplantation, with no discernible differences in cancer characteristics between utilized and non-utilized donors.

Keywords: donor cancer, kidney donation, registry-based study, allograft failure, utilization

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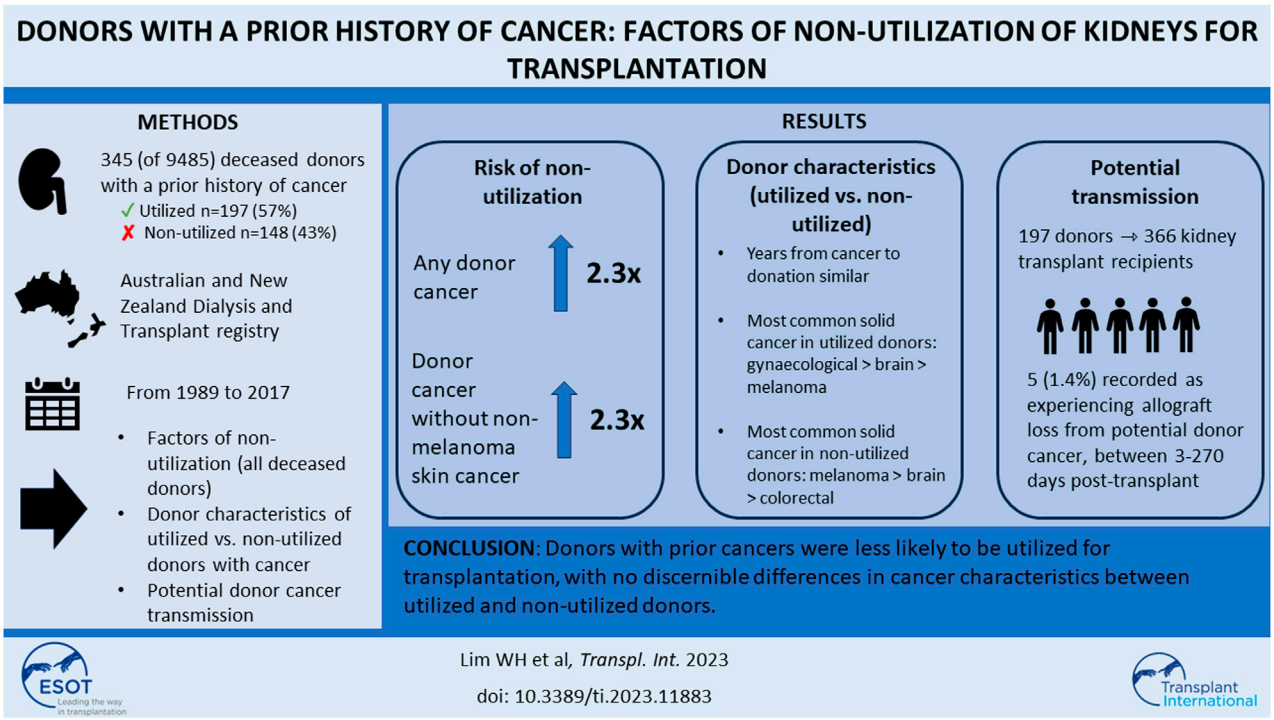
INTRODUCTION

The ongoing shortage of donor organs to match the increasing demand has prompted organ donor programs to consider higher risk donors, such as those with a prior cancer history, for transplantation. Acceptance of organ donors with a cancer history for transplantation may vary according to recipient characteristics, the expected recipient survival after transplantation and types and stages of prior donor cancer(s).



GRAPHICAL ABSTRACT

Donor cancer transmission is an infrequent but potentially life-threatening complication in kidney transplantation. The risk of disease transmission is rare, but the frequency of donor-transmitted cancer is difficult to quantify because of potential reporting bias, and granular details of the donor history may be lacking at the time of procurement. Registry-based studies have reported the frequency of donor-transmitted cancers post-solid organ transplants was between 0 and 6 cases per 10,000 solid organ transplants, but varied according to the donor cancer type [1–8]. Prior research have found the most frequent donor-transmitted cancers were kidney cancers and melanomas. Once transmitted, the risk of death was highest among recipients of donor-transmitted lung cancer, melanoma and cancers of the central nervous system [6, 8–10]. However, these data are reliant on the published literature and registry analyses without knowledge of the specific details regarding the histological types, stages and treatment responses of these cancers. Knowledge of the reasons for non-utilization will provide guidance to assist clinicians and patients in future decision-making processes when considering transplantation of organs from these donors.

Given the ongoing uncertainty concerning the likelihood of cancer transmission from donors with prior cancers coupled with the significant patient morbidity and mortality associated with donor-transmitted cancers [9], there are considerable centre and country variations in clinical decision making regarding donor utilization for transplantation. In addition, the actual non-utilization rate of organ donors with cancer remains unclear. In this study, we aimed to determine the likelihood and factors of donor non-utilization. We also defined the risks and outcomes of transplant recipients who received kidneys from donors with a prior cancer history.

MATERIALS AND METHODS

Study Population

All intended and actual deceased donors (i.e., consented for donation) in Australia and New Zealand between 1989 and 2017 from the Australia and New Zealand Organ Donation (ANZOD) and Australia and New Zealand Dialysis and Transplant (ANZDATA) registries were included in this study. For deceased donors with a past history of any cancers (including solid cancers, haematological cancers and non-melanoma skin cancers [NMSC]), data for these prior cancers were extracted from the registries. In addition, kidney transplant recipients (and their matched donors with prior cancer history) who had lost their kidney allografts from donor cancer were also identified from the registries. Data of allograft loss in kidney transplant recipients who have received deceased donor kidneys without a prior cancer history in the same time period were also extracted. An intended organ donor was a person for whom the donation work was initiated and a formal written consent for organ donation was undertaken, but did not become an actual donor (i.e., kidneys were not retrieved); whereas an actual donor was a

person for whom the organ retrieval procedure had commenced for the purpose of transplantation, even if organs were not utilized for transplantation.

The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism." This study was approved by the Human Research Ethics Committee of the University of Western Australia.

Data Collection

Deceased donor characteristics included age, sex, ethnicity, primary cause of death, donation pathway [donation after neurological determination of death (DNDD) and donation after circulatory determination of death (DCDD)], comorbid conditions (diabetes, hypertension, smoking history and prior hepatitis C exposure), terminal estimated glomerular filtration rate (eGFR, in mL/min/1.73 m [2]) and donation era. Donor cancer details extracted from the registries included date of cancers, site(s), histology and treatment(s) (if data were available).

Exposure

The study groups were categorized into non-utilized (either intended donors or actual donors whose kidneys were retrieved but were not utilized for transplantation) and utilized (actual donors whose kidneys were retrieved and were utilized for transplantation) donors.

Study Outcomes

The primary outcome was the risk of non-utilization of the donor kidneys. If only one kidney was utilized (and the other kidney was not utilized), the donor was considered as being utilized. The characteristics of donors with prior cancer history were described, stratified by utilized and non-utilized donors. We also determined the risk and outcomes of cancer transmission among recipients who received kidneys from donors with known prior cancers. In this study, donor cancer transmission was defined as allograft loss reported to ANZDATA registry as being attributed to donor cancer. Consequently, allograft loss from donor cancer may include donor cancers that were transmitted at time of organ donation, cancers that were derived from donor cells and circumstances where a decision was made (by clinicians and recipients) to "terminate" allograft function following detection of donor cancer. The registry does not verify the accuracy of the reporting or require evidence that cancer cells were of donor origin, and it does not collect the exact reason for the reported allograft loss from donor cancer by the transplanting centres.

Statistical Analyses

Baseline characteristics were expressed as number (proportion), median [interquartile range (IQR)] and mean (standard deviation, SD) where appropriate; with comparisons between utilized and non-utilized donors examined using chi-square test, Kruskal-Wallis test and t-test, respectively. The association between donor cancer history (with and without inclusion of donors with prior NMSC) and non-utilization was determined using logistic regression models, with the estimates expressed as unadjusted and adjusted odds ratio (OR) and 95% confidence intervals (95% CI). Covariates included in the multivariable models were selected according to known biological relationships with outcomes and included donor age, donor hypertension, donor diabetes status, donor smoking history, donor hepatitis C viral status [nucleic acid test (NAT)], donor ethnicity, donor terminal eGFR, donor death pathway (DNBD or DCDD) and era. Two sensitivity analyses were undertaken. First, characteristics of specific cancer subtypes were described and compared between 3 groups of intended donors, actual donors with and without kidneys utilized for transplantation. Second, an analysis restricting to actual donors (i.e., intended donors were excluded) was undertaken to examine the association between donor cancer history and non-utilization, with adjustment of donor covariates as the main model.

To define the risks and outcomes of donor cancer transmission, we compared the characteristics between donors that were utilized and those that were not utilized for transplantation. We focussed on pre-specified donor cancer types including melanoma, brain, breast, kidney and bladder, gynaecological, prostate and haematological cancers using descriptive analysis. We determined the risk of cancer transmission and subsequent allograft loss in recipients who received a donor with prior cancer history. We also described the donor and recipient characteristics of allograft loss from potential donor cancer in kidney transplant recipients who have received deceased donor kidneys without a prior cancer history. All analyses were undertaken using Stata (version 15.1 StataCorp LP, College Station, TX).

RESULTS

The study cohort is shown in **Figure 1**. There were 9,485 intended and actual deceased donors, of these, 1,645 (17%) were not utilized for kidney transplantation. **Table 1** shows the donor characteristics of kidneys from utilized and non-utilized donors. The mean (SD) age was higher (49 [18] vs. 41 [18]) and a greater proportion had diabetes (12% vs. 3%), were current smokers (42% vs. 38%), had a history of hypertension (38% vs. 20%) and positive hepatitis C virus NAT (5% vs. <0.1%) compared with utilized donors. The primary causes of donor death attributed to cerebral hypoxia/ischaemia or infarct was 37% in non-utilized donors compared to 19% in utilized donors.

Of 8,620 actual donors, 780 (9%) were not utilized for transplantation. Non-utilized actual donors were more likely to have diabetes (12% vs. 3%), have a positive hepatitis C virus NAT (4 vs. <0.1%), and were more likely to be DCDD donors (17% vs. 11%) compared to utilized actual donors. A greater proportion of actual donors were not utilized for transplantation in the more recent era compared to the earlier eras (1989–1998: 4%, 1999–2007: 7%, 2008–2017: 13%).

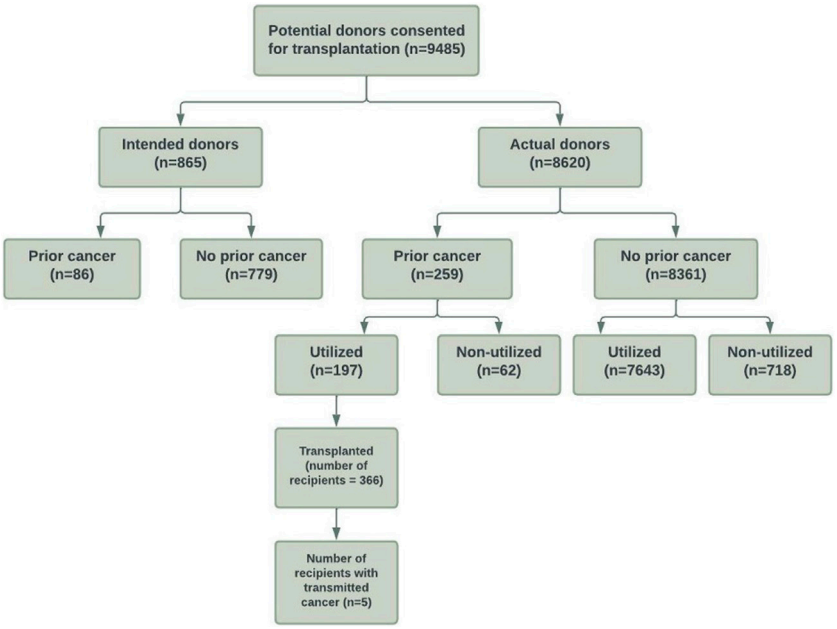


FIGURE 1 | Flow diagram of the study cohort of consented intended and actual deceased donors with and without prior cancer history in Australia and New Zealand between 1989 and 2017.

Donors With Prior Cancer History

Of the intended and actual donors, 345 (4%) donors had a prior history of cancers (254 [3%] donors with solid/haematological cancers and 91 [1%] with NMSC). Of these donors with prior cancer history, 197 (57%) donors were utilized for transplantation.

Of the actual donors, 259 (3%) donors had a prior history of cancers (190 [2%] donors with solid/haematological cancers and 69 [1%] with NMSC). Of these donors, 62 (24%) donors were not utilized. If restricted to donors with solid/haematological cancers, 49 of 190 (26%) donors were not utilized for transplantation. **Supplementary Table S1** shows the cancer types of "Other" cancers.

Association Between Donor Prior Cancer History and Non-utilization of Donor Kidneys

Compared to donors without a cancer history, the adjusted OR (95% CI) for non-utilization among donors with any prior cancer was 2.29 (1.68, 3.13). Other donor factors associated with an increased risk of non-utilization included older donor age, current smokers, DCDD donor status, prior history of donor hypertension or diabetes, lower terminal donor eGFR and positive donor hepatitis C NAT (**Figure 2**; **Supplementary Table S2**). The adjusted OR for non-utilization among donors with only a prior history of solid/haematologial cancers was 2.33 (1.59, 3.41).

In the sensitivity analysis restricting to actual donors (intended donors excluded, n = 865), the adjusted OR (95% CI) for non-utilization of donors with any prior cancer history was 2.36 (1.88, 3.53) and was 2.53 (1.57, 4.08) for donors with only a prior history of solid/haematologial cancers (**Figure 2**; **Supplementary Table S2**).

Characteristics of Donors With Prior Cancer History

There were 345 (4% of study cohort) donors with a prior history of cancer, of these, 91 (26%) had a prior history of NMSC and 254 (74%) donors a prior history of solid organ or haematological malignancy (including malignant melanoma). The median (IQR) time from donor cancer diagnosis to consent for donation was 5.8 (0.7, 11.9) years for non-utilized intended/actual donors, and 7.5 (1.4, 16.2) years for utilized actual donors. Of the 91 donors with prior NMSC, kidneys from 56 (62%) donors were utilized for transplantation, respectively. Of the 254 donors with prior cancers other than NMSC, 113 (44%) donors were not utilized for transplantation. **Table 2** shows the characteristics of the utilized and non-utilized donors for transplantation. The majority of the donors were of White backgrounds, and the proportion of donors with comorbidities was similar in both utilized and non-utilized groups. NMSC was the most frequent prior cancer type for both utilized and non-utilized potential donors. Among non-utilized potential donors, the most frequent prior cancer types were melanoma, colorectal, brain and prostate cancers.

Donor Cancer Characteristics in Selected Prior Cancer Types

Table 3 shows the cancer types and treatment strategies among potential donors with prior cancers, stratified by donor utilization

TABLE 1 | Baseline characteristics of the study cohort.

	Deceased donor utilization status		
	Non-utilized donors (*n* = 1,645)	**Utilized donors (*n* = 7,840)**	***p*-value**
Donor characteristics			
Female (n, %)	647 (39.3)	3,351 (42.7)	0.01
Age (years, mean [SD])	49.5 (18.0)	41.5 (17.9)	<0.01
Ethnicity (n, %)			<0.01
White	1,460 (88.7)	7,283 (92.9)	
Australian Aboriginals/TSI	36 (2.2)	79 (1.0)	
Asians	100 (6.1)	297 (3.8)	
New Zealand Māoris	19 (1.2)	91 (1.2)	
Others	30 (1.8)	90 (1.1)	
Donor cancer history			
Any cancer (including NMSC)	148 (9.0)	197 (2.5)	<0.01
Any non-NMSC cancer	113 (6.9)	141 (1.8)	<0.01
Donor comorbid conditions			
BMI (kg/m^2, mean [SD])	27.2 (6.1)	25.8 (5.7)	<0.01
Missing data (n, %)	64 (3.9)	375 (4.8)	
Diabetes (n, %)			<0.01
None	1,345 (81.8)	6,571 (83.8)	
Yes	198 (12.0)	264 (3.4)	
Missing data	102 (6.2)	1,005 (12.8)	
Hypertension (n, %)			<0.01
None	966 (58.7)	5,398 (68.9)	
Yes	615 (27.4)	1,376 (17.5)	
Unknown/missing data	64 (3.9)	1,066 (13.6)	
Smoking history (n, %)			<0.01
None	542 (32.9)	2,960 (37.8)	
Former	362 (22.0)	1,201 (15.2)	
Current	684 (41.6)	2,592 (33.1)	
Unknown/missing data	57 (3.5)	1,087 (13.9)	
Hepatitis C virus NAT (n, %)			<0.01
Negative	1,566 (95.2)	7,834 (99.9)	
Positive	79 (4.8)	6 (0.1)	
Donation pathway characteristics			
DCDD (n, %)	691 (42.0)	861 (11.0)	<0.01
Cause of death (n, %)			<0.01
Cerebral infarct/hypoxia	601 (36.5)	1,518 (19.4)	
Intracranial haemorrhage	621 (37.8)	3,401 (43.4)	
Traumatic brain injury	244 (14.8)	2,069 (26.4)	
Others	179 (10.9)	852 (10.8)	
Donor terminal eGFR [mL/min/1.73 m [2], mean (SD)]	78.8 (42.1)	93.6 (34.4)	0.78
Missing data (n, %)	780 (47.4)	132 (1.7)	
Era of donation (n, %)			<0.01
1989–1998	97 (5.9)	2,268 (28.9)	
1999–2007	228 (13.9)	1,932 (24.6)	
2008–2017	1,320 (80.2)	3,640 (46.5)	

Data expressed as number (%) or as mean [standard deviation (SD)]. TSI, Torres Strait Islander; eGFR, estimated glomerular filtration rate; NAT–nucleic acid test; DCDD, donation after circulatory determination of death; NMSC, non-melanoma skin cancer.

status. The time from cancer occurrence to donor consent/organ donation varied by prior cancer types, but these time periods were similar for both utilized and non-utilized donors. The median (IQR) duration between time of cancer diagnosis to the time of consent for organ donation were 9.0 (4.9, 14.3) years for donors with prior colorectal cancer, 20.0 (10.3, 24.9) years for donors with prior breast cancer, 10.1 (5.4, 17.1) years for donors with prior melanoma, 4.1 (2.8, 8.0) years for donors with prior prostate cancer, 1.0 (<0.1, 9.6) years for donors with prior brain cancer and 13.0 (6.4, 20.7) years for donors with prior gynaecological cancers.

The treatment strategies for the prior cancers were similar between utilized and non-utilized actual donors; with majority of donors with prior melanoma, colorectal, breast, prostate and gynaecological cancers had surgery or chemo-radiotherapy. Among the 31 intended donors with prior brain cancers, 11 (35%) had high grade gliomas/glioblastoma multiforme or medulloblastoma. Of these 11 donors, 5 (45%) donors were utilized for transplantation. Of the 14 donors with colorectal cancer, 7 (50%) cancers were adenocarcinoma, of which 2 (29%) were utilized for transplantation. Of the 21 donors with breast cancer, 14 (67%) donors were utilized for transplantation. Of the

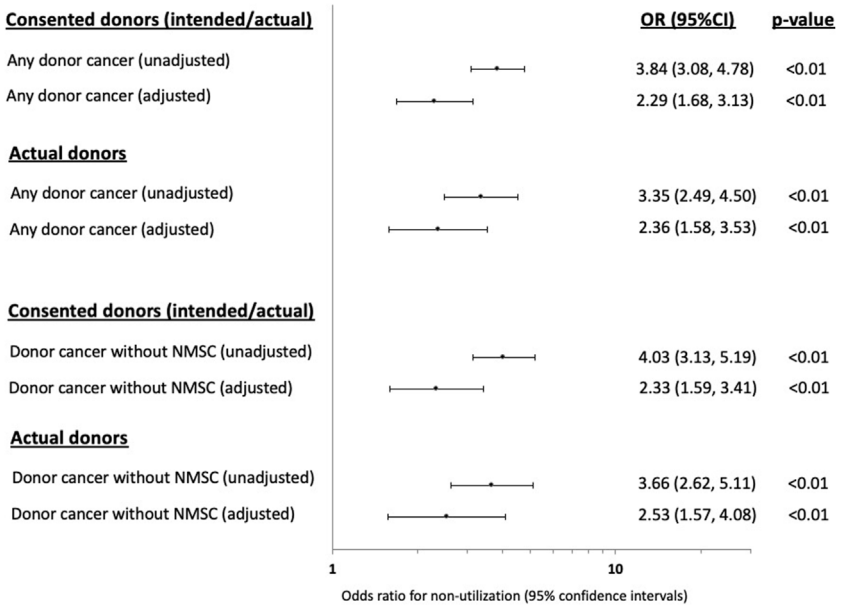


FIGURE 2 | Forest plots showing the unadjusted and adjusted odds ratio (OR) with 95% confidence intervals (95% CI) of the associations between prior donor cancer history [with and without a prior history of non-melanoma skin cancer (NMSC)] and non-utilization of kidneys from intended and actual donors.

38 donors with gynaecological cancers, 30 (79%) donors were utilized for transplantation (of which 13 cancers were cervical cancers and 15 cancers were unknown/not reported).

In a sensitivity analysis examining the cancer characteristics of intended and utilized and non-utilized actual donors, times from donor cancer to organ donation were lower in intended donors compared to actual donors for melanoma, colorectal and breast cancer, but these were not statistically significant (**Supplementary Table S3**).

Risk of Cancer Transmission in Donors With Prior Cancers

Three-hundred and sixty-six recipients received kidneys from 197 donors with a prior cancer history, with a median allograft follow-up period of 4 years. Of these recipients, 5 (1.4%) were recorded as experiencing allograft loss from four donors with prior cancer. All 4 donors died from intracranial haemorrhage. There were 2 donors with a prior history of non-Hodgkin lymphoma, 1 donor with prior malignant melanoma and 1 donor with prior kidney cancer. All 5 recipients were reported to be alive at the end of follow-up (31st December 2017), with allograft loss reported to occur between 3–270 days post-transplant. Donor kidney cancer transmission occurred in one recipient who received a kidney from a donor with a prior kidney cancer at 8 months post-transplant. Two recipients were reported to have lost their allografts from donor cancer but the type of cancer (in the allograft) was not recorded in the registry (**Table 4**).

Over the study period, of the 14,671 kidney transplant recipients who have received kidneys from donors without a known prior cancer history, there were 12 recipients who were reported to experience potential donor cancer-related allograft loss from 10 deceased donors (i.e. 10/7,643 donors without a prior history of cancer at the time of organ donation or 0.1%). Intracranial haemorrhage was the cause of death in 5 donors. The exact cause of allograft loss (or whether a cancer was detected in the allograft) was not reported to the registry (**Supplementary Table S4**).

DISCUSSION

In this large registry study spanning almost three decades, we have shown that donors with a prior cancer history were less likely (by approximately 2-fold) to be utilized for kidney transplantation compared to donors who did not have prior cancer. While cancer history influenced the likelihood of utilization of consented donor kidneys, there were other important donor factors such as terminal kidney function and donor comorbidities that clinicians would consider for non-utilization. Although donors with a prior cancer history comprised of only 4% of all utilized donors, over 50% of these donors (with prior cancer history) were utilized for transplantation. The time from diagnosis to organ donation varied by cancer types, with average duration of between 4 (or less) years for brain, prostate and kidney cancers; and between 10 and 20 years for melanoma, colorectal and breast cancers. There were a total of 5 (1%) reported cases of donor cancer transmission over a median follow-up time of 4 years from 366 recipients who have received kidneys from 197 donors with a prior history of cancer, suggesting transplant clinicians follow a relatively conservative approach in accepting higher risk donors. Of those with transmitted disease, all recipients experienced allograft loss, with 3 of 5 cases reported to have cancer detected in the allograft. None of the recipients died as a result of cancer

TABLE 2 | Characteristics of utilized and non-utilized deceased donors with prior cancer history.

		Non-utilized donors (*n* = 148)	Utilized donors (*n* = 197)	*p*-values
Donor demographics	Age (mean, SD)	58.9 (15.3)	56.9 (12.1)	0.171
	Age (median, IQR)	61.0 (52.0, 69.0)	60.0 (48.0, 67.0)	0.062
	Female (n, %)	66 (44.6)	107 (54.3)	0.074
	Race (n, %)			0.515
	White	141 (95.2)	193 (98.0)	
	Asian	4 (2.8)	2 (1.0)	
	Aboriginal	3 (2.0)	2 (1.0)	
	Comorbid condition (n, %)			
	Diabetes	19 (12.9)	13 (6.6)	0.129
	Hypertension	68 (45.9)	69 (35.0)	0.071
	Smoking (former/current)	80 (54.1)	122 (61.9)	0.400
	Hepatitis C virus NAT positive	2 (1.4)	0 (0.0)	0.384
Prior donor cancer history	Cancer types (n, %)			
	Melanoma	22 (14.9)	18 (9.1)	
	Brain	12 (8.1)	19 (9.6)	
	Colorectal	11 (7.4)	3 (1.5)	
	Haematological cancers	6 (4.1)	6 (3.0)	
	Breast	7 (4.7)	14 (7.1)	
	Thyroid gland	3 (2.0)	8 (4.1)	
	Female gynaecological	8 (5.4)	30 (15.2)	
	Prostate	11 (7.4)	14 (7.2)	
	Kidney/Bladder	10 (6.8)	11 (5.6)	
	Respiratory	2 (1.4)	1 (0.5)	
	NMSC	35 (23.6)	56 (28.4)	
	Others/unknown	21 (14.2)	17 (8.6)	
	Years from cancer to donation			
	Mean (SD)	8.7 (12.0)	11.0 (14.4)	0.113
	Median (IQR)	5.8 (0.7, 11.9)	7.5 (1.4, 16.2)	0.287
Donation history	Donor status (n, %)			<0.001
	Actual donor	62 (41.9)	197 (100.0)	
	Intended donor	86 (58.1)	0 (0.0)	
	"Intended" DNDD	88 (59.5)	155 (78.7)	<0.001
	Causes of death (n, %)			
	Intracranial haemorrhage	75 (50.7)	112 (56.9)	
	Cerebral infarct	12 (8.1)	20 (10.2)	
	Cerebral hypoxia/ischaemia	29 (19.6)	25 (12.7)	
	Traumatic brain injury	18 (12.2)	20 (10.2)	
	Others	9 (6.0)	19 (9.7)	
	Missing data	5 (3.4)	1 (0.3)	
	Year of donation (n, %)			0.437
	1989–1999	0 (0.0)	3 (1.5)	
	2000–2005	18 (12.2)	19 (9.6)	
	2006–2010	20 (13.5)	32 (16.3)	
	2011–2015	60 (40.5)	83 (42.1)	
	2016–2017	50 (33.8)	60 (30.5)	

Data expressed as number (%), mean [standard deviation (SD)] or as median [interquartile range (IQR)]. NAT, nucleic acid test; DNDD, donation after neurological determination of death; NMSC, non-melanoma skin cancer.

transmission. Based on the current dataset, we could only speculate donor origin cancers were either transmitted with the allograft or were derived from the allograft. However, given that allograft loss (attributed to donor cancer) in these 5 cases occurred early post-transplant (range 3–270 days), it is therefore less likely that these donor cancers (presumed to cause allograft loss) represent *de novo* (new) recipient-derived cancer.

The rate of non-utilization of donor organs for kidney transplantation varies between countries and donor quality, with rates of donor discards reported up to 20% [11, 12]. Several cohort studies have examined predictive factors for non-utilization and these included older donor age, female donors, hepatitis B and C seropositive status, higher terminal serum creatinine concentration and donor comorbidities, such as hypertension, diabetes and smoking history [13–15]. It is important to emphasize that the metrics and terminologies of utilization and non-utilization of donor kidneys are inconsistent across studies, and therefore reliable comparisons between regions and countries could not be made with confidence. In the United States, it is standard practice to recover kidneys before acceptance by individual units. On the contrary, in countries such as the United Kingdom and Australia, recovery of kidneys is contingent upon the acceptance and allocation of the kidneys for transplantation [16, 17]. In our study, we have found that

TABLE 3 | Specific cancer subtypes of utilized and non-utilized donors with prior cancer history.

	Non-utilized donors (*n* = 148)	Utilized donors (*n* = 197)	*p*-values
Donor cancer types			
Melanoma (n)	22	18	0.79
Sites (n)			
Skin	20	16	
Non-skin	0	1	
Unknown	2	1	
Treatment			
Surgery	18	14	
None/Others	1	2	
Unknown	3	2	
Years to donation[a]	10.2 (5.4, 15.9)	10.1 (5.4, 28.5)	
Brain (n)	12	19	0.27
Types			
Astrocytoma	4	8	
Low-grade glioma	1	2	
High grade glioma/GBM	3	5	
Medulloblastoma	3	0	
Meningioma	1	0	
Others	0	4	
Treatment			
Surgery	9	5	
Radiotherapy	1	1	
Chemotherapy	0	1	
None	2	9	
Unknown/others	0	3	
Years to donation[a]	2.1 (0.1, 20.9)	0.7 (0.1, 8.8)	
Colorectal (n)	11	3	0.14
Types			
Adenocarcinoma	5	2	
Unknown	2	0	
Carcinoid	1	0	
Others	3	1 (carcinoma-in-situ)	
Treatment			
None	2	0	
Surgery	6	3	
Chemotherapy/radiotherapy	0	0	
Unknown/others	3	0	
Years to donation[a]	7.5 (3.7, 14.3)	14.1 (10.1, 26.7)	
Breast (n)	7	14	0.16
Types			
Adenocarcinoma	4	2	
Invasive ductal carcinoma	2	2	
Ductal carcinoma *in situ*	1	1	
Unknown	0	9	
Treatment			
None	1	0	
Surgery	4	11	
Chemotherapy/radiotherapy	1	1	
Unknown/others	1	2	
Years to donation[a]	10.3 (4.6, 21.7)	20.3 (17.8, 25.3)	
Prostate (n)	11	14	0.16
Types			
Adenocarcinoma	6	12	
Others	3	0	
Unknown	2	2	
Treatment			
None	2	1	
Surgery	6	10	
Chemotherapy/radiotherapy	1	2	
Unknown/others	2	1	
Years to donation[a]	3.1 (0.2, 7.9)	5.4 (3.3, 11.0)	

(Continued on following page)

TABLE 3 | (*Continued*) Specific cancer subtypes of utilized and non-utilized donors with prior cancer history.

	Non-utilized donors (*n* = 148)	Utilized donors (*n* = 197)	*p*-values
Kidney/bladder (n)	10	11	-
Types			
RCC	7	8	
Papillary cancer (kidney)	1	0	
Kidney oncocytoma	1	1	
Bladder (urothelial/TCC)	1	2	
Treatment			
None	9	8	
Surgery	1	2	
Unknown/others	0	1	
Years to donation[a]	-	0.8 (0.3, 1.1)	
Gynaecological (n)	8	30	0.22
Types			
Cervical cancer (SCC/adenocarcinoma)	5	13	
Cervical cancer *in situ*	0	0	
Uterine	0	2	
Others/Unknown	3	15	
Treatment			
None	0	1	
Surgery	4	22	
Chemotherapy/radiotherapy	1	0	
Unknown/others	3	7	
Years to donation[a]	21.4 (9.5, 24.8)	11.0 (6.4, 18.1)	
Haematological (n)	6	6	0.87
Types			
Leukaemia	1	2	
Lymphoma	5	4	
Treatment			
None	3	3	
Surgery	0	0	
Chemotherapy/radiotherapy	1	2	
Unknown/others	1	1	
Years to donation[a]	0.9 (-, 6.6)	3.6 (0.8, 16.5)	

[a]*Represents median [interquartle range (IQR)] years to donation using available recorded data. GBM, glioblastoma multiforme; RCC, renal cell cancer; DCIS, ductal carcinoma-in-situ, TCC, transitional cell cancer.*

TABLE 4 | Characteristics of kidney transplant recipients with allograft loss attributed to donor cancer.

Donor cancer transmission (year)	Prior donor cancer (type)[a]	Donor age	Cause of donor death	Recipient cancer type/site (days from transplant if known)*	Time to allograft loss in days
1 (1998)[a]	Yes (NHL)	72	Intracranial haemorrhage	Lymphoma in allograft	11
2 (1998)[a]	Yes (NHL)	72	Intracranial haemorrhage	Lymphoma in allograft	10
3 (2001)	Yes (melanoma)	47	Intracranial haemorrhage	None	155
4 (2003)	Yes (renal cell cancer)	56	Intracranial haemorrhage	Adenocarcinoma in allograft (255)	270
5 (2008)	Yes (NHL)	68	Intracranial haemorrhage	None	3

[a]*Same donor (for cases 1&2). NHL, non-Hodgkin lymphoma. *Note all recipients remain alive at the end of survey period.*

donor cancer history was associated with at least a 2-fold greater risk of non-utilization. This finding is consistent with current literature [18], but other characteristics including histological types, stage, prior treatment and duration since cancer treatment, may have influenced the decision-making process. However, these details were not routinely recorded or were inadequately captured in the registries. In addition, the exact reasons of not accepting kidneys from consented donors with a prior cancer history are not collected by ANZDATA and ANZOD registries. We speculate that there may be many reasons including cancer and non-cancer transplant-related factors. However, donors with a prior cancer history who were not consented for organ

donation are not routinely captured by the registries and therefore an accurate metric of the total number of donors with a prior cancer history being assessed for possible organ donation is unavailable. The introduction of new OrganMatch clinical data system in Australia from 2019 has allowed the capture of pre-specified reasons for non-utilization of donor organs but these data are not yet available for analysis.

The outcomes of donor cancer transmission in kidney transplantation were summarized and presented in previous literature. In a systematic review of 69 studies (case reports, case series and registry data) of 104 donor-transmitted cancer cases, kidney cancer, melanoma, lymphoma and lung cancer were the four most common donor-transmitted cancers, with less than 1 in 2 kidney transplant recipients surviving beyond 2 years. Donor-transmitted melanoma and lung cancer were associated with the poorest outcome, whereas for donor-transmitted kidney cancer, almost 75% survived beyond 2 years [9]. An updated systematic review in 2020 showed similar findings with donor-transmitted melanoma and lung cancer associated with the poorest recipient prognoses (5 years survival of 43% and 19%, respectively), whereas donor-transmitted kidney cancer and lymphomas had the most favorable recipient prognoses (5 years survival of 93% and 63%, respectively) [8]. A framework for evaluating donors with prior cancers has been proposed by a malignancy subcommittee, part of the *ad hoc* Disease Transmission Advisory Committee (DTAC) of the Organ Procurement and Transplantation Network/United Network for Organ Sharing (OPTN/UNOS). The use of organs from donors with cancers deemed as intermediate (estimated frequency of donor cancer transmission of 1%–10%) or high risk (estimated frequency >10%) are generally not recommended, whereas those at minimal (estimated frequency <0.1%) or low risk (estimated frequency 0.1%–1%) can be considered with appropriate informed consent [19]. Although donors with more aggressive donor cancers such as melanoma, lung cancer and higher stage breast and colon cancers are deemed unsuitable, these risks must be balanced against the projected survival gain for potential recipients. However, the risks of transmission for less common donor cancers are often difficult to define. For example, the risk of transmission of donors with prior central nervous sytem cancers, such as glioblastoma multiforme, is uncertain, with prior studies of such cases showing no conclusive evidence of transmission [3, 5, 20–22]. In our study, of the 141 actual donors with prior solid or haematological cancers (excluding those with NMSCs) whose kidneys were utilized for transplantation, 32% of the donor cancers were gynaecological and prostate cancers, with a lesser proportion of utilized kidneys from donors with prior colorectal, haematological and breast cancers, likely reflecting the general risk tolerance of clinicians when allocating these donor kidneys for transplantation. There were no noticeable differences in the time periods from cancer to organ donation, cancer histological types and treatment for selected donor cancers for intended and actual donors, suggesting that the selection of donors with cancers for consent has been carefully considered. It is important to emphasize that the actual risk of cancer transmission from donor to recipients cannot be determined with certainty in this study because situations where donors with a prior cancer history and did not result in cancer transmission are not captured by the registries. Under-reporting of prior cancer history (of donors) and the possibility of donor cancer transmission is likely. Furthermore, verification of disease transmission using detailed donor-HLA typing or other molecular techniques to ensure the tumour cells are of donor origin are not required by the registries. Consequently, we are unable to provide detailed information regarding the cancer type and the reason of allograft loss from donor cancer in this study.

The evidence that underpinned the current recommendation for donor acceptance are based on case reports and series aligned with expert opinions, and therefore are of low to very low quality. Nevertheless, the decision to accept kidneys from donors with prior cancer history is often contingent on the perceived risks (of potential donor cancer transmission) and benefits (to the potential transplant candidates) of utilizing these kidneys for transplantation. The type, stage, adequacy of prior anti-cancer treatment and follow-up, and the interval from cancer diagnosis to donation must be carefully considered on a case-by-base basis. Specifically, the clinicians will need to balance between the potential risk of "undetected or unrecognised" donor cancer recurrence (and therefore the possibility of donor cancer transmission) against the risk of death without transplantation. However, the recipients' wish must be respected and informed consent must be obtained. A shared decision making process between the recipients and health professionals, which involve consideration of the patients and their families' persectives, preferences and circumstances must be valued.

While the precise risk of transmission from donor to recipient of any given cancer is usually unknown, there has been an attempt in broadly categorizing the possible risk of transmission based on the cancer type and stage, its metastatic potential, and its patterns of recurrence in both the transplant and non-transplant setting [19, 23, 24]. It is imperative that all jurisdisctions and donation agencies maintain a register of outcomes of transplants from donors with cancer. These registers should include a well-defined, minimum set of outcomes that are acceptable to patients and may include all cancer characteristics such as tumour histology, stage, cancer genetics and management strategies. Reports on donor transmission events and their outcomes must be published regularly. A global repository that collects high-risk donor details, including those with prior cancer and combined with minimum and real-time data entry requirements with sufficient follow-up periods is essential to inform recommendations to guide informed decisions regarding utilization of these donor organs for transplantation.

This study has a number of potential limitations. Selection, reporting, confounding and information biases are inherent to this observational analysis. These limitations could have hampered accurate estimation of the risk and outcomes of donor transmitted cancers. Inconsistent reporting of follow-up times, the lack of treatment specific details and cancer stage may have precluded the understanding of the actual risk of cancer transmission of organs from donors with prior cancers history with varying clinical, histological, genetic and treatment characteristics.

In this study, we have shown that prior cancer history is a key factor for donor non-utilization. This study also highlights the need to improve data collection relating to the clinical decision-making process of the acceptance and utilization of organs from donors with prior cancer history, as well as the need for accurate records

of donor-transmitted cancers in organ transplantation. The establishment of global repositories, combined with minimum and real-time data entry requirements with sufficient follow-up periods are essential to inform recommendations to guide informed decisions regarding utilization of these higher risk donor organs.

DATA AVAILABILITY STATEMENT

Data extraction from the registry need ethics approval for study investigators to handle the data (as per requirement of the registry's privacy laws). However, with ethics approval, this or other datasets can be requested from ANZDATA registry. Requests to access the datasets should be directed to request@anzdata.org.au.

ETHICS STATEMENT

The studies involving humans were approved by University of Western Australia. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because because prior consents for inclusion in the ANZDATA registry, including using deidentified data for research, have already been provided.

AUTHOR CONTRIBUTIONS

WL and GW designed the study, WL, EO, and GW analysed and interpreted the initial data. All authors participated in drafting the paper and interpreted final data.

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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.2023.11883/full#supplementary-material

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