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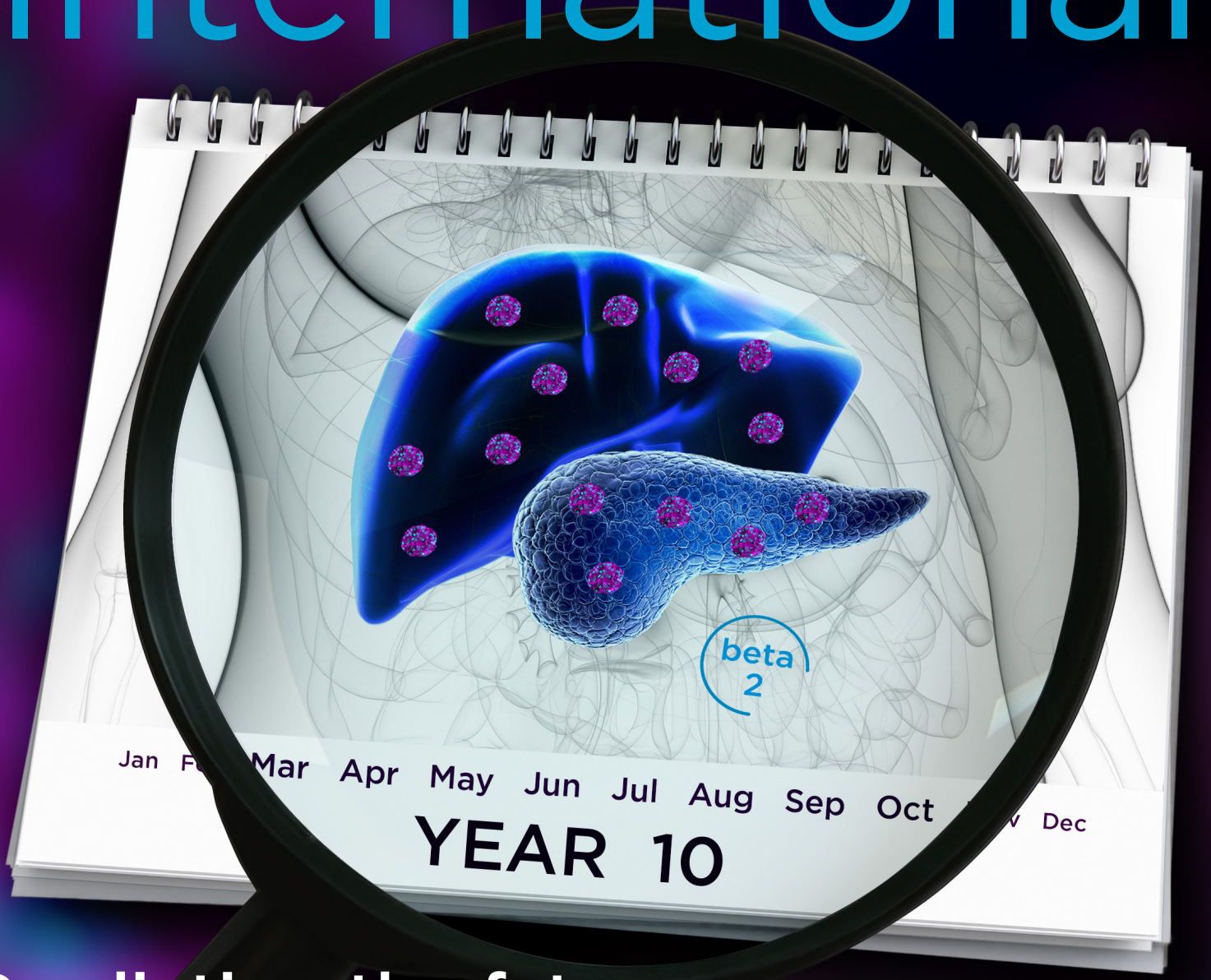

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Original Research

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TRANSPLANT TRIAL WATCH
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Transplant Trial Watch

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Keywords: organ donation, randomised controlled trial, kidney transplantation, immunosuppresion, tacrolimus

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

Randomized Intervention to Assess the Effectiveness of an Educational Video on Organ Donation Intent Among Hispanics in the New York Metropolitan Area.

by Pekmezaris, R., et al. World Journal of Transplantation 2023; 13(4): 190–200.

Aims
The aim of this study was to evaluate whether an educational video was effective in improving organ donation intent among Hispanic New York residents.

Interventions
Participants were randomised to either view a short educational video on organ donation prior to the survey or to view the same video following the survey.

Participants
365 Hispanic New York City (NYC) residents.

Outcomes
The main outcomes of interest were to assess the impact of the emotional video on willingness to donate, and to identify driving factors for organ donation.

Follow-Up
N/A.

CET Conclusion
This randomised study from New York recruited adult Hispanic residents and delivered an online survey to elicit their knowledge and views on organ donation. Participants were randomised to watch an emotive video on deceased donation either before answering the survey, or after. The authors found

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that participants who watched the video before answering the survey showed more willingness to register as a donor (OR 2.05) and greater awareness as to how to sign up. The study is well designed and interesting, demonstrating how simple information provision may impact donation decisions in diverse populations. It is worth noting that the study did not measure actual registrations, just intent, and future studies should look at impact on actual registration rates as a closer proxy to real-world benefit.

Jadad Score
1.

Data Analysis
Strict intention-to-treat analysis.

Allocation Concealment
No.

Trial Registration
N/A.

Funding Source
Not reported.

RANDOMISED CONTROLLED TRIAL 2

Comparison of 2 Immunosuppression Minimization Strategies in Kidney Transplantation: The ALLEGRO Trial.

by van den Born, J. C., et al. Transplantation 2023 [record in progress].

Aims
The aim of this trial was to compare standard immunosuppression with two immunosuppression minimisation strategies in *de novo* kidney transplant recipients.

Interventions
Participants were randomised to one of three groups: the early steroid withdrawal arm, the standard-dose tacrolimus arm, and the tacrolimus minimisation arm.

Participants
295 *de novo* kidney transplant recipients.

Outcomes
The primary outcome was kidney function at 24 months posttransplantation. The secondary outcomes were patient survival, treated rejection, kidney failure, discontinuation of study medication for more than 6 weeks, and treatment failure.

Follow-Up
24 months.

CET Conclusion
This multicentre trial from the Netherlands randomised *de novo* renal transplant recipients to one of three immunosuppression strategies—standard care, early steroid withdrawal or tacrolimus minimisation at 6 months. The study was designed to demonstrate non-inferiority in renal function at 24 months, and met the primary endpoint, with no difference seen between the three groups. There was a higher incidence of acute rejection in the early steroid withdrawal group, but no increase in DSA formation. In general, study design is good although unblinded, with centralised randomisation and intent-to-treat analysis. Withdrawal rate was around 25% in each arm at 24 months. Inclusion criteria are fairly broad for an immunosuppression minimisation study, allowing recipients up to 80 years of age, PRA up to 75% and first or second transplants. One notable exclusion criteria was for type 1 diabetic recipients; the authors do not provide a rationale for this.

Jadad Score
3.

Data Analysis
Strict intention-to-treat analysis.

Allocation Concealment
Yes.

Trial Registration
ClinicalTrials.gov—NCT01560572.

Funding Source
Industry funded.

CLINICAL IMPACT SUMMARY

Whilst there have been relatively few randomised trials of novel immunosuppressant strategies in renal transplantation in recent years, there has been a lot of interest in modified protocols that aim to minimise the adverse effects of immunosuppressant agents. Most studies have focussed on minimising either corticosteroid use or calcineurin inhibitor exposure, as these have the potential to have greatest impact on long-term outcomes. Corticosteroid avoidance or minimisation appears to reduce the risk of metabolic complications (hypertension, high cholesterol and new onset diabetes) at the expense of slightly higher risk of early steroid sensitive acute rejection [1]. Calcineurin inhibitor withdrawal or tapering studies vary in their

approach, with or without substitution with an mTOR inhibitor. Meta-analysis shows that CNI withdrawal or avoidance may increase the risk of acute rejection, but the reduced nephrotoxicity improves short-term graft survival and decreases the risk of hypertension [2]. However, heterogeneity in the published literature and a lack of long-term outcome reporting means that most centres still employ a long-term CNI strategy for renal recipients.

A recent, multicentre, randomised ALLEGRO study from the Netherlands attempts to address some of these issues [3]. In this open label study, *de novo* kidney transplant recipients were randomised to either standard immunosuppression (basiliximab, tacrolimus, MMF and steroids) or minimisation. Two different minimisation strategies were tested: early steroid withdrawal (at day 3) or late tacrolimus reduction (at month 6). Patients were followed for 24 months. The study followed a non-inferiority design, with a difference of 10 mL/min or less in eGFR at month 24 being defined as non-inferior. Both minimisation arms were shown to be non-inferior for the primary endpoint. As seen in previous studies (and meta-analyses) there was an increased incidence of acute rejection in the steroid withdrawal arm (but no increase in DSA formation), with a reduction in incidence of new onset diabetes and lower serum cholesterol. Unlike previous studies, there was no increase in rejection following late CNI minimisation.

The study design is robust, with central randomisation and use of an intent-to-treat analysis. Inclusion criteria for immunosuppression trials are often very restrictive and unrepresentative, so the authors should be commended for including a wide range of recipients up to 80 years of age, PRA of up to 75% and repeat transplants, improving real-world generalisability. Perhaps slightly oddly, the authors excluded recipients with pre-existing type 1 diabetes, but did not provide a rationale for this decision.

Whilst this is a large, well conducted study, the findings are not particularly novel and are in keeping with the existing literature. They do demonstrate, however, that late CNI reduction is safe and feasible. Longer-term follow-up of participants in the study would be useful to demonstrate the effects of a reduction in metabolic risk and/or nephrotoxicity on longer-term graft and patient survival.

Clinical Impact

3/5.

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 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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NEWS AND VIEWS
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The Dawn of a New Era in Kidney Transplantation: Promises and Limitations of Artificial Intelligence for Precision Diagnostics

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Keywords: artificial intelligence, kidney transplant, machine learning, deep learning, transplantation

Announced as "the most revolutionary technology in decades" [1], artificial intelligence (AI) allows for the analysis and extraction of insights from huge clinical datasets. By using AI algorithms, healthcare professionals expect to gain valuable insights into patient outcomes, identify predictive factors, and develop personalized approaches for each individual. In addition, AI also holds the promise of streamlining clinical workflows, supporting real-time decision making, and enabling more efficient use of healthcare resources. As AI technology continues to develop and infiltrate new fields of our society every day, we wanted to propose a critical appraisal and try to define, among its numerous possible applications in transplant medicine, the ones that have the capability to address existing gaps and solve unmet needs.

The widespread introduction of AI in transplant nephrology has been prompted by the ever-increasing complexity and volume of information, as well as the existence of multiple nephrology registries around the world. Since the first kidney transplant, we have witnessed a shift in therapeutic goals to achieve. Initially, most efforts were focused on obtaining good short-term outcomes. This was accomplished by refining surgical techniques, researching and learning about more effective preservation solutions, and improving immunosuppression protocols. As a result, the use of kidney transplantation as a therapeutic procedure has spread rapidly (becoming, *de facto*, a victim of its own success, with growing waiting lists), and the focus has had to shift towards long-term success. In contrast with short-term outcomes, the number and diversity of variables impacting the survival of graft and patient in the long-term (including recipient's innate and adaptive alloimmune responses, recurrence of the initial disease, nephrotoxicity of the immunosuppressive drugs, infections, cancer, etc.) [2] complexify the decision-making process, largely explaining the relative stagnation of kidney transplantation outcomes over the last few decades [3]. Here, AI could play a crucial role since this technology unveils a tremendous potential to improve immunological donor/recipient matching, kidney graft organ preservation, ischemic/reperfusion profiles, and pharmacokinetic post-transplant surveillance, which all have long term impacts (**Figure 1**). In addition, AI algorithms are also theoretically capable of identifying patterns and signatures indicative of rejection or generating personalized risk scores for individual patients by integrating multiple data sources including donor and recipient demographics, clinical variables, genetic testing, laboratory results, and histopathological findings.

Biopsies provide key prognostic information for the health of allografts [4–6], which is essential for choosing the appropriate therapeutic interventions and predicting long-term outcomes. The complex and time-consuming process of histopathological analysis for kidney allograft biopsies relies

News & Views

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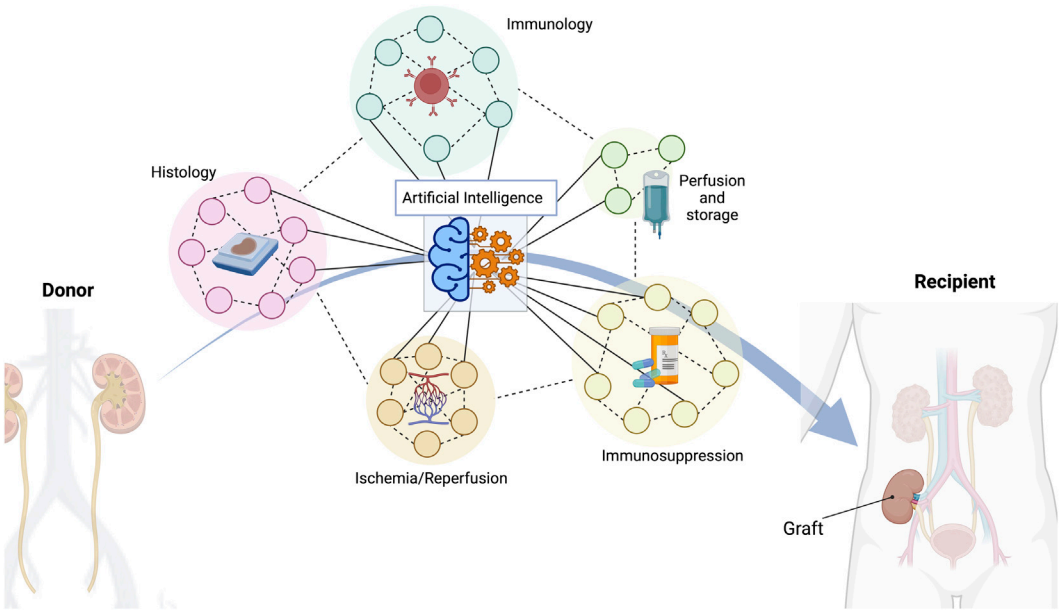

FIGURE 1 | A multitude of different indicators are crucial to achieve a successful long-term outcome in kidney transplantation. Histopathological features, donor/recipient immunology matching characteristics, different types of organ perfusion and storage, several types of immunosuppressive regimes, or ischemia/reperfusion consequences are just some of the most important variables to consider. Artificial intelligence has the capacity to manage all these data and provide the best possible solution.

heavily on the expertise of renal pathologists, which represents a significant bottleneck due to its limited availability [7]. Even when accessible, interobserver variability and the subjective nature of traditional histopathological assessment can result in diagnostic inaccuracies and misclassifications, which in turn impact clinical decision-making [7]. AI and machine learning (ML) have emerged as promising solutions to these problems, increasing the amount of information that can be collected while decreasing workload, and increasing reproducibility of the biopsy evaluation [4]. Recent studies have demonstrated the promise of AI-based solutions in the field of kidney histopathology. In 2019, Hermsen et al. [8] proposed the first convolutional neural network (cNN) applied to kidney biopsies. By using whole slide images of stained kidney transplant biopsies, the convolutional neural network (cNN) was effectively trained to perform multi-class segmentation of kidney tissue sections. It showed excellent accuracy in tissue classification, particularly in the detection of glomeruli, and demonstrated strong associations between visually scored histological elements and network-derived measurements. This research has laid a solid foundation for AI-driven quantitative investigations in renal histopathology, facilitating the integration of deep learning into everyday diagnostics. Similarly, research conducted by Ginley et al. [9] outlined the successful application of ML and image analysis algorithms to classify biopsy samples from patients with diabetic nephropathy, demonstrating substantial concordance with classifications made by three different pathologists. This study highlighted the potential of computational methods, emphasizing that these tools can provide consistent, precise interpretation of renal biopsies, thereby improving clinical diagnostic precision and providing new insights into disease progression and prognosis.

In 2022, Kers and colleagues performed a retrospective, multicenter analysis on 5,844 kidney allograft slide images from 1948 patients. CNNs were trained to categorize biopsies as normal, rejection, or other diseases. A cross-validation and an external real-world cohort (counting 1,847 and 101 patients, respectively) have been used as validation. Results showed concordance for biopsies classified as normal (AUC 0.87 [CI 0.85–0.88]), as disease (AUC 0.87 [0.86–0.88]), as other diseases AUC (0.75 [0.72–0.77]), or as rejection (AUC 0.75 [0.73–0.76]). This study showed that deep learning-based classification of transplant biopsies could support pathological diagnostics of kidney allograft rejection [10]. Lastly, Yi et al focused on using AI to classify histological kidney abnormalities to use as indicators of graft loss [11]. More specifically, a deep learning algorithm was designed to improve prediction of renal allograft failure by developing a pipeline that accurately identifies and quantifies pathology related to interstitial fibrosis, tubular atrophy, and inflammation. Once the algorithm was trained on renal graft biopsies, the deriving digital features correlated significantly with existing scoring systems. Moreover, the Interstitial and Tubular Abnormality Score (ITAS) in baseline samples and the Composite Damage Score in post-transplant biopsies were highly predictive of graft loss, outperforming conventional scores or clinical predictors. Although promising, all of these examples of automated image analysis platforms are not yet ready for routine clinical implementation and several hurdles need to be overcome [12, 13].

Taking one step back, not using machine learning for image analysis but for automating the rules of the Banff Classification applied to individual lesion scores from pathology reports, Yoo et al. [14] respond to the demand for more reliable and uniform

classification of kidney transplant biopsies. This system utilizes an algorithm that encodes Banff 2019 classification rules. The algorithm is embedded in an accessible, user-friendly online tool that categorizes cases into the different Banff diagnostic groups. The authors compared the system's diagnostic accuracy, repeatability and efficiency with those of experienced pathologists from 20 transplant referral centers from Europe and North America. In the group of adult kidney transplant recipients, the Banff Automation System reassigned 83 of 279 cases of antibody-mediated rejection and 57 of 105 cases of T-cell-mediated rejection, applying the Banff rules strictly and thus more correctly than the expert pathologists did, possibly because, in day-to-day routine, pathologists draft their report before some key clinical information are available (DSA screening, etc.). A key finding of the study was the association between the system's correction of diagnostic inaccuracies and improved assessment of long-term risks to allograft outcomes. Based on these results the authors claimed that this system has the potential to streamline study comparisons and reduce healthcare costs by preventing misdiagnoses.

In the same line [15], provides an innovative AI-based approach to merging histological and clinical data. In this work, the authors aim to overcome the heterogeneity in the interpretation of kidney allograft biopsies by applying ML-based interpretation of pathological lesions, which is improved by combining it with clinical data. This strategy strives to shed light on clinical "cloudy" situations where pathologic condition (e.g., rejection) is never really described as "absent/present," but as a constantly changing state. 935 biopsies were read by an expert panel of pathologist and transplant physicians. The resulting ML diagnostic classifier was then put to the test on three distinct biopsy cohorts for a total of 4,693 biopsies. The ML classifier showed remarkable consistency, achieving over 90% accuracy in predicting and diagnosing T cell-mediated rejection, antibody-mediated rejection, and interstitial fibrosis-tubular atrophy. It also showed superior performance when compared to a computer-based decision algorithm that strictly adhered to the Banff rules without taking clinical context into account. Therefore, the use of AI, integrated with clinicopathological features, can significantly improve diagnostic efficiency. Notably, the classifier showed perfect accuracy in categorizing six cases previously highlighted in a Banff Working Group survey [13] involving 72 pathologists and 95 clinicians, which demonstrated that the human participants deviated from the reference diagnosis in 26% and 35% of cases for pathologists and clinicians, respectively.

Next to the evaluation of biopsies and rejection diagnosis, other applications of AI in kidney transplantation are emerging. After kidney transplantation, recipients are monitored intensively. Every transplant center faces the challenges of a rigorous follow-up with early detection of post-transplant complications and effective management of immunosuppression. In this context, AI is increasingly becoming implemented, particularly in overseeing immunosuppression regimes via pharmacokinetics analysis and in predicting recipient pharmacokinetic behavior [16–18]. The multi-faceted nature and the inter-individual variability of immunosuppression management poses significant challenges due to the need for individualized treatment plans and vigilant monitoring to prevent rejection episodes or adverse therapy effects. Also, ML algorithms trained on historical patient data can predict individual responses to different immunosuppressive drugs, helping clinicians determine the optimal dosages and combinations of these drugs. In addition, AI tools can help monitor patient adherence to medication regimens and alert clinicians to any deviations. This can be invaluable in an area where non-adherence can have devastating consequences. For example [17], developed dose prediction algorithms to forecast recipient tacrolimus dose after kidney transplantation. This study enrolled 1,045 kidney transplant patients. Different ML models [including multiple linear regression (MLR), artificial neural network (ANN), and regression tree (RT)] were applied and evaluated. Among all ML-models, the RT model showed outperformance in both cohorts [derivation cohort 0.71 (0.67–0.76); validation cohort 0.73 (0.63–0.82)]. Moreover, RT exceeded the MLR model by 4%. This frontline paper was the first to propose using ML to predict tacrolimus stable dose. More recently [19], developed ML prediction models (Xgboost) to estimate tacrolimus inter-dose AUC based on a limited number of blood concentrations and predictors. Two different cohorts of patients have been analyzed following twice-a-day and once-a-day dosing. Every model was subjected to data division, allocating 75% for the training set and 25% for the test set. Xgboost models in the training set that exhibited the lowest Root Mean Square Error (RMSE) in a ten-fold cross-validation experiment were then assessed in the test set as well as six independent full pharmacokinetic datasets from kidney, liver, and heart transplant recipients. Xgboost models demonstrated excellent AUC estimation capabilities in the test datasets, with relative bias under 5% and relative root mean square error (RMSE) below 10%. Furthermore, these models outperformed the Maximum A Posteriori (MAP) Bayesian estimation in the six independent full pharmacokinetic datasets.

Despite the promises of AI-driven follow-up after kidney transplantation, a number of hurdles must be overcome before these systems can be implemented in clinical practice. First, to minimize biases and improve generalizability, the performance of AI algorithms is heavily dependent on the quality and quantity of data used for training [15]. Second, the integration of AI systems into clinical workflows requires validation in real-world settings and consideration of ethical, legal, and regulatory aspects [20, 21]. Additionally, it is important to remember that AI-driven histological classification systems should not be viewed as a replacement for expert renal pathologists but rather as a complementary tool that can enhance their diagnostic abilities. The combination of human expertise and AI-driven approaches can lead to improved diagnostic accuracy and better-informed clinical decision-making, which will ultimately benefit patients and the broader transplantation community [22, 23].

The application of AI is still in its early stages and, aside from ethical or privacy issues, the current hype for this technology should not overshadow AI's intrinsic limitations. The scientific method is based on a six-step cycle: observe, define a question, predict, collect data, analyze data, and draw conclusions [24]. AI can only improve the prediction phase but lacks the potential to generate data or new hypotheses autonomously. In addition, in the prediction phase, AI should not simply be identified as an "oracle technology" that will correctly predict the outcome of an experiment. AI could create new correlations, but not causal links [25]. A full understanding of how the predictions of the "oracle" are arrived at is an integral part of scientific understanding, and

therefore AI should be integrated with causal inference reasoning to be fully exploited in the future. Moreover, existing AI systems that base their predictions only on associations in data are highly vulnerable to any changes in the way these variables are related [25]. That is why, at this stage, AI cannot replace the nuanced and complex decision-making skills inherent in transplant medicine.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

AP conceptualized and wrote the manuscript. MN wrote and reviewed the manuscript. OT wrote and reviewed 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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Primary Graft Function and 5Year Insulin Independence After Pancreas and Islet Transplantation for Type 1 Diabetes: A Retrospective Parallel Cohort Study

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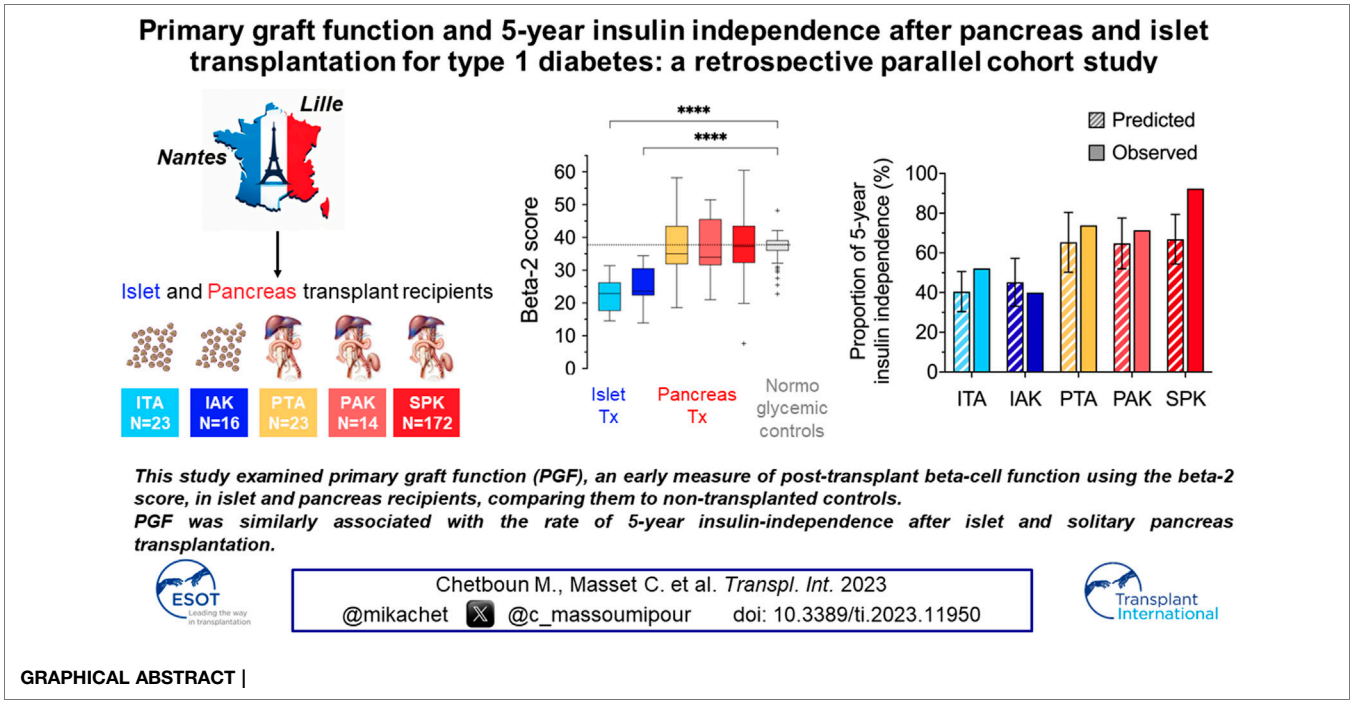
In islet transplantation (ITx), primary graft function (PGF) or beta cell function measured early after last infusion is closely associated with long term clinical outcomes. We investigated the association between PGF and 5 year insulin independence rate in ITx and pancreas transplantation (PTx) recipients. This retrospective multicenter study included type 1 diabetes patients who underwent ITx in Lille and PTx in Nantes from 2000 to 2022. PGF was assessed using the validated Beta2-score and compared to normoglycemic control subjects. Subsequently, the 5 year insulin independence rates, as predicted by a validated PGF-based model, were compared to the actual rates observed in ITx and PTx patients. The study enrolled 39 ITx (23 ITA, 16 IAK), 209 PTx recipients (23 PTA, 14 PAK, 172 SPK), and 56 normoglycemic controls. Mean[SD] PGF was lower after ITx (ITA 22.3[5.2], IAK 24.8[6.4], than after PTx (PTA 38.9[15.3], PAK 36.8[9.0], SPK 38.7[10.5]), and lower than mean beta-cell function measured in normoglycemic control: 36.6[4.3]. The insulin independence rates observed at 5 years after PTA and PAK aligned with PGF predictions, and was higher after SPK. Our results indicate a similar relation between PGF and 5 year insulin independence in ITx and solitary PTx, shedding new light on long-term transplantation outcomes.

Keywords: insulin independence, prediction, islet transplant, pancreas allograft, primary graft function

Abbreviations: PGF, Primary Graft Function; T1D, Type 1 Diabetes; PTx, Pancreas transplantation; ITx, Islet transplantation; ITA, Islet Transplantation Alone; IAK, Islet After Kidney transplantation; PTA, Pancreas Transplantation Alone; PAK, Pancreas After Kidney; SPK, Simultaneous Pancreas-Kidney.



GRAPHICAL ABSTRACT |

INTRODUCTION

Type 1 diabetes is caused by the autoimmune destruction of pancreatic beta-cells, leading to a complete deficiency of insulin secretion [1]. While exogenous insulin therapy remains the standard treatment, allogeneic transplantation of either whole pancreas organs or isolated pancreatic islets have emerged as validated therapeutic approaches in patients with severe forms of type 1 diabetes (T1D). The choice between pancreas (PTx) or islet transplantation (ITx) depends on various factors, including recipient characteristics, risk of immunosuppressive regimen and associated comorbidities [2–6].

In PTx, the vascularized transplanted organ rapidly restores endogenous insulin production, resulting in a substantial improvement in glycemic control, sustained insulin independence over years and the potential for regression of diabetic degenerative complications, including nephropathy lesions [7]. In patients with end stage renal failure, simultaneous pancreas-kidney transplant (SPK) was also linked to enhanced patient survival [8]. On the other hand, the transplantation of a vascularized pancreas requires a major surgical procedure which carries specific risks, such as bleeding, infection, and vascular thrombosis. Stringent patient selection is therefore crucial to minimize risks and ensure successful outcomes [9–11].

ITx entails only a minimally invasive procedure consisting of the infusion of few milliliters of isolated pancreatic islets into the portal vein, typically using a radiological or mini-surgical approach [3, 12–14], resulting in limited risks.

Although partial islet graft function is sufficient to suppress severe hypoglycemia [15], multiple islet infusions are often required to achieve sustained insulin independence [16–18].

Overall, PTx results in better long-term metabolic results than ITx [19–22], with the best long term outcome being reported after SPK. The reasons underlying these discrepancies are not fully elucidated. Assessing and predicting long-term graft function is an important objective for optimizing patient outcomes.

In the field of ITx, long-term graft survival has been related to the early estimate of transplanted beta-cell function, also named primary graft function (PGF) [16, 23]. A recent global study analyzing 1210 islet recipients from the international Collaborative Islet Transplant Registry [17], confirmed this tight relation between primary graft function, estimated 1 month after last islet infusion with the Beta2-score, a validated index of beta-cell function [24], and the overall 5 year success of ITx. Importantly, this association was independent of graft characteristics such as the number of islet infusions, the total transplanted islet mass, and also of the immunosuppressive regimen. These findings designate primary graft function as a robust early endpoint, which can be used to predict long-term outcomes in ITx [17]. In contrast, the evaluation of primary graft function (within first weeks after surgery) and its relation with long-term success (i.e., insulin independence) has not been explored in PTx recipients.

The primary objective of the present study was therefore to analyze and compare the potential association of primary graft function estimated soon after transplantation, and the 5 year rate of insulin independence in patients receiving an ITx and for the first time in patients receiving PTx.

PATIENTS AND METHODS

Study Design

This retrospective multicentre cohort study was designed to estimate primary graft function in patients who received beta-cell replacement with either PTx or ITx, and to analyze its relation with the rate of 5 year insulin independence. In addition, we also compared primary graft function in transplanted patients with beta-cell function estimated in non-transplanted normoglycemic individuals.

Study Population

Pancreas and Islet Transplantation Recipients

We enrolled participants from two single-center cohorts of ITx and PTx in whom all variables required to calculate primary graft function were available within weeks after transplantation, and a follow-up of at least 5 years.

PTx was performed at Nantes University Hospital between 2000 and 2022. Recipients aged from 18 to 65 years old were included if they received pancreas transplantation alone (PTA), pancreas after kidney (PAK) or simultaneous pancreas and kidney transplantation (SPK), had a functional pancreas graft, and available variables to calculate the Beta-2 score (HbA1c mostly available after the third post-operative month), and a follow-up of at least 5 years. Procurement of pancreases, for both PTx and ITx, was obtained from ABO-compatible/MHC-unmatched brain-dead deceased donors with a negative T-cell cross-match. Whole organ pancreas was transplanted following procurement (i.e., less than 12 h) using a duodeno-enteric anastomosis, either with or without Roux-en-Y. Portal or systemic venous diversion was performed. Kidney transplantation was performed according to standard surgical procedure [25]. The induction immunosuppressive strategy consisted of a T-cell depleting agent (anti-thymocyte globulin for 5 days) and TNF-alpha inhibitor Etanercept (since 2017), tacrolimus and antitiproliferative agent mycophenolate mofetil or mycophenolic acid, all at standard and recommended doses. Steroids were administered for only 7–10 days.

ITx was performed at Lille University Hospital between 2003 and 2017, as previously described [14]. Briefly, recipients were patients with C-peptide negative type 1 diabetes, aged from 18 to 65 years old who received an islet transplantation alone (ITA) or after kidney transplantation (IAK) in the context of three prospective trials (ClinicalTrials.gov Identifier: NCT00446264/NCT01123187 [16] and NCT01148680 [26]) and a follow-up of at least 5 years. Islets were isolated within 12 h following pancreas procurement and cultured for up to 72 h prior to transplantation [27]. ITx consisted of two to three sequential intraportal islet infusions within 3 months, with the aim of reaching adequate metabolic control (i.e., HbA1c ≤ 6.5% without severe hypoglycemia) without exogenous insulin. No re-transplantation was performed during the follow-up even when the patient had lost his islet graft. Access to portal vein was obtained under general anesthesia by percutaneous transhepatic catheterization of a peripheral portal branch under ultrasound guidance or by a surgical mini-invasive laparotomy with vascular approach of a proximal mesenteric vein. Heparin (35 units/kg of recipient body weight) was added to the final human islet preparation, gently infused by gravity with portal pressure monitoring as previously described [16, 23]. Participants received Interleukin-2 receptor antagonist (Daclizumab^{TM}) induction with sirolimus and tacrolimus maintenance (trials NCT00446264 and NCT01123187) [16]. Participants from trial NCT01148680 [26] received induction with TNF-alpha inhibitor (Etanercept^{TM}), T-cell depleting agent (anti-thymocyte globulin) for first infusion or with Interleukin-2 receptor antagonist for second or third infusions followed by maintenance therapy using tacrolimus and antiproliferative agents (mycophenolate mofetil).

Controlled Non-transplanted Population

In addition, we also analyzed data from normoglycemic adult individuals enrolled in two prospective cohorts (OBEDIAB, ClinicalTrials.gov Identifier: NCT00688974; and ABOS, ClinicalTrials.gov Identifier: NCT01129297), at Lille University Hospital between 2004 and 2022 for surgery. Participants with a body mass index comprised between 18 and 40 kg/m2 and normal glucose control (fasting plasma glucose <5.6 mM/L, 2 h plasma glucose <7.8 mM:L, HbA1c<5.7%), in whom the four variables required to calculate the Beta-2 score were available at the baseline visit, were included in the present study.

Data Collection

Recipient, donor and transplantation characteristics were collected in the ITx and PTx cohort prior to transplantation. Including recipient age, sex, body mass index (BMI), pre-transplant glycemic status, immunosuppressive regimens, graft characteristics. The total islet mass transplanted was expressed in islet-equivalent (i.e., one islet-equivalent corresponds to the tissue volume of one spherical islet with a diameter of 150 µm [28]). Allogeneic immunization prior to transplantation was evaluated by complement-dependent lymphocytotoxicity assay prior to 2007 and by the LABScreen Mixed Luminex flow bead assay (One Lambda^{TM}) after 2007 and preformed donor-specific antibodies (DSA) were defined as positive if minimum mean fluorescent intensity (MFI) was equal to or greater than 500 in ITx and 1000 in PTx recipients.

Study protocols were approved by the Institutional Review Board and were previously published [16, 23, 25, 26, 29]. PTx data were extracted from the French Nantes DIVAT cohort approved by the French CNIL (n°914184). The quality of the DIVAT data bank is validated by an annual cross-center audit and has been reviewed by the appropriate ethics committee in accordance with the ethical standards laid down in the Declaration of Helsinki 2000 as well as the Declaration of Istanbul 2008. The database was locked on July 1, 2023. The implementation of the database refers to the standard operating procedures established in accordance with the European Data Protection Directive (95/46/EC) and, upon its entry into force, Regulation (EU) 2016/679, also referred as the General Data Protection Regulation (GDPR), with the French CNIL concerning the processing of personal data in clinical studies. Data were de-identified before analysis in order to respect confidentiality. A signed informed consent was obtained from all ITx, PTx and OBEDIAB/ABOS patients.

Exposure of Interest

The study exposure of interest was primary graft function, an early estimation of the functional beta-cell mass after transplantation. In ITx, primary graft function was assessed as previously described, 1 month after the last islet infusion (2–6 months after first islet infusion) [16, 17]. In PTx, since HbA1c level was rarely measured before the end of the third month after surgery, primary graft function was assessed at this time period. In all cases, primary graft function was estimated with the Beta-2 score, a continuously validated variable (in which 0 represents no beta-cell function) calculated using a fasting blood sample based on values of fasting C-peptide (nmol/L), fasting blood glucose (mmol/L), HbA1c (%), and daily exogenous insulin needs per kg of body weight (IU/kg per day) [24]. In the OBEDIAB/ABOS cohort, beta-cell function was similarly estimated with the Beta-2 score using the fasting values of C-peptide, blood glucose, and HbA1c measured during a 75 g oral glucose tolerance test prior to surgery and allowed to classify the glucose tolerance disorder of each patient according to the criteria of the American Diabetes Association. In this population only normoglycemic controls were included in the present study.

Outcome

The success of transplantation was defined as insulin independence, i.e., no exogenous insulin needs for a minimum of 14 consecutive days, assessed 5 years after transplantation.

Statistical Analysis

Quantitative variables were expressed as means ± standard deviation in cases of normal distribution or medians (interquartile range, IQR) otherwise. Categorical variables were expressed as numbers (percentage). Normality of distributions was assessed using histograms and the Shapiro-Wilk test.

Pre-transplant recipient and transplantation characteristics were described for three different subgroups: ITA/IAK, PTA/PAK, and SPK. Beta-2 score and its determinants, fasting serum C-peptide, and HbA1c, were described for different subgroups in ITx, PTx recipients, and in OBEDIAB/ABOS individuals and continuous variables were compared using the One-way Welch ANOVA test. Note that only patients with a functional pancreatic graft at 3 months were analyzed in this study (per-protocol analysis, excluding patients with primary graft failure), whereas all islet-transplanted patients had a functioning graft at 1 month and were included in the analysis (intention-to-treat analysis).

For each subgroup of recipients (ITA, IAK, PTA, PAK, and SPK), we calculated the mean observed 5-year rate of insulin independence. For this analysis, only patients transplanted between 2000 and 2018 were analyzed. We estimated the mean predicted 5 year rate of insulin independence using an online calculator based on PGF [30]. As previously outlined [30], this calculator solely depending on the value of the primary graft function was constructed and validated using a cohort of islet recipients and predicts diverse outcomes validated in ITx [17].

All statistical analyses were performed using SAS Studio Statistics (version 3.81) and Prism GraphPad (Version 10.0.1) software.

RESULTS

Characteristics of the Study Population

Among 476 patients who benefited from PTx in Nantes (377 SPK/43 PAK/56 PTA), 209 recipients did not meet the inclusion criterion of the study, mainly because the lack of available HbA1c and/or C-peptide at 3 months after transplantation ($n = 207$), or because of early graft loss ($n = 60$). The individuals excluded for missing values showed no clinically relevant differences when compared to the included recipients (**Supplementary Table S1**). Baseline and transplantation characteristics of the study participants are described in **Table 1**. Of these recipients, 172 (82%) underwent SPK, 23 (11%) received PTA, and 14 (7%) received PAK transplantation.

All 39 patients who underwent ITx in Lille during the study period (16 IAK/23 ITA) were enrolled. The baseline recipient's and transplantation characteristics are provided in **Table 1**. Among them, 28 (72%) received three islet cell infusions, while 11 (28%) received two infusions. A total of 106 infusions of human islets were carried out, with recipients experiencing a median overall transplantation duration of 2.7 months (IQR 1.6–4.1). There were no further infusions conducted throughout the follow-up period. The median total islet mass transplanted was 13.6 thousand islet-equivalents per kg of body weight (IQR 11.7–15.9).

A total of 56 non-transplanted normoglycemic individuals were included in this study. Of these, 43 (77%) were women, their median age was 41 (IQR 34–48) years, and their median BMI was 37.6 (IQR 27.0–38.9) kg/m^2

Primary Graft Function

Mean[SD] primary graft function estimated with the Beta-2 score in ITA, IAK, PTA, PAK, and SPK recipients was 22.3[5.2], 24.8 [6.4], 38.9[15.3], 36.8[9.0], and 38.7[10.5], respectively. Mean beta-cell function estimated with the Beta-2 score in normoglycemic controls was 36.6[4.3]. As displayed in **Figure 1A**, the mean values of primary graft function in ITA and IAK recipients were significantly lower than the mean beta-cell function measured in normoglycemic controls ($p < 0.0001$). Conversely, the mean value of primary graft function in PTx recipients with a surviving graft and the mean beta-cell function measured at the time of enrolment in normoglycemic controls were similar.

The mean fasting C-peptide levels were significantly higher in the controls compared to ITA ($p < 0.0001$) and IAK ($p = 0.001$), but they were significantly lower compared to SPK ($p < 0.0001$) and similar to those of PTA ($p = 0.643$) and PAK ($p = 0.310$) (**Figure 1B**).

Of note, the overall HbA1c values of normoglycemic controls did not significantly differ from those in IAK, PTA, PAK and SPK recipients but were significantly lower compared to ITA ($p = 0.191$) (**Figure 1C**).

Five-Year Insulin Independence

Among the 39 islet-transplanted recipients, two never achieved insulin independence, and 22 patients (56.4%) were not insulin independent at 5 years. At the last follow-up, 32 ITx recipients out of 39 had a functional graft (serum C-peptide ≥0.3 ng/mL).

TABLE 1 | Recipient, graft and transplantation characteristics in the Islet transplantation cohort.

	ITA/IAK *n* = 39	PTA/PAK *n* = 37	SPK *n* = 172
Pre-transplantation recipient's characteristics			
Female gender, n (%)	20 (51%)	18 (49%)	63 (37%)
Age (years), mean (SD)	45 (8)	42 (±9)	40 (±7)
Body mass index (kg/m^2)	24 (±3)	25 (±4)	23 (±3)
HbA1c (%)	8.2 (±1.0)	9.4 (±2.6)	8.3 (±1.5)
Preformed donor specific antibody	1 (3%)	2 (6%)	14 (10%)
Transplantation characteristics			
Islet transplantation			
Number of islet infusions	2.7 (±0.5)		
Time between first and last infusion, months	2.7 (1.6–4.1)		
Total islet mass transplanted, 10^3 IEQ/kg of recipient weight	13.6 (11.7–15.9)		
Total tissue volume (mL)	12.9 (9.7–14.9)		
Islet purity[a] (%)	47 (44–54)		
Islet viability[a] (%)	93 (91–96)		
Pancreas transplantation			
Female donor		12 (32%)	59 (34%)
Donor age (years)		32 (±14)	33 (±11)
Donor body mass index (kg/m^2)		23 (±3)	23 (±3)
Cold ischemia time (min)		603 (±161)	658 (±177)
Immunosuppression			
T-cell depleting agent induction	11 (28%)	35 (95%)	161 (95%)
Calcineurin inhibitor	39 (100%)	37 (100%)	167 (98%)
m-TOR inhibitor	28 (72%)	37 (100%)	3 (2%)
Corticosteroid therapy	0 (0%)	32 (86%)	153 (90%)

Recipient, and transplantation characteristics are reported as n (%), mean (SD), or median (IQR) as appropriate.

[a]*The overall islet purity and viability were the weighted median (IQR) of the two or three islet infusions transplanted by the volume of each preparation. The total tissue volume was the sum of the volume of each infused preparation in the recipient.*

ITA, islet transplant alone; IAK, islet after kidney; PTA, pancreas transplant alone; PAK, pancreas after kidney; SPK, simultaneous pancreas kidney; m-TOR inhibitor, mammalian target of rapamycin inhibitor.

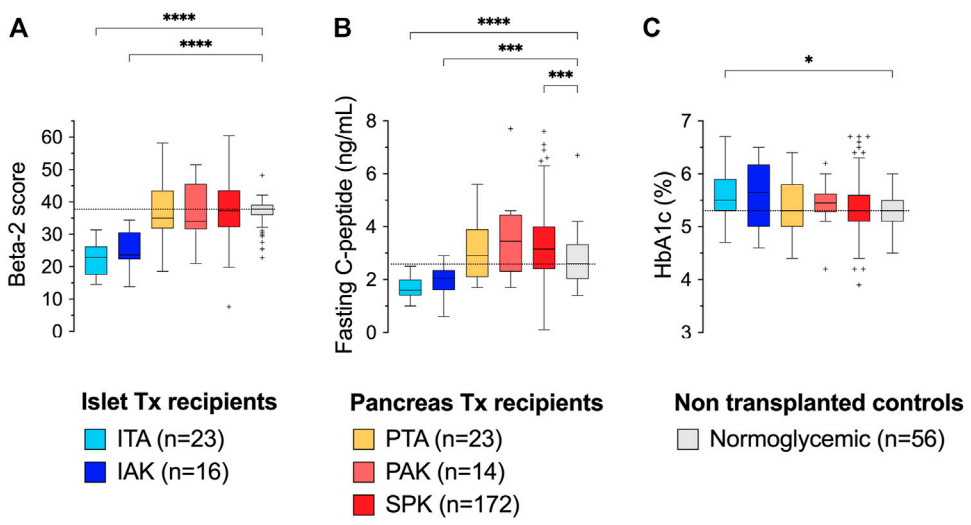


FIGURE 1 | Beta-2 score **(A)**, fasting serum C-peptide **(B)** and HbA1c **(C)** values in islet recipients, pancreas recipients and non transplanted control individual The distribution is represented in the form of a box plot using the Tukey method, where the line in the middle of the box is drawn at the median, the box limits represent the 25th and 75th percentiles, and the whisker limits are represented from the value of the 25th percentile minus 1.5 times the interquartile range (IQR) to the value of the 75th percentile plus 1.5 times the IQR. Outliers are represented individually. *p* values ≤0.001 are summarized with an asterisk. Groups were compared with Welch ANOVA tests. Symbol meaning: $p \leq 0.05$ (*); $p \leq 0.01$ (**); $p \leq 0.001$ (***); $p \leq 0.0001$ (****) ITA, Islet Transplantation Alone; IAK, Islet After Kidney transplantation; PTA, Pancreas Alone; PAK, Pancreas After Kidney; SPK, Simultaneous Pancreas-Kidney.

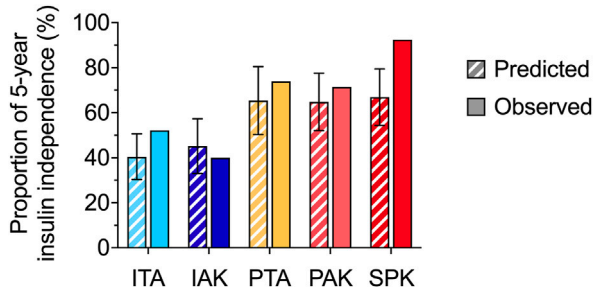


FIGURE 2 | Predicted and observed proportion of 5 years insulin independence among islet and pancreas recipient with initial graft function The mean (95% confidence interval) of predicted 5 year rate of insulin independence (hatched bar) and the observed 5 year rate of insulin independence (solid bar) are reported for the various recipient subgroups. IA, Islet Transplantation Alone; IAK, Islet After Kidney transplantation; PA, Pancreas Alone; PAK, Pancreas After Kidney; SPK, Simultaneous Pancreas-Kidney.

Among the 209 recipients who received PTx and had a functional pancreas graft at 3 months, 23 patients (11.0%) had lost insulin independence during the 5 years follow-up. Of note, 12.5% of the overall cohort of PTx recipients experienced a graft loss before 3 months and were therefore excluded from the present analysis (60 out of 476 pancreas recipients).

Relation Between Primary Graft Function and 5 Year Outcome

We used the PGF-based calculator available online [30] to estimate the mean (95% CI) proportion of patients in each subgroup with 5 year insulin independence, as illustrated in **Figure 2**, the proportion of insulin-independent patients observed at 5 years remained within the prediction confidence interval determined by the calculator in islet and solitary pancreas recipients but not in SPK. Indeed, in this subgroup of patients, the observed rate of 5 year insulin-independence was significantly higher than the rate predicted with the PGF-based calculator.

DISCUSSION

In the current study, we analyzed the early post-transplant beta-cell function, referred to as primary graft function, in islet transplantation and pancreas transplantation, and examined its relationship with the 5 year rate of insulin independence across all transplantation modalities.

Our study demonstrated that primary graft function values were comparable between ITA and IAK, as well as between PTA, PAK, and SPK. However, mean value of primary graft function was significantly lower in ITx recipients compared to PTx recipients. Primary graft function values in ITx recipients were also significantly lower than the beta-cell function observed in normoglycemic controls. In contrast, pancreas transplant recipients exhibited primary graft function values similar to beta-cell function values in normoglycemic controls.

Notably, serum C-peptide levels in normoglycemic controls were higher than in the ITA and IAK groups. However, these levels were similar to those in solitary pancreas recipients but lower than in SPK recipients. Every islet and pancreas recipient exhibited marked improvements in HbA1c levels, aligning with the American Diabetes Association's recommended targets, compared to their pre-transplant values. Additionally, mean HbA1c values were not significantly different between the various types of transplantation, except for ITA, where recipients exhibited significantly higher values.

Secondly, our findings indicated that for PTA and PAK recipients, the calculator's predictions of 5 year insulin independence rates, which were based solely on primary graft function, were relatively precise. In contrast, the calculator tended to underestimate the outcomes in SPK recipients.

These results are in line with a recent study demonstrating the independent linear association between primary graft function and various 5 year outcomes of ITx [17], including graft function, insulin independence, adequate glucose control, and overall transplantation success assessed with the Igls 2.0 criteria [31]. To our knowledge, the present study is the first to extend these results in the context of PTx. These findings indicate that the difference in long-term outcomes of PTx and ITx are likely attributable to the superior initial function of islets that survive the transplantation of a vascularized pancreas, in contrast to isolated islets infused in the portal vein. Of note, all subgroups transplanted with a vascularized pancreas had similar primary graft function. The 5 year insulin independence rate observed in patients who simultaneously received a kidney from the same donor (SPK) was, however, superior than in those who received a solitary pancreas (PTA/PAK). This difference between SPK and solitary pancreas transplant was also reported in the International Pancreas Transplant Registry and related to the reduction of immunologic graft loss [32]. In a study on SPK recipients, synchronous pancreas and kidney rejection occurred in 73%, kidney-only rejection occurred in 23% and pancreas-only rejection occurred in only 3% of biopsies [33]. Taken together, these results suggest a positive impact of monitoring kidney function for early detection and treatment of the overall allogenic immune response. Diagnosing immune rejection remains therefore challenging in solitary pancreas or islet transplantation [34].

Several limitations need to be considered when interpreting our study. First, the retrospective design of the study and the limited sample size for certain groups could have introduced selection bias. A prospective study in a larger cohort of patients could yield more robust and generalizable results. Additionally, data were collected from two parallel single center cohorts, which could introduce variations in patient selection and follow-up protocols. Multicenter studies with standardized protocols could help mitigate this potential bias and strengthen the study's findings.

Second are the method and timing used to estimate primary graft function. Several composite indexes have been proposed to estimate beta-cell function [35]. We chose here to use the Beta-2 score, a simple and continuous score validated in ITx [24]. The

use of more sophisticated tests to estimate primary graft function, such as dynamic tests of insulin secretory reserve, could have refined the prediction of long-term outcomes [36]. As previously described, primary graft function was assessed 1 month after the last islet infusion [17], which corresponds to the necessary time for full revascularization of islets transplanted in the liver [37]. In practice, this also resulted in a mean duration of 4.3 [2.8] months after the first islet infusion. The optimal timing for assessing PGF after the transplantation of a vascularized pancreas is unknown. Here, we used 3 months for this was the earliest data available in the study's participants.

Of note, PTx recipients who experienced graft failure before that date (60 cases) had to be excluded from this retrospective study, since 5 year follow-up data were not available, resulting in a twelve percent overestimation of the reported 5 year rate of insulin independence after PTx. This exclusion of early pancreatic graft failures may be debatable. However, since our main objective was to evaluate the predictive value of early beta-2 score in functional pancreas transplant recipients for long-term graft function, we assume this exclusion did not introduce bias into our study's analysis and conclusions. It is also important to note that half of the eligible pancreas transplant recipients were excluded from the analysis due to missing data. Nonetheless, as the included and excluded groups were comparable (**Supplementary Materiel**), we assume these exclusions did not introduce bias into our analysis.

Finally, it should be noted that organ allocation rules differ for islet and pancreas recipients in France and many countries. This practically favors the use of organs from donors with lower BMI and younger age in pancreas Tx. This potential selection bias, may have contributed to higher primary graft function observed in pancreas Tx recipients.

In summary, this study showed that the beta cell function restored in patients with Type 1 diabetes following islet Tx, even after multiple infusions, remains generally inferior to the levels observed in recipients of pancreas Tx and to those measured in control individuals. Our results also suggest that this difference in PGF likely explains the difference in 5 year rate of insulin independence generally observed between islet and pancreas Tx. Overall, this study suggests for the first time, a potential use of primary graft function as an early predictor of long-term outcome of PTx, principally PTA and PAK. Optimal primary graft function indicates better graft function and a higher likelihood of maintaining long-term insulin independence. However, to better understand this predictive role, further research is needed in the context of PTx. Prospective, larger scale, long-term studies remain warranted to distinguish the respective role of primary graft function and confounding factors, such as recipient allogeneic and autoimmune reactions, and the effects of immunosuppressive treatments.

In conclusion, the present study supports the value of primary graft function in the management of type 1 diabetes patients undergoing beta-cell replacement with various modalities, such as PTx, ITx, or other insulin-secreting cell transplantation.

DATA AVAILABILITY STATEMENT

Datasets presented in this article are available upon reasonable request to corresponding authors.

ETHICS STATEMENT

The studies involving humans were approved by European Data Protection Directive (95/46/EC). 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

MC, CM, DC, and FP contributed substantially to the conception and design of the study, the acquisition of data, or the analysis and interpretation. MC and CM conducted the data analysis. MC, CM, DC, and FP drafted the article. MC, CM, DC, and FP reviewed/edited the manuscript. MC, CM, MM, FD, VG, VR, TH, CB, LS, CK, GB, JB, GK, IC, AH, MG, CG, JD, KL, AJ, MH, RC, JK-C, M-CV, DC, and FP contributed to the interpretation of data and critical revision of the article. FP and DC are the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11950/full#supplementary-material

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ORIGINAL RESEARCH
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Donor Proteinuria and Allograft Function in Kidney Transplantation: Short- and Long-Term Results From a Retrospective Cohort Study

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Donor proteinuria (DP) is a common but rarely evaluated aspect of today's kidney transplant allocation process. While proteinuria after kidney transplantation is a risk factor for impaired graft function and survival, the long-term effects of DP in kidney transplantation have not yet been evaluated. Therefore, this study aims to investigate the impact of DP on the long-term outcome after kidney transplantation. A total of 587 patients were found to be eligible and were stratified into two groups: (1) those receiving a graft from a donor without proteinuria (DP–) and (2) those receiving a graft from a donor with proteinuria (DP+). At 36 months, there was no difference in the primary composite endpoint including graft loss and patient survival (log-rank test, $p = 0.377$). However, the analysis of DP+ subgroups showed a significant decrease in overall patient survival in the group with high DP ($p = 0.017$). DP did not adversely affect patient or graft survival over 36 months. Nevertheless, this work revealed a trend towards decreased overall survival of patients with severe proteinuria in the subgroup analysis. Therefore, the underlying results suggest caution in allocating kidneys from donors with high levels of proteinuria.

Keywords: kidney transplantation, patient survival, graft survival, allocation, proteinuria

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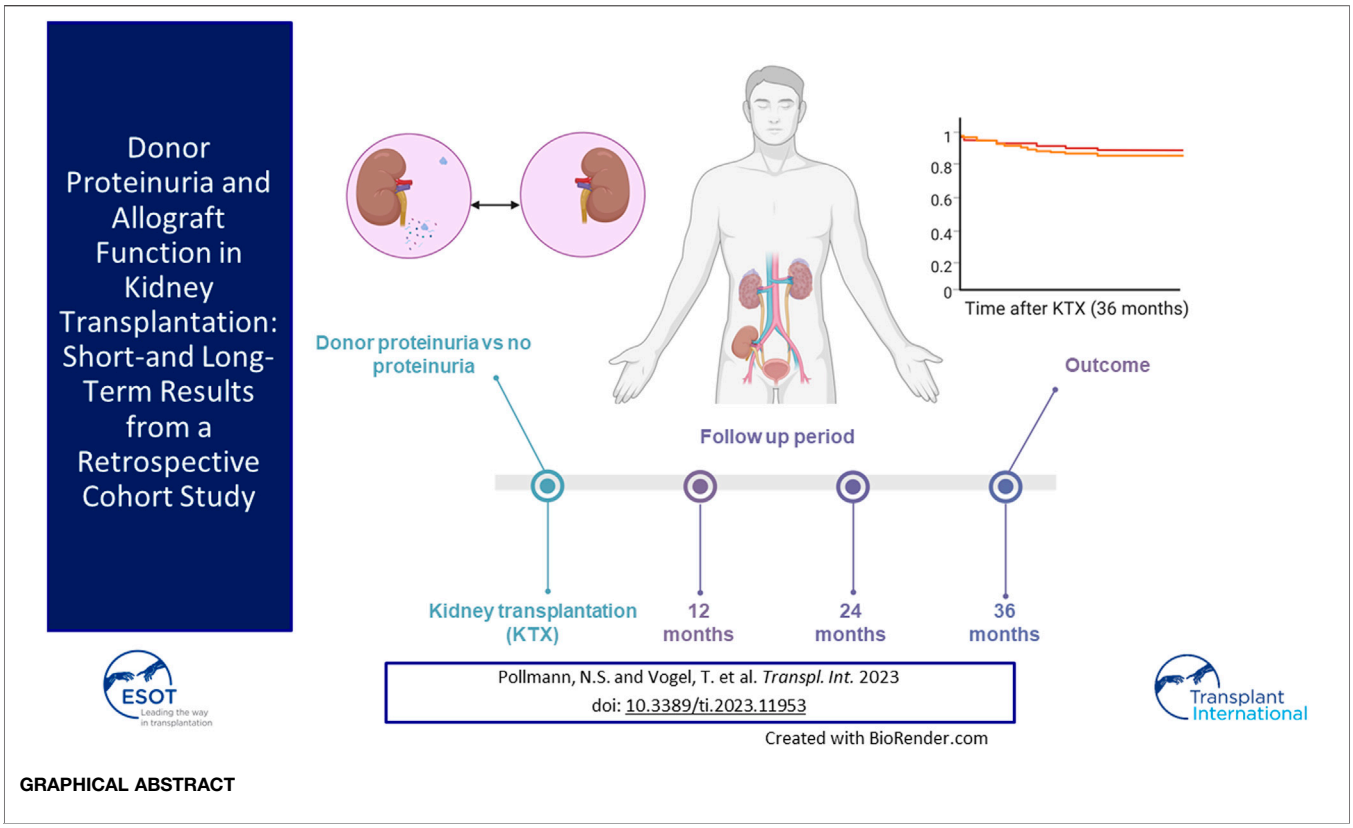
INTRODUCTION

Donor shortage remains the cardinal problem of modern transplant medicine, especially in kidney transplantation (KTX). To address this ever-growing issue, multiple approaches have been taken to increase the donor pool, but the number of patients waiting for a suitable organ still exceeds the number of potential donors. Hence, the acceptance of marginal organs continues to increase [1]. Undoubtedly, these kidneys have a higher susceptibility to ischemia-reperfusion injury, combined with an undeniable risk of inferior long-term graft function [2]. These developments highlight the importance of a patient-based allocation with the characterization of harmful and harmless donor conditions.

Proteinuria is a common diagnosis after KTX and has been identified as an independent risk factor for inferior graft function and reduced graft survival after KTX [3–5]. Proteinuria can be diagnosed by a quantitative measurement of urine albumin or protein-to-creatinine ratio, as well as by albumin or protein excretion (PE) rate. In addition, a semiquantitative measurement with



GRAPHICAL ABSTRACT

urine dipsticks can be used, as described by Kidney Disease: Improving Global Outcomes (KDIGO) and Chronic Kidney Disease (CKD) guidelines [6]. The prevalence of proteinuria in kidney transplant recipients ranges from 7.5% to 45% [7]. However, proteinuria is much more prevalent in organ donors, with low- and high-grade proteinuria occurring in 35.1% and 74.1% of allocated kidneys, respectively [8]. Yet, there are no official guidelines regarding donor proteinuria (DP) in kidney allocation, and the long-term impact of DP as an independent risk factor has not yet been validated. In consequence, DP can influence allocation decisions, with the inherent risk of declining suitable organs. This is especially important as most countries are experiencing a shortage of organ donors, resulting in long waiting times for patients on transplant lists. Therefore, declining a potentially suitable organ is negligent. On the contrary, proteinuria may indicate chronic kidney disease [9], and in kidney recipients, proteinuria is associated with reduced graft function and impaired 5 years graft survival. Patients with proteinuria have a survival rate of only 69%, compared to 93% for patients without proteinuria [10]. In kidney recipients with proteinuria, a decreased overall survival was observed compared to KTX recipients without proteinuria [11]. Additionally, patients with proteinuria have a 2.45-times increased risk of a cardiovascular event, such as ischemic heart disease, cerebrovascular disease, or peripheral vascular disease [7]. Multiple risk factors for posttransplant proteinuria have recently been defined, including a female donor, a male recipient, patients with acute rejection, donor age, and donor cardiovascular death [12, 13]. However, the effect of DP on proteinuria in the recipient has not yet been evaluated. This study aims to analyze the impact of DP on long-term (36 months) outcomes after KTX, specifically focusing on patient and graft survival.

MATERIALS AND METHODS

Study Design and Study Population

This study was conducted as a retrospective single-center cohort study with follow-up of 36 months. All patients who underwent deceased-donor KTX at the University Hospital Münster, Germany, between 2006 and 2016 were screened for inclusion. Children under the age of 18, recipients of combined organ transplants, and recipients of living donations were excluded from this study. A total of 1,122 patients were initially screened, of whom 535 were excluded due to missing donor or recipient data or meeting the exclusion criteria (**Figure 1**). The remaining 587 eligible patients who met the inclusion criteria (deceased donation, age >18, complete dataset for recipient and donor) were further stratified into two groups: (1) patients who received a graft from a donor with proteinuria (DP+) and (2) patients who received a graft from a donor without proteinuria (DP−). Additionally, a subgroup analysis of the DP+ group was conducted, including four grades of DP severity: (+), +, ++, and +++. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. The

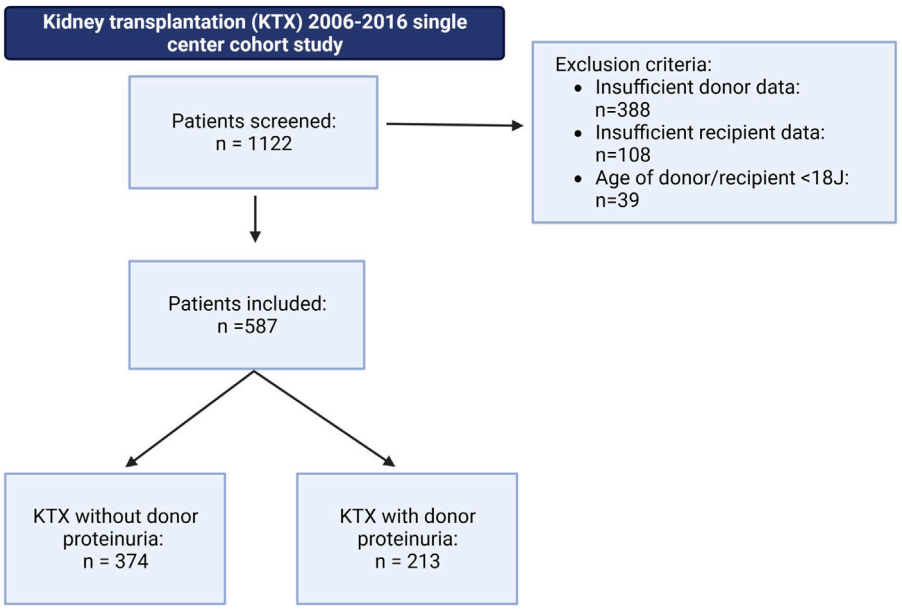


FIGURE 1 | Patient selection within the underlying retrospective cohort study, including a 36 months follow-up. A total of 587 patients met the following inclusion criteria: kidney transplantation after deceased donor donation; donor or recipient age above 18 years; and complete donor and recipient dataset. Patients were stratified into two groups: (1) patients receiving a graft from a donor with proteinuria (DP+) and (2) patients receiving a graft from a donor without proteinuria (DP–).

local ethics committee approved the study (Ethik-Kommission der Ärztekammer Westfalen-Lippe und Westfälischen Wilhelms-Universität, permit number: 2021-283-f-S). Written informed consent was not required as the study was a retrospective chart review. All data used in the final analysis were de-identified.

Patient Cohort and Outcome Parameters

All grafts were procured on behalf of Eurotransplant (ET), and only grafts procured from donors after brain death were used. Donor characteristics were obtained from the Eurotransplant Network Information System (ENIS). Donor characteristics included age, sex, body mass index (BMI), cardiopulmonary resuscitation (CPR) and duration of CPR (in minutes), presence of comorbidities (hypertension, smoking, or diabetes mellitus), ischemia time, kidney donor risk index (KDRI), and kidney donor profile index (KDPI). In addition, the donor variables included extended criteria donor (ECD) status, which was defined as age ≥60 years or 50–59 years with at least two of the following conditions: a history of hypertension, a serum creatinine (sCr) level of 1.5 mg/dL, and a cerebrovascular cause of death. Other variables considered were use of vasopressors during donor evaluation, length of stay in the intensive care unit prior to donation, highest and most recent (at time of procurement) sCr levels (in µmol/L) during donor evaluation, cytomegalovirus (CMV) status, and human leukocyte antigen (HLA) mismatch. Complete donor urine findings were analyzed, including urine leukocytes, urine epithelial cells, urine bacteria, urine casts, urine erythrocytes, urine glucose, and the presence of proteinuria, measured by semiquantitative dipstick analysis. Recipient data were collected retrospectively from a prospective clinical database. Demographic recipient variables included age, sex, dialysis vintage, history of hypertension, and the reason for end-stage kidney disease (ESKD).

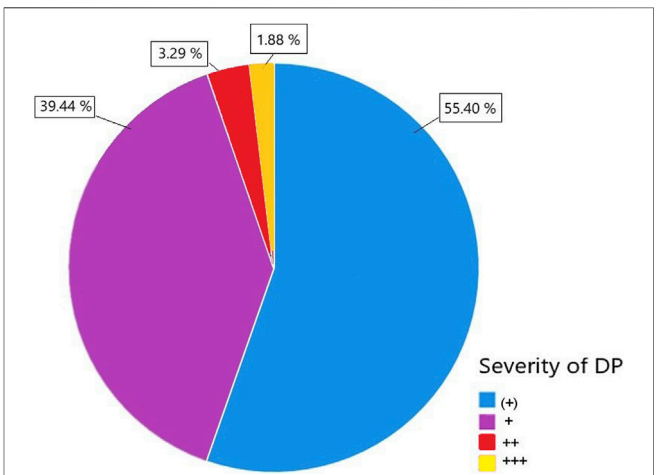


FIGURE 2 | The severity of donor proteinuria (DP) was categorized into four levels: DP $^{(+)}$, DP^{+}, DP^{++}, and DP^{+++}, specified by semiquantitative urine measurement.

Outcome Measures

Blood and urine samples were collected at various time points during the routine follow-up. Samples were taken immediately after the postoperative period, as well as at 3 (baseline), 6, 12, 24, and 36 months after KTX. The primary endpoint was a composite endpoint (event-free survival) that included graft loss and patient

TABLE 1 | Donor characteristics.

Variable	DP − *n* = 374	DP + *n* = 213	*p*-value
Age (years, mean ± SD)	54.6 ± 14.8	55.7 ± 15.3	0.401[a]
Sex (n, % males)	181 (48.4)	108 (50.7)	0.607[b]
Body mass index (kg/m^2, median (IQR))	26.0 (24.0; 28.0)	27.0 (24.0; 30.0)	**0.012**[c]
Serum Creatinine at procurement (µmol/l median (IQR))	70.70 (5.00, 97.20)	79.60 (54.80, 122.45)	**0.007**[c]
Cardiopulmonary resuscitation (n, %)	79 (21.1)	49 (23.0)	0.605[b]
Duration of cardiac arrest (min, median (IQR))	15.0 (10.0; 45.0)	20.0 (10.0; 55.0)	0.555[c]
Hypertension (n, %)	121 (32.4)	77 (36.1)	0.365[b]
Diabetes mellitus (n, %)	30 (8.0)	23 (10.8)	0.295[b]
Smoking (n, %)	144 (38.5)	89 (41.7)	0.483[b]
Cold ischemia time (h, median, (IQR))	10.1 (7.4; 13.4)	10.1 (7.5; 13.3)	0.923[c]
Warm ischemia time (min., median, (IQR))	35.0 (30.0; 40.0)	32.0 (28.0; 40.0)	0.860[c]
Kidney donor profile index (median, (IQR))	70.0 (49.0; 92.0)	72.0 (49.0 94.0)	0.128[c]
Kidney donor risk index (median, (IQR))	1.2 (1.0; 1.6)	1.2 (1.0; 1.8)	0.062[c]
Expanded criteria donors (n, %)	234 (41.7)	101 (57.7)	<**0.001**[b]
Perioperative vasopressors (n, %)	2 (0.8)	3 (0.9)	0.359[b]
Time at intensive care unit prior to donation (days, median, (IQR))	3.0 (2.0; 6.0)	3.0 (2.0; 6.5)	0.428[a]
Diuresis prior to donation (ml/h, median (IQR))	166.7 (115.9; 229.0)	129.2 (91.6; 204.0)	<**0.0001**[a]
Cytomegalovirus risk status[d]			0.534[e]
Low (*n,%*)	132 (35.3)	75 (35.2)	
Intermediate (*n, %*)	91 (24.3)	44 (20.7)	
High (*n, %*)	151 (40.4)	94 (44.1)	
Human leukocyte antigen mismatch[f]			0.837[e]
0 (n, %)	62 (16.6)	32 (15.0)	
1–3 (n, %)	197 (52.7)	117 (54.9)	
4–6 (n, %)	115 (30.7)	64 (30.0)	
Urine leukocytes (n, %)	79 (21.12)	69 (32.39)	**0.003**[b]
Urine epithelial cells (n, %)	23 (6.15)	22 (10.32)	0.076[b]
Urine bacteria (n, %)	35 (9.36)	26 (12.21)	0.325[b]
Urine casts (n, %)	10 (2.67)	17 (7.81)	**0.007**[b]
Urine erythrocytes (n, %)	127 (33.96)	110 (51.64)	<**0.001**[b]
Urine glucose (n, %)	65 (17.4)	62 (29.1)	<**0.001**[b]

Results are presented as mean ± standard deviation (SD), median with interquartile range (IQR) or relative frequency.
[a]*Student's t-test.*
[b]*Fisher's exact test.*
[c]*Mann-Whitney U test.*
[d]*Cytomegalovirus (CMV) risk status based on donor (d) and recipient (r) status low = d-/r-, intermediate: d-/r+ or d+/r+, high: d+/r-.*
[e]*Chi square test.*
[f]*Number of cumulative human leukocyte antigen mismatches.*
Significant values are highlighted bold for clarity.

survival. It was estimated using the Kaplan-Meier methodology and compared using log-rank testing. Graft loss was defined as the need to reinitiate dialysis. Secondary outcome parameters included renal function, as measured by the estimated glomerular filtration rate (eGFR; mL/h/1.73 kg^2, estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD EPI) formula)), PE per day (mg/d), and urine protein/creatinine ratio (UPCR; mg/g creatinine). Other outcome measures included primary non-function (PNF, defined as the need for continued dialysis within 90 days after KTX), DGF (defined as any need for dialysis within the first week after KTX), biopsy-proven acute rejection, new onset of diabetes after transplantation, and cardiovascular events (including myocardial infarction, angina pectoris, coronary artery revascularization, or congestive heart failure) after transplantation.

Statistical Analysis

Normally distributed continuous variables are shown as the mean with standard deviation (SD), and not normally distributed continuous variables are presented as the median with interquartile range (IQR). Groups were compared using Student's t-test for normally distributed data, Mann-Whitney U test for not normally distributed data, and Fisher's exact test for categorical variables. The Kolmogorov-Smirnov test was used to analyze the distribution of continuous variables. Recipient kidney function parameters were analyzed using a mixed model for repeated measurements. The data for the variables UPCR and PE were logarithmically transformed before analysis. Comparisons of serum and urine parameters within each group were performed using a one-way analysis of variance (ANOVA). Additionally, within each time point, the DP+ group was compared to the DP− group. All *p*-values were adjusted using the Holm-Šídák method. Results are presented as the median and a 95% confidence interval. The probability of event-free survival, which includes patient survival and the probability of graft loss, was estimated using the Kaplan-Meier methodology, and all three endpoints were compared using the log-rank test (for *p*-values ≤0.05). Cox proportional hazards regression models were fitted to determine the influence of donor variables (proteinuria, age, cold ischemia time, CPR, sCr at

TABLE 2 | Recipient characteristics.

Variable	DP − *n* = 374	DP + *n* = 213	*p*-value
Age (mean ± SD)	56.42 ± 12.39	57.00 ± 12.27	0.585[a]
Gender (n, % males)	226 (60.4)	135 (63.4)	0.537[b]
Dialysis vintage (month, median, (IQR))	66.0 (37.5; 93.5)	73.0 (43.0; 93.0)	0.403[c]
Hypertension before Transplantation (n,%)	362 (96.8)	200 (93.9)	0.113[b]
Diagnosis of end stage renal disease (n,%)			0.313[d]
Glomerulonephritis	106 (28.3)	50 (23.5)	
Diabetic nephropathy	28 (7.5)	25 (11.7)	
Hypertensive nephropathy	7 (1.9)	6 (2.8)	
Obstructive nephropathy	6 (1.6)	1 (0.5)	
Fokal segmental glomerulosklerosis	38 (10.2)	25 (11.7)	
Interstitial nephritis	14 (3.7)	8 (3.8)	
Vasculitis	12 (3.2)	3 (1.4)	
Chronic pyelonephritis	10 (2.7)	7 (3.3)	
Alport syndrome	5 (1.3)	4 (1.9)	
Autosomal dominant polycystic kidney disease 2	54 (14.4)	30 (14.1)	
Benign Nephrosclerosis	3 (0.8)	2 (0.9)	
Other	28 (7.5)	16 (7.5)	

Results are presented as mean ± standard deviation (SD), median with interquartile range (IQR) or relative frequency.
[a]*Student's t-test.*
[b]*Fisher's exact test.*
[c]*Mann-Whitney U test.*
[d]*Chi square test.*

procurement, hypertension, diabetes mellitus) on event-free survival, patient survival, graft loss, as well as reduced renal function (transformed to a dichotomous endpoint of eGFR </> 30 mL/h/1.73 kg). Hazard ratios (HR) and 95% confidence intervals (CI) were calculated. All statistical analyses and graphics were performed using IBM SPSS® Statistics 24 for Windows (IBM Corporation, Somers, NY, USA) and GraphPad Prism 10 software for Windows (GraphPad Software, CA, USA).

RESULTS

A total of 587 patients met the inclusion criteria. This cohort was further stratified based on the presence of proteinuria in the donor. Out of the total patients, 213 (36.3%) received a DP+ graft, while 374 patients (63.7%) received a DP− organ (**Figure 1**). Within the DP+ cohort, the majority had low grade proteinuria (55.4%) followed by mild grade proteinuria (39.4%). Only a small fraction had moderate proteinuria (3.3%) or high-grade proteinuria (1.9%), as indicated by semiquantitative measurement (**Figure 2**).

Both groups showed similar demographic donor characteristics (**Table 1**). However, DP− donors had a slightly higher BMI (26.0 vs. 27.0, $p = 0.012$) and higher sCr level at the time of procurement (0.760 µmol/L vs. 0.555 µmol/L, $p = 0.007$) (**Table 1**). In addition, the frequency of ECD donors was significantly higher in the DP+ cohort (57.75%) compared to the DP− cohort (41.71%) (**Table 1**) ($p < 0.001$). Moreover, DP+ donors had significantly lower diuresis during donor evaluation (DP+: 129.2 mL/h, DP−: 166.7 mL/h) ($p < 0.001$). When analyzing urine parameters, the frequency of positive findings for urine leukocytes, urine casts, urine erythrocytes, and urine glucose was significantly higher in the DP+ cohort (**Table 1**). There were no significant differences regarding baseline demographic recipient characteristics between the DP+ and DP− groups (**Table 2**).

The combined endpoint of patient and graft survival, specifically the probability of event-free survival, did not differ significantly between both groups (DP+: 83.5% event-free survival; DP−: 85.5% event-free survival; $p = 0.379$) (**Figure 3A**). This indicates that DP did not negatively affect long term outcomes after KTX. In addition, patient survival was comparable ($p = 0.124$), with 89.8% for DP+ patients and 93.3% for DP− recipients (**Figure 3B**). There was an equally low probability of graft loss in both cohorts, with 9.0% in the DP− group and 7.9% in the DP+ group ($p = 0.642$) (**Figure 3C**). Therefore, the results suggest that neither long-term patient survival, nor long-term graft loss was impaired by DP.

When analyzing post-transplant renal function, it was observed that the DP− and DP+ cohorts had similar eGFR at 3, 6, 12, 24, and 36 months after KTX (**Figure 4A**). However, longitudinal analysis within each cohort revealed a significantly higher eGFR (compared to baseline) 6 months after KTX in the DP− cohort ($p = 0.005$). Additionally, in the DP+ cohort, the eGFR at 24 months after KTX was significantly higher than at the 3 months baseline ($p = 0.010$) (**Figure 4**).

The comparison of post-transplant UPCR revealed decreasing values for both groups over time. Both the DP− and DP+ cohorts showed a significant decrease in UPCR at 12, 24, and 36 months after KTX compared to the 3 months baseline (**Figure 4B**). In addition, the overall urine PE in the DP− group was significantly lower at 6, 12, 24, and 36 months compared to the baseline at 3 months. In contrast, the DP+ group showed a significant decrease in urine PE 12 months after KTX compared to the 3 months baseline. Overall, the DP+ group showed lower values for PE and UPCR compared to the DP− group, but these differences were not statistically significant (**Figure 4C**).

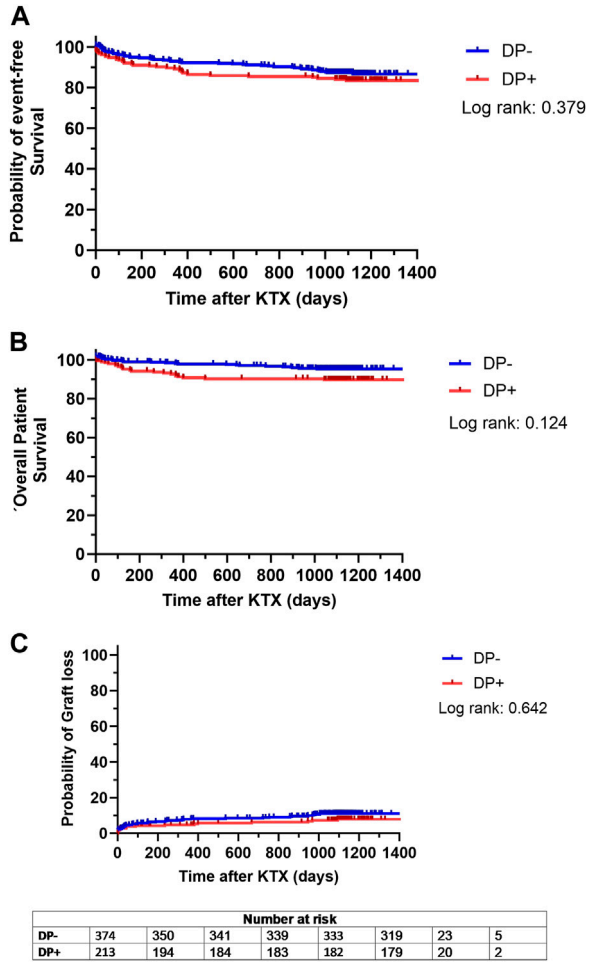


FIGURE 3 | Kaplan Meier analysis for probability of **(A)** event-free survival, defined as combined patient and graft survival, **(B)** overall patient survival and **(C)** probability of graft loss separated for patients receiving a graft from a donor with proteinuria (DP+) and patients receiving a graft from a donor without proteinuria (DP–). Survival rates of DP+ (red lines) and DP– (blue lines) recipients following kidney transplantation (KTX) were estimated by Kaplan-Meier methodology and compared by log-rank test.

Analysis of secondary endpoints showed no significant difference between the DP+ and DP– cohorts for the incidence of DGF, PNF, biopsy-proven rejection, new onset of diabetes after transplantation, or cardiovascular events after transplantation (**Table 3**).

To explore independent donor-associated risk factors, univariate and multivariate Cox regression models were used for the following endpoints: event-free survival (including patient and graft survival), patient survival, graft survival, and marginal renal function (eGFR <30 mL/h/1.73 m^2). Regarding event-free survival, both univariate and multivariate analyses showed no significant association with proteinuria, cold ischemia time, CPR, sCr at procurement, or diabetes mellitus (**Table 4**). However, donor age was found to be significantly associated with a reduced probability of event-free survival in both univariate analysis (HR: 1.05 [1.03–1.08], $p < 0.001$) and multivariate analysis (HR: 1.05 [1.03–1.08], $p < 0.001$) (**Table 4**). Donor age was also found to negatively affect patient survival (HR: 1.04 [1.01–1.06], $p = 0.002$) (**Table 5**), graft survival (HR: 1.04 [1.03–1.06], $p < 0.001$) (**Table 6**), and renal function (HR: 1.03 [1.02–1.05], $p < 0.001$) (**Table 7**), all in the multivariate analysis, respectively. In addition, hypertension was found to be associated with a reduced probability of event-free survival (HR: 1.92 [2.00–3.36], $p = 0.022$) in the univariate analysis (**Table 4**). It was also associated with reduced graft survival (HR: 1.62 [1.06–2.5], $p = 0.025$) (**Table 6**) and marginal renal function (HR: 1.60 [1.06–2.40], $p = 0.025$) (**Table 7**).

To further investigate whether the severity of DP would impact the outcome after KTX, a subgroup analysis was conducted within the DP+ group. When stratified for DP severity, the probability of event-free survival did not differ significantly among the $DP^{(+)}$, DP^{+}, and DP^{++} groups (83.7%, 84.5%, and 100%, respectively) (**Figure 5A**). However, the DP^{+++} cohort showed a tendency towards decreased event-free survival compared to the other subgroups, although this difference remained statistically insignificant (50.0%, $p = 0.151$) (**Figure 5A**). In addition, the overall patient survival of the $DP^{(+)}$, DP^{+}, and DP^{++} cohorts were comparable (89.6%, 92.4%, and 100%) (**Figure 5B**). A significant decrease in overall patient survival was observed in the DP^{+++} cohort compared to the other subgroups (50.0%, $p = 0.017$) (**Figure 5B**). The probability of graft loss was equally low in the $DP^{(+)}$, DP^{+}, DP^{++}, and DP^{+++} groups ($p = 0.709$) (**Figure 5C**).

With respect to the excretory renal parameters, a similar range was observed within the subgroups over time. The DP^{+++} cohort showed an overall trend of reduced eGFR. However, this reduction was only significant 24 months after KTX ($p < 0.0001$). In addition, PE was increased in the DP^{+++} group at three and 6 months after KTX (536.7 mg/gr Cr and median = 443.2 mg/gr Cr, respectively), but this increase did not reach statistical significance (**Supplementary Figure S1**). The analysis of UPCR showed relatively low parameters in the $DP^{(+)}$, DP^{+}, and DP^{++} groups.

DISCUSSION

Proteinuria is a well-described feature after KTX, but its impact on graft and patient outcomes remains uncertain, making it an undefined factor in the kidney allocation process. Therefore, this study investigated long-term outcomes in KTX patients, stratified based on donors with and without proteinuria. Additionally, the underlying investigation aimed to evaluate DP as a potential risk factor for post-transplant proteinuria. Proteinuria is known to be a prognostic factor for poor long-term outcomes, including reduced patient and graft survival as well as an increased risk for cardiovascular events after KTX [7]. This study established that within a 36 months post-transplant follow-up period, DP was not associated with impaired patient or graft survival or impaired graft function. Our results affirm the previous findings of Kuhn et al. [8], who demonstrated that there was no effect of DP in KTX on graft survival or function within 12 months after surgery [8].

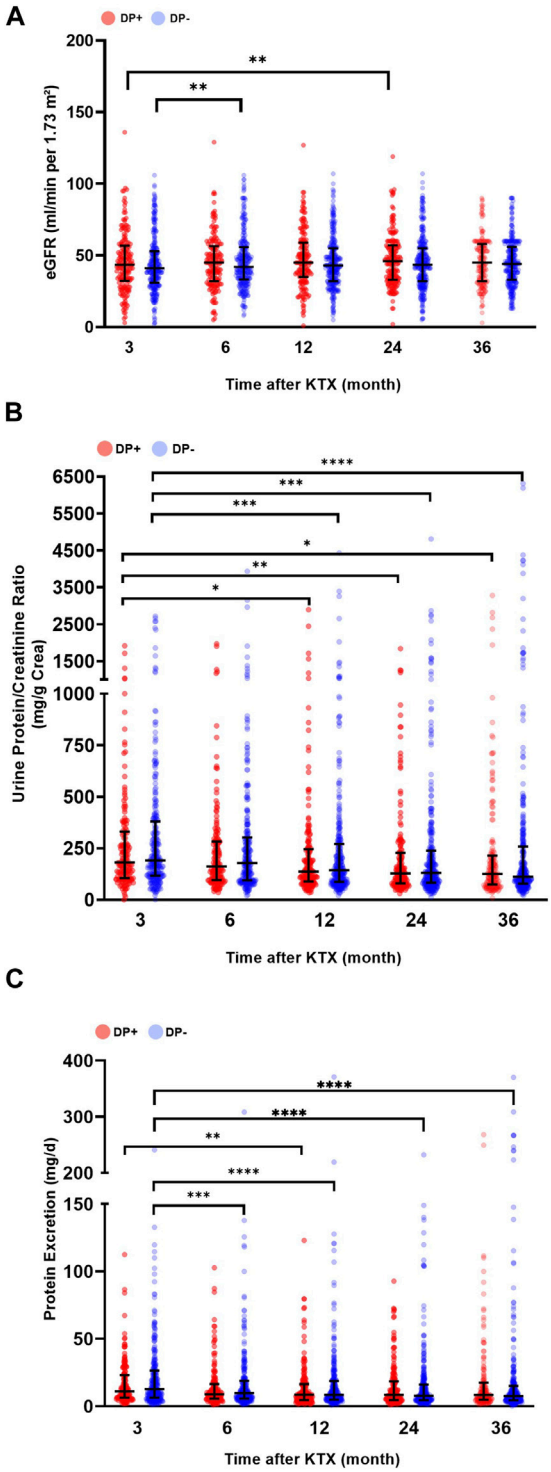


FIGURE 4 | Post-transplant graft function and urine protein excretion. Serum and urine parameters of patients receiving a graft from a donor with proteinuria (DP+) and patients receiving a graft from a donor without proteinuria (DP–). **(A)** Estimated glomerular filtration rate (eGFR mL/min/1,73 m^2), **(B)** urine protein/creatinine ratio (mg/g creatinine; UPCR), and **(C)** urine protein excretion (mg/d; PE) after kidney transplantation (KTX). Comparisons of serum and urine parameters within each group were

(*Continued*)

FIGURE 4 | performed using a one-way analysis of variance (ANOVA). The data for the variables UPCR and PE were logarithmically transformed before analysis. Within each time point, the DP+ group was compared to the DP– group. All *p*-values were adjusted using the Holm-Šídák method. A *p*-value less than 0.05 was considered statistically significant, $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$; $^{****}p \leq 0.0001$.

It has been thoroughly established that KTX is associated with improved survival, reduced morbidity, and increased quality of life when compared to long-term dialysis [14]. However, KTX is facing an ever-growing obstacle due to the declining number of donated organs. Thus, the shortage of donors demands the optimal utilization of every potentially suitable organ. Critical assessment of donor organ quality in deceased donor KTX includes evaluating urine findings. Among the challenges of analyzing urine findings in deceased donors is that pathological findings may not always indicate preexisting chronic kidney disease. This is also true for proteinuria in donors, which could be caused by trauma, intense exercise, dehydration, fever, or a urinary tract infection. At the same time, DP could be the result of a glomerular disorder, including focal segmental glomerulosclerosis, glomerulonephritis, diabetic or hypertensive nephropathy, and vasculitis [15]. Thus, when DP is included in the decision-making process of donor selection, the involved surgeons and nephrologists are at risk of either declining a suitable graft or accepting a graft with structural kidney damage. To address this dilemma, this study aimed to investigate the impact of DP on outcomes after KTX. For this purpose, 578 patients were enrolled in the study and closely monitored at our interdisciplinary KTX clinic. As this investigation was conducted at a single center, we were able to utilize nearly complete datasets for analyzing the long-term effects.

Both donors and recipients showed similar baseline demographic variables in the DP+ and DP– groups. Nevertheless, some differences were observed with less favorable features, including a higher rate of ECD grafts in the DP– donor group compared to the DP+ cohort. On the other hand, DP– donors showed a lower BMI and a higher eGFR prior to KTX compared to DP+ donors. The higher rate of ECD kidneys in the DP– cohort may have influenced the results of this study in favor of DP+ donors. However, since the KDPI and KDRI were similar in both groups and the eGFR rates were initially higher in DP– grafts, the difference may be less significant.

DP was not associated with impaired event-free survival and did not affect patient survival or the likelihood of graft loss. This demonstrates that DP did not negatively affect long term outcomes after KTX and thus, transplantation of grafts from donors with low-grade DP is safe with regard to short- and long-term outcomes. In both the univariate and multivariate analyses of donor characteristics, DP was not identified as a risk factor for any of the three defined endpoints.

As indicated by both UPCR and PE parameters, DP was not associated with post-transplant proteinuria over time after KTX. Both UPCR and PE parameters concordantly decreased within both experimental groups over the observed time. However, the increment in PE development was stronger in the DP– group compared to the DP+ group. Nevertheless, DP was not associated with high PE and UPCR values. In fact, the DP+ group showed even smaller PE and UPCR values compared to the DP– group. Therefore,

TABLE 3 | Secondary endpoints.

	DP − *n* = 374	DP + *n* = 213	*p*-value
Primary nonfunction (n, %)	21 (5.6)	11 (6.6)	0.718[a]
Delayed graft function (n, %)	87 (23.3)	52 (24.4)	0.763[a]
Biopsy proven acute rejection (n, %)	183 (48.9)	101 (47.4)	0.731[a]
New onset of diabetes after transplantation (n, %)	44 (11.8)	29 (13.6)	0.603[a]
Cardiovascular event after transplantation (n, %)	37 (9.9)	24 (11.3)	0.673[a]

Results are presented as relative frequency.
[a]*Fisher's exact test.*

TABLE 4 | Cox regression model of event-free survival.

	Univariate		Multivariate	
Donor characteristics	***p*-Value**	**HR (95% CI)**	***p*-Value**	**HR (95% CI)**
Proteinuria (yes/no)	0.651	0.87 (0.48–1.58)	0.554	0.83 (0.45–1.53)
Age (years)	<**0.001**	1.05 (1.03–1.08)	<**0.001**	1.05 (1.03–1.08)
Cold ischemia time (hours)	0.988	1.00 (0.93–1.07)	0.316	1.04 (0.97–1.11)
Cardiopulmonary resuscitation (yes/no)	0.539	0.80 (0.39–1.64)	0.846	1.08 (0.51–2.28)
Last serum creatinine (μmol/L)	0.302	1.00 (0.99–1.00)	0.364	1.00 (0.99–1.00)
Hypertension (yes/no)	**0.022**	1.92 (1.10–3.36)	0.471	1.25 (0.69–2.26)
Diabetes mellitus (yes/no)	0.063	2.05 (0.96–4.38)	0.460	1.35 (0.61–2.99)

Hazard ratios (HR) and 95% confidence intervals (CI).
Significant values are highlighted bold for clarity.

TABLE 5 | Cox regression model of patient survival.

	Univariate		Multivariate	
Donor characteristics	***p*-Value**	**HR (95% CI)**	***p*-Value**	**HR (95% CI)**
Proteinuria (yes/no)	0.130	1.57 (0.88–2.83)	0.229	1.44 (0.80–2.62)
Age (years)	**0.001**	1.04 (1.02–1.06)	**0.002**	1.04 (1.01–1.06)
Cold ischemia time (hours)	0.499	1.03 (0.95–1.10)	0.141	1.06 (0.98–1.14)
Cardiopulmonary resuscitation (yes/no)	0.296	0.65 (0.29–1.46)	0.421	0.71 (0.30–1.65)
Last serum creatinine (μmol/L)	0.327	1.00 (1.99–1.01)	0.257	1.00 (1.00–1.01)
Hypertension (yes/no)	0.063	1.74 (0.97–3.13)	0.396	1.31 (0.71–2.42)
Diabetes mellitus (yes/no)	0.552	1.33 (0.52–3.36)	0.942	0.97 (0.37–2.53)

Hazard ratios (HR) and 95% confidence intervals (CI).
Significant values are highlighted bold for clarity.

TABLE 6 | Cox regression model of graft survival.

	Univariate		Multivariate	
Donor characteristics	***p*-Value**	**HR (95% CI)**	***p*-Value**	**HR (95% CI)**
Proteinuria (yes/no)	0.378	1.21 (0.79–1.86)	0.588	1.13 (0.73–1.75)
Age (years)	<**0.001**	1.04 (1.02–1.06)	<**0.001**	1.04 (1.03–1.06)
Cold ischemia time (per hour)	0.269	1.03 (0.98–1.08)	**0.018**	1.06 (1.01–1.12)
Cardiopulmonary resuscitation (yes/no)	0.189	0.68 (0.34–1.21)	0.471	0.80 (0.44–1.46)
Last serum creatinine (μmol/L)	0.760	1.00 (1.00–1.01)	0.555	1.00 (1.00–1.01)
Hypertension (yes/no)	**0.025**	1.62 (1.06–2.48)	0.563	1.14 (0.73–1.79)
Diabetes mellitus (yes/no)	0.182	1.54 (0.82–2.90)	0.705	1.14 (0.59–2.20)

Hazard ratios (HR) and 95% confidence intervals (CI).
Significant values are highlighted bold for clarity.

this study indicated that DP was not associated with post-transplant proteinuria after KTX. The analysis of eGFR in the DP+ group over 36 months showed significantly higher values at 12 months after KTX compared to the 3 months time point.

Concordantly with findings of previous studies, donor age was associated with a higher risk of impaired overall patient survival, death-censored graft survival, and event-free survival within this investigation [16, 17]. In addition, the underlying study

TABLE 7 | Cox regression model of renal function.

Donor characteristics	Univariate		Multivariate	
	p-Value	HR (95% CI)	*p*-Value	HR (95% CI)
Proteinuria (yes/no)	0.378	1.21 (0.79–1.86)	0.588	1.13 (0.73–1.75)
Age (years)	<**0.001**	1.04 (1.02–1.06)	<**0.001**	1.04 (1.03–1.06)
Cold ischemia time (per hour)	0.269	1.03 (0.98–1.08)	**0.018**	1.06 (1.01–1.12)
Cardiopulmonary resuscitation (yes/no)	0.189	0.68 (0.39–1.21)	0.471	0.80 (0.44–1.46)
Last serum creatinine (μmol/L)	0.760	1.00 (1.00–1.00)	0.555	1.00 (1.00–1.01)
Hypertension (yes/no)	**0.025**	1.62 (1.06–2.48)	0.563	1.14 (0.73–1.79)
Diabetes mellitus (yes/no)	0.182	1.54 (0.82–2.90)	0.705	1.14 (0.59–2.20)

Hazard ratios (HR) and 95% confidence intervals (CI).
Significant values are highlighted bold for clarity.

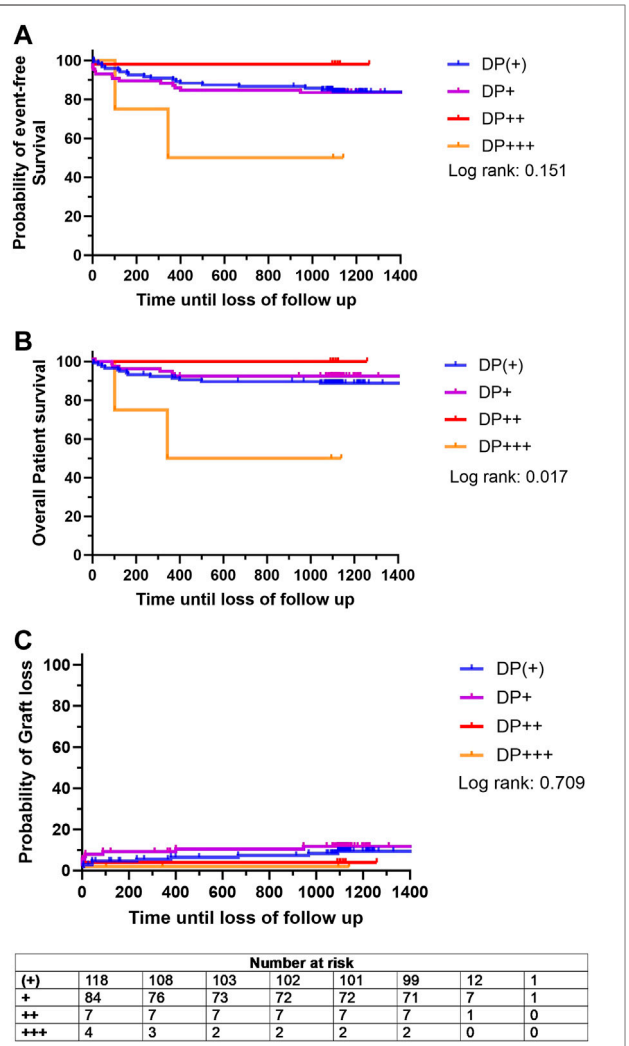


FIGURE 5 | Kaplan Meier analysis for probability of **(A)** event-free survival, defined as combined patient and graft survival, **(B)** overall patient survival and **(C)** probability of graft loss stratified based on the degree donor proteinuria (DP): DP $^{(+)}$ (green line), DP^{+} (purple line), DP^{++} (red line), and DP^{+++} (yellow line). Survival rates were estimated by Kaplan-Meier methodology and compared by log-rank test.

confirmed that donor hypertension is a risk factor for impaired graft survival and event-free survival [18, 19]. According to current literature, these results show that higher donor ages and hypertension negatively affect overall patient survival [20]. Therefore, the validity of the underlying results can be assumed.

The semiquantitative measurement of proteinuria within this study, using urine dipsticks, undoubtedly represents one main limitation of this investigation. It is important to note that with proper quantification in the donor, one could better extrapolate how a high degree of UPE would impact post-transplant graft function. Furthermore, urine dipstick measurements should be interpreted with caution because they correlate poorly with the albumin-to-creatinine ratio (ACR), have low sensitivity and specificity, and have not yet been evaluated in renoprotective randomized controlled trials [19]. On the other hand, this method is used for kidney allocation, and specialized transplant surgeons select suitable kidney grafts based on semiquantitative measurements of proteinuria. Despite its drawback, dipstick analysis correlates with end-stage renal disease and is a widely used screening parameter [19]. In addition, this study confirmed recent findings on DP in KTX, even for a long-term period of up to 36 months after transplantation. Similar to the previous investigation on DP KTX [8], the underlying study did not investigate DP as a combined risk factor in ECD kidneys. Therefore, future investigations should outline the possibility that DP could be a combined risk factor in elderly donors (e.g., ≥60 years) with diabetes and nicotine abuse. In addition, it would be helpful to correlate the degree of DP with pre-transplant or implantation biopsies to better test the hypothesis that severe DP is indicative of structural kidney damage in the donor.

Interestingly, the subgroup analysis of the DP+ group revealed a significant decrease in overall patient survival in the group with high DP ($p = 0.017$). The results indicate an adverse effect of high-grade DP on long-term patient survival and are further supported by the observation of a reduced eGFR in the DP group. As stated earlier, proteinuria after KTX is a well-known risk factor for impaired graft survival [10]. This study indicates that donors with high levels of proteinuria might have an impact on the long-term graft performance in KTX.

However, our findings suggest that low-grade DP does not imply a risk of long-term complications or an influence on graft survival.

Nevertheless, we highlight the need for further research on DP with respect to high proteinuria in quantitative urine measurements. Additionally, there is a need for further testing of donors at risk, particularly those who are older (e.g., ≥60 years) or have diabetes.

CONCLUSION

In conclusion, this retrospective cohort study of 587 patients investigated the impact of DP from a 36 months perspective. No effect on patient or graft survival was observed in low-grade DP. This indicates that transplantation of grafts from donors with low-grade DP is safe with regard to short- and long-term outcomes. Nevertheless, differences in the secondary endpoint analysis revealed a trend towards decreased patient survival and eGFR values in DP+ patients, especially in subgroups with severe proteinuria. Therefore, the underlying results suggest caution when allocating kidneys from donors with high levels of proteinuria.

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 Ethik-Kommission der Ärztekammer Westfalen-Lippe und Westfälischen Wilhelms-Universität, permit number: 2021-283-f-S. 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

NP and TV contributed equally to this work including patients collection, statistics, graphics and writing. DG, LP, SK, PH, FK, HM, SR, and AP revised the manuscript, helped with advice for statistics and graphics. FB supervised the study and provided critical feedback.

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.2023.11953/full#supplementary-material

SUPPLEMENTARY FIGURE S1 | Post-transplant graft function and urine protein excretion in patients receiving a graft from a donor with proteinuria (DP). Subgroups were stratified based on the degree of DP: (+), +, ++, and +++. **(A)** Estimated glomerular filtration rate (eGFR mL/min/1,73 m^2), **(B)** urine protein/creatinine ratio (mg/g creatinine; UPCR), and **(C)** urine protein excretion (mg/d; PE) after kidney transplantation (KTX). Comparisons of serum and urine parameters within each group were performed using a one-way analysis of variance (ANOVA). The data for the variables UPCR and PE were logarithmically transformed before analysis. All p-values were adjusted using the Holm-Šídák method. A p-value less than 0.05 was considered statistically significant, $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$; $^{****}p \leq 0.0001$.

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The Incidence of Antibody-Mediated Rejection Is Age-Related, Plateaus Late After Kidney Transplantation, and Contributes Little to Graft Loss in the Older Recipients

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It is not known whether antibody-mediated rejection (ABMR) is age-related, whether it plateaus late after transplantation, and to what extent it contributes to graft loss in older recipients. Patients transplanted between 2010 and 2015 ($n = 1{,}054$) in a single center had regular follow-up until January 2023. Recipients were divided into age groups at transplantation: 18–39 years ("young"), 40–55 years ("middle age"), and >55 years ("elderly"). Ten years after transplantation the cumulative % of recipients with ABMR was 17% in young, 15% in middle age, and 12% in elderly recipients ($p < 0.001$). The cumulative incidence of ABMR increased over time and plateaued 8–10 years after transplantation. In the elderly, with a median follow-up of 7.5 years, on average 30% of the recipients with ABMR died with a functional graft and ABMR contributed only 4% to overall graft loss in this group. These results were cross-validated in a cohort of recipients with >15 years follow-up. Multivariate cox-regression analysis showed that increasing recipient age was independently associated with decreasing risk for ABMR. In conclusion, the cumulative risk for ABMR is age-dependent, plateaus late after transplantation, and contributes little to overall graft loss in older recipients.

Keywords: kidney transplantation, age, elderly, antibody-mediated rejection, graft survival

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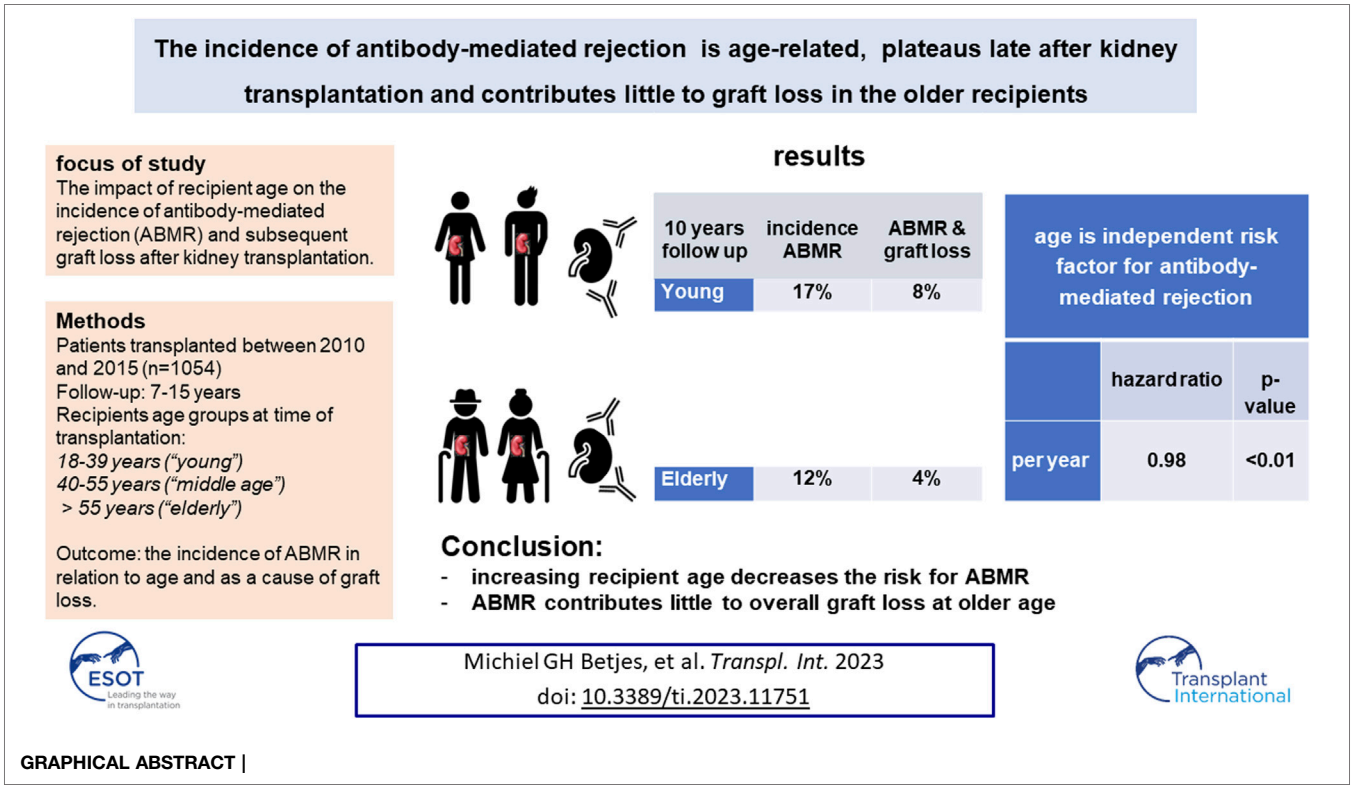
Abbreviations: ABMR, antibody-mediated rejection; CDC, complement dependent cytotoxicity; C4d, complement C4d; DSA, donor-specific antibodies; HLA, human leukocyte antigen; IFTA, interstitial fibrosis and tubular atrophy; PRA, panel reactive antibodies; TCMR, T cell-mediated rejection.



GRAPHICAL ABSTRACT |

INTRODUCTION

In recent years, the classification of causes of long-term kidney allograft loss other than death have shown a paradigm shift. Chronic allograft nephropathy [1, 2] has been replaced as a concept by redefined and regularly updated pathology criteria (Banff criteria). This includes the categories of (chronic-active) antibody-mediated rejection (ABMR) and interstitial fibrosis with tubular atrophy (IFTA), which are now recognized as major causes of graft loss across all age-categories [3]. With regard to the long-term outcome, in particular, ABMR is now recognized as a major cause of graft loss other than death [4–6].

Unfortunately, published data on histological proven causes of graft loss in the long-term are relatively scarce. Recently, we reported on biopsy results before graft failure occurred in a cohort of recipients with very long-term follow up of at least 15 years up to 24 years [7]. The results showed that death is an important competitive risk factor for (chronic-active) ABM-related graft loss and gave an indication that the cumulative incidence of c-aABMR plateaued out at around 15 years after transplantation. This finding is of interest as it mirrors the plateau in the incidence of TCMR which is usually observed 1–5 years after transplantation, depending on recipient age [8, 9]. This phenomenon is explained by the development of donor-specific hypo responsiveness (DSH) on the level of T cell alloreactivity, which is mediated by activation-induced cell death of donor-specific alloreactive T cells [10, 11]. DSH allows for a lower intensity of immune suppression in the first months after transplantation, as is part of the protocol in the majority of transplantation centers. The occurrence of a parallel DSH for ABMR could be the rationale for a change in immune suppression much longer after transplantation. In addition, increasing age is associated with decreased functionality of the immune system leading to a decreased risk for TCMR [12–15]. Whether age also decreases the risk for ABMR is not known but in our previous study a trend towards a lower cumulative incidence of ABMR in elderly recipients was observed [7]. Such a phenomenon would indicate that in this age group reduction of the immune suppression could be realized at an earlier point in time after transplantation, which will result in less side effects such as infections. As elderly recipients are the fastest growing group of kidney transplant recipients [16], it is important to have knowledge about the risk for ABMR over time after transplantation and the relative contribution to graft loss in this age group.

Given the long-term follow up of our previous study, the initial immune suppressive medication differed from the current standard with tacrolimus, MMF, and steroids, as 35% were treated with ciclosporin instead of tacrolimus and <10% received anti-CD25 induction therapy. Another difference was the relatively low number of elderly recipients (n = 230 aged >55 years). For this reason, we analyzed a larger and more recent cohort of recipients transplanted between 2010 and 2015, in order to have at least 7 years of follow-up data. The primary objective was to study the incidence of ABMR over time. We subsequently characterized age of recipient as a variable for

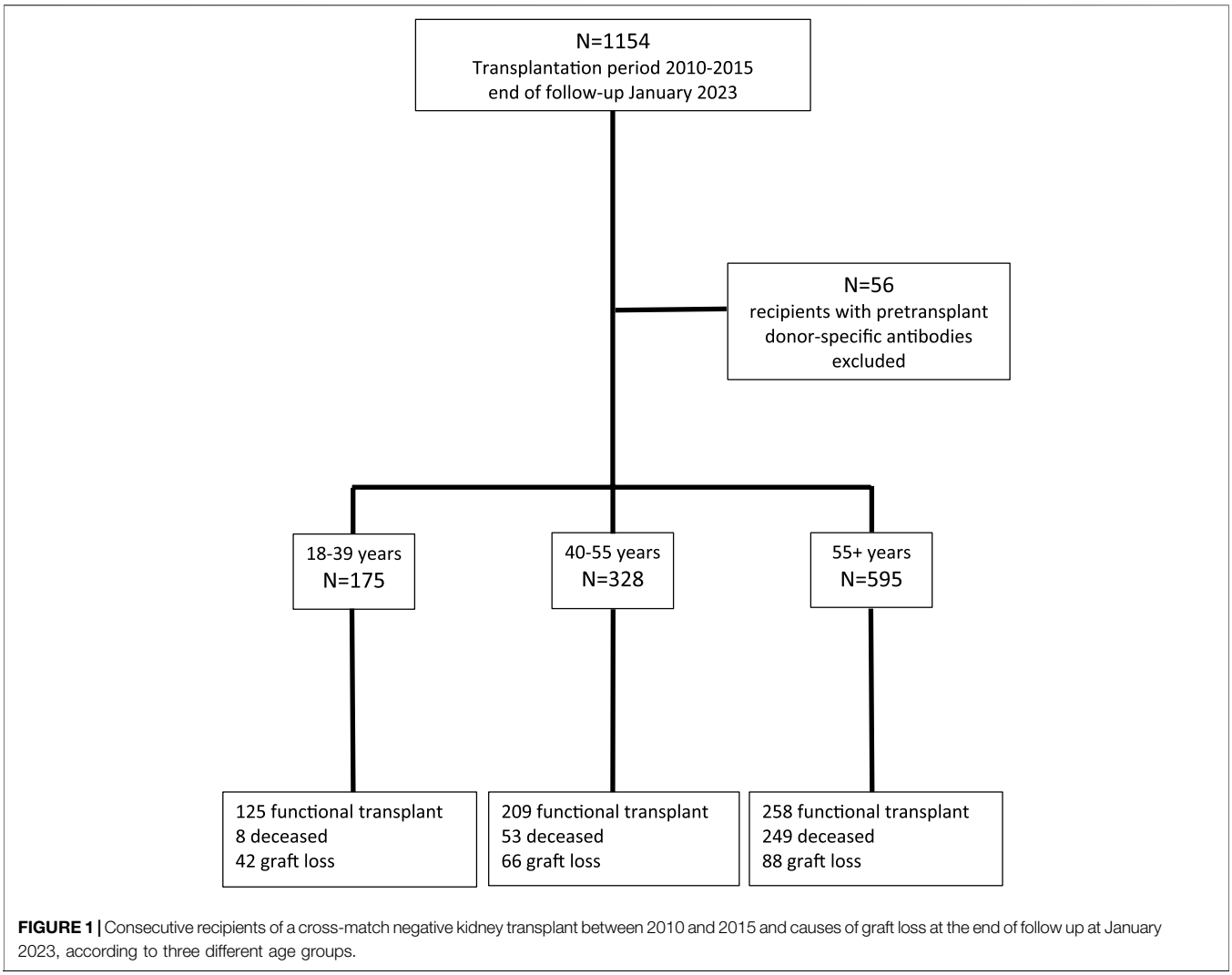


FIGURE 1 | Consecutive recipients of a cross-match negative kidney transplant between 2010 and 2015 and causes of graft loss at the end of follow up at January 2023, according to three different age groups.

ABMR incidence and the relative contribution of ABMR to overall graft loss.

PATIENTS AND METHODS

This study included all consecutive kidney transplantations performed between January 2010 and December 2015 at the Erasmus Medical Center in the Netherlands. The last follow-up date for data analysis was 1 January 2023. Recipients were seen at least once a year at our out-patient clinic and clinical data were registered in a national database (Netherlands Organ Transplant Registry). **Figure 1** shows the flow chart of patients included for analysis. The patients transplanted in the presence of HLA-specific DSA within this period have been described before and were excluded in the current analysis [17].

All other transplantations were performed with a negative complement-dependent cytotoxicity cross-match with both current and historic sera and ABO blood group-incompatibility was not an exclusion criterion. Induction therapy was basiliximab and T cell depletion by anti-thymocyte globulin or alemtuzumab was given in the minority of cases (**Table 1**). Rituximab was given in cases of ABOi transplantation in combination with IVIG and immune absorption and from 2014 onwards rituximab was replaced by alemtuzumab [18]. The standard immune suppressive medication protocol was based on tacrolimus (aiming for predose concentrations of 10–15 ng/mL in weeks 1–2, 8–12 ng/mL in weeks 3–4, and 5–10 ng/mL, thereafter) combined with mycophenolate mofetil (starting dose of 1 g b.i.d., aiming for predose concentrations of 1.5–3.0 mg/L) and glucocorticoids. All patients received 50 mg prednisolone b.i.d. intravenously on days 0–3. Thereafter, 20 mg oral prednisolone was started and subsequently tapered to 5 mg at month 3 and thereafter stopped within 3 months.

Data of the current cohort were compared with a previous cohort of kidney transplantations at our center in the period 1995–2005 which has been described in detail previously [7, 9]. For comparison with the current study cohort, only kidney transplant recipients without pre-transplant HLA-specific DSA were included (n = 573).

The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as

TABLE 1 | Kidney transplant recipients transplanted in the absence of HLA-specific DSA stratified into three age groups at time of transplantation; period January 2010—December 2015.

	"Young" 18–39 years *n* = 175	"Middle age" 40–55 years *n* = 328	"Elderly" >55 years *n* = 595
Mean age recipient in years (SD)	30.7 (6.0)	48.6 (4.4)	65.3 (5.7)
Mean age donor in years (SD)	47.7 (13.9)	51.2 (13.4)	55.4 (14.6)
Recipient male/female ratio	48/52%	47/53%	45/55%
Follow-up in years, median	9.0	8.8	7.5
Deceased/living donor kidney	22/78%	30/70%	41/59%
DBD type[a]	11%	15%	20%
DCD type[a]	11%	15%	21%
Delayed graft function	11%	17%	23%
Never functioning graft[b]	2%	3%	3%
Pre-emptive transplantation	39%	34%	32%
Cold ischaemia time in hours	4.8 ± 5.7	5.6 ± 6.1	6.8 ± 6.4
Retransplantation	27%	18%	9%
PRA[c] > 5%	13%	13%	8%
HLA mismatches (median)			
Class I	1	1	1
Class II	1	1	1
Induction therapy	90%	95%	96%
Anti-IL-2 receptor antibody	81%	86%	90%
T cell depleting antibody	5%	5%	2%
Rituximab[d]	5%	5%	4%
Initial maintenance immune suppression			
Steroids	100%	100%	100%
Tacrolimus/ciclosporin	97/1%	96/0%	97/0%
MMF/azathioprine	98/1%	98/1%	99/1%
Everolimus	0%	1%	0%

[a]*DBD, deceased by brain death; DCD, deceased by circulatory death.*
[b]*The category "never functioning graft" includes all kidney transplants that have never functioned sufficiently to allow discontinuation of dialysis.*
[c]*PRA, panel reactive antibodies (above 5% indicates the presence of cytotoxic anti-HLA antibodies in recipient's serum).*
[d]*Rituximab was given as induction therapy to blood group ABO-incompatible transplantations.*

outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism" and in accordance with the declaration of Helsinki. All patients gave written informed consent for participating in the Netherlands Organ Transplant Registry database, and for assessing additional information on DSA measurements, approval by the institutional review board of the Erasmus Medical Center (MEC-2021-0357) was obtained.

All renal biopsies were *for cause* and were performed in cases of progressive loss of graft function. No DSA surveillance or kidney biopsy protocol was in place. The initial biopsy reviews were rescored following the 2018 Banff reference guide [3]. For analysis, biopsies meeting histological criteria for ABMR with or without positive C4d staining, but without detectable DSA, were scored as ABMR for the current study as described in detail previously [19] and used in prior publications [20–22]. All cases of ABMR, as well as the late cases, were treated with pulse methylprednisolone and intravenous immunoglobulins (1–2 g/kg bodyweight) with additional plasmapheresis in early ABMR. Alemtuzumab was administered as second-line treatment in a small number of patients [19].

Identification of Anti-HLA Donor-Specific Antibodies

In the case of a positive screening, this was followed by antibody identification by SAB assay of either Lifecodes or OneLambda. For the Lifecodes SAB test, data were analyzed using MATCHIT! Antibody software version 1.3.1 (Immucor) and cut-offs were bead-specific in combination with a raw MFI of more than 750. For OneLambda, data were analyzed using HLA FUSION antibody software version 3.4.18 (One Lambda). For the SAB assay, in 56% of recipients a OneLambda kit was used and in 44% an LifeCodes kit.

Outcomes and Variables

For data analysis, the outcome of the kidney biopsy was further categorized as previously published [7] in five categories: TCMR, ABMR, recurrence kidney disease, diagnosis of *de novo* kidney disease, and interstitial fibrosis with tubulus atrophy (IFTA). In cases of graft failure, the diagnosis of the *for cause* kidney biopsy was used to categorize the type of graft failure if no other clinical event could explain the loss of kidney function.

The other graft loss categories were a clinical diagnosis of cause for graft failure and "unknown" if no biopsy was performed and a clinical diagnosis for allograft failure could not be made (**Table 2**). Primary non-function is the category of grafts that never had function after transplantation due to (histological or clinical suspected) acute tubular necrosis (ATN).

Three age groups were established based on recipient age at time of transplantation:18–39 years ("young age"), 40–55 years ("middle age"), and >55 years old ("elderly").

Statistical Analysis

Differences in patient, donor, and transplant characteristics were assessed by the Fisher's exact test for categorical variables and

TABLE 2 | Kidney allograft outcomes, according to recipient age at time of transplantation, at follow-up January 2023.

	"Young" 18–39 years *n* = 175	"Middle age" 40–55 years *n* = 328	"Elderly" >55 years *n* = 595	*p*-value
Death with functioning graft	8 (4.6%)	51 (15.5%)	250 (42.0%)	<0.001
Number of graft loss other than death	42 (24.0%)	66 (20.0%)	88 (14.8%)	<0.001
Cause of graft loss				
All rejections	27 (64.3%)	37 (56.1%)	34 (38.6%)	<0.001
TCMR[a]	13 (30.9%)	11 (16.7%)	10 (11.3%)	<0.01
ABMR[a]	14 (33.3%)	26 (39.4%)	24 (27.2%)	ns
Interstitial fibrosis/tubulus atrophy	4 (9.4%)	3 (4.5%)	8 (9.1%)	ns
Recurrence of original disease	4 (9.5%)	7 (10.6%)	10 (11.4%)	ns
Kidney injury/disease[b]	3 (7.2%)	5 (7.6%)	18 (20.4%)	ns
Peri-operative complications	3 (7.1%)	(9.1%)	5 (5.7%)	ns
Unknown	1 (2.4%)	7 (10.6%)	9 (10.2%)	ns
Primary non-function	0 (0.0%)	1 (1.5%)	4 (4.5%)	ns

[a]*TCMR, T cell-mediated rejection; ABMR, antibody-mediated rejection.*

[b]*Kidney injury/disease is the category including events or diseases causing irreversible kidney injury leading to graft loss, the category "unknown" indicates that no kidney biopsy was performed and no clinical cause of graft loss was established. ns, not significant (p > 0.05).*

Mann-Whitney U test for continuous variables. All *p*-values were 2-tailed.

Death censored graft loss and incidence of graft loss according to cause were assessed by Kaplan-Meier survival analysis with log-rank statistics for differences between strata. Univariate Cox proportional hazards analysis was used to identify clinical and demographic variables as given in **Table 1** for association with rejection and graft survival. Variables with a *p*-value of <0.1 were considered for stepwise forward regression to calculate hazard ratios and corresponding confidence intervals. Interaction terms that met statistical significance ($p < 0.05$) were included in the multivariate model. Statistical analysis was performed with software IBM SPSS statistics 21.

RESULTS

Baseline Characteristics and Graft and Recipient Survival Per Age Category

The majority of recipients are within the elderly group, with a median age of 65 years, which is in line with the general trend of increasing numbers of elderly patients receiving a kidney transplant (**Table 1**). Over 90% of recipients were treated with the current standard protocol of immune suppression consisting of anti-CD25 induction followed by triple immune suppression with tacrolimus as calcineurin inhibitor of choice. Known age-related differences in type of donor kidney (fewer living donor kidneys in the elderly), number of re-transplantations (more in the young patients) are also present in this study cohort (**Table 1**). With the growing number of elderly patients being transplanted, follow-up differed per age group and the medians were, respectively, 9.0, 8.8, and 7.5 years after transplantation for young, middle age, and elderly recipients. At last follow up in January 2023, 71.4%, 63.7%, and 43.3% of young, middle age, and elderly recipients, respectively, were alive with a functioning graft (**Figure 1**). The frequency of death with a functioning graft ranged from 4.6% in the young to 41.8% in the elderly (**Table 2**). For graft loss other than death (24.0% for young, 20% for middle age, and 14.6% for elderly recipients), rejection constituted the major cause of graft loss in every age group; 64.3% in the young, 56.1% in the middle age, and 38.6% in the elderly group (**Table 2**). The frequency of graft loss categorized as "unknown" was low in all age groups and was on average 1.2%.

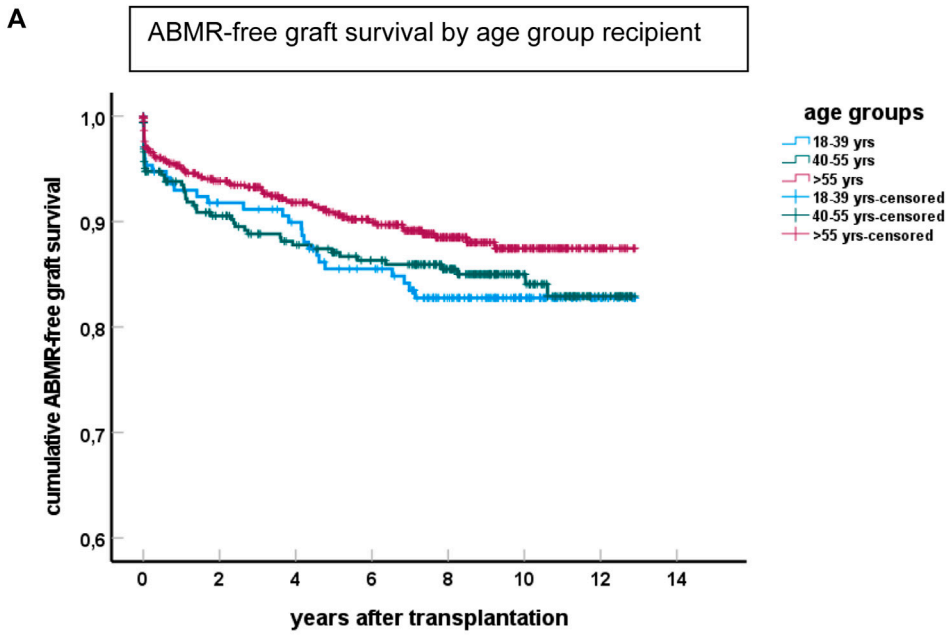
As expected [23], malignancies, infection, and cardiovascular disease constituted the three main causes of death at follow-up in all age groups with a dominance of cardiovascular disease in elderly patients (**Supplementary Table S1**).

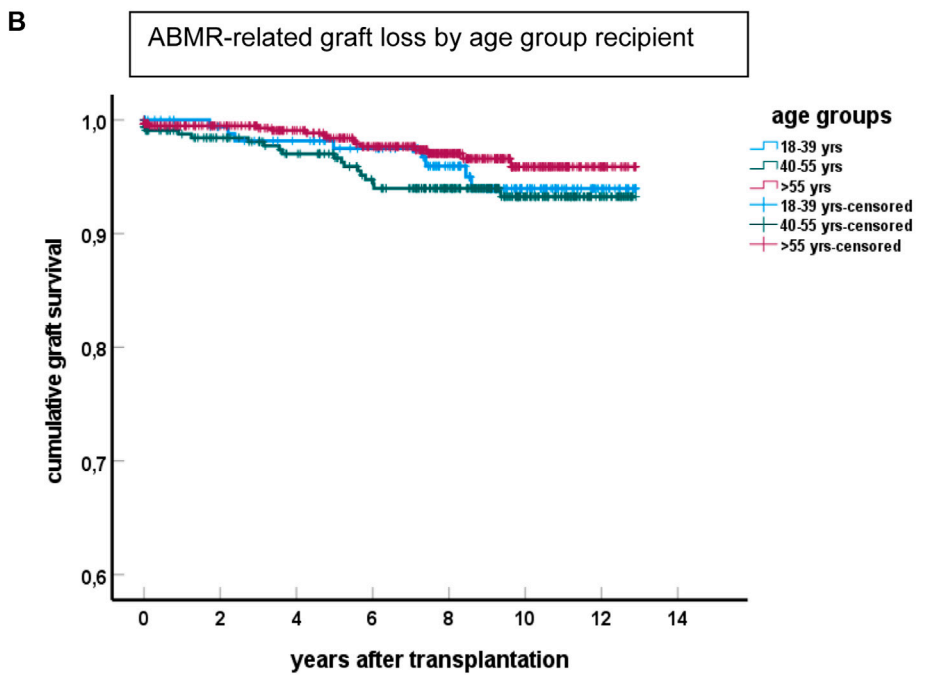
The incidence of ABMR plateaus at 10 years after transplantation and is negatively associated with age of the recipient

The total number of recipients with a diagnosis of ABMR was 135 and the cumulative risk for AMBR increased steadily until about 8–10 years after transplantation, after which only very few new cases were diagnosed. HLA-specific DSA were detected in the serum at the time of diagnosis in 53% of cases with ABMR histology (71% in the young, 49% in the middle age, and 58% in the elderly group, $p > 0.1$ for difference between groups). The % of C4d positivity in the biopsies diagnosed as ABMR was on average 43% with also no differences related to age.

The cumulative incidence of ABMR at 10 years after transplantation was 12% in the elderly, 15% in the middle age, and 17% in the young group (**Figure 2**). The cumulative incidence per 10 years of recipient age showed only a marginal difference for age groups 55–64 and 65 years or older (**Supplementary Figure S1**). The average yearly incidence of AMBR between 1 and 6 years after transplantation was 1.1%. The relationship between recipient age and ABMR incidence was confirmed by uni- and multivariate logistic regression analysis (**Table 3**). Apart from the recipient's age, the number of HLA-DR mismatches, PRA positivity, and type of donor remained significantly associated with the incidence of ABMR in multivariate Cox regression analysis.

ABMR-related graft loss was recorded in 64 cases (24 elderly recipients, of which one was related to nivolumab and lowering immune suppression because of cancer). At last follow-up, 8%, 7.9%, and 4% of young, middle age, and elderly patients,





18-39 yrs	175	161	153	141	110	60	19	0
40-55 yrs	328	292	270	245	200	99	31	0
>55 yrs	595	515	454	382	249	110	25	0

FIGURE 2 | Kaplan-Meier analysis of ABMR and graft survival for different age groups. **(A)** Shows ABMR-free graft survival ($p = 0.06$ for difference between groups) and **(B)** the survival curves for ABMR-related graft loss censored for death and other causes of graft failure. The numbers of recipients in follow-up at different time points after transplantation are shown beneath the bottom figure.

TABLE 3 | Antibody-mediated rejection after kidney transplantation: uni- and multivariate Cox regression analysis.

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	***p*-value**	**HR (95% CI)**	***p*-value**
Age recipient per year	0.98 (0.97–0.99)	0.025	0.98 (0.97–0.99)	0.009
Age donor per year	1.01 (0.99–1.01)	0.35	—	—
Kidney donor DD vs. LD[a]	1.48 (1.05–2.09)	0.024	1.61 (1.12–2.30)	0.009
Cold ischemia time in hours	1.03 (1.01–1.06)	0.007	—	—
T cell depletion induction	1.00 (1.00–1.00)	0.257	—	—
PRA positive[b]	3.08 (2.06–4.60)	<0.001	2.69 (1.76–4.12)	<0.001
HLA class I mismatches	1.20 (0.94–1.53)	0.13	—	—
HLA class II mismatches	1.29 (1.01–1.65)	0.039	—	—
Total HLA class I and II mismatches	1.11 (1.01–1.24)	0.038	1.19 (1.06–1.34)	0.002

[a]*DD, deceased donor; LD, living donor.*
[b]*PRA, panel-reactive antibodies in CDC-screening above 5%.*

respectively, had lost their graft because of ABMR ($p = 0.04$ young vs. elderly recipients). Age had no effect on the cumulative graft loss in DSA or C4d positive subgroups of ABMR (as analyzed by KM curves of the age groups and logistic regression analysis, data not shown) Subsequently, ABMR incidence and ABMR-related graft loss were compared with a previously published cohort with a minimum follow-up of 15 years [7]. The study cohort transplanted between 2010–2015 showed a relatively earlier time of diagnosis of ABMR. Incidence of ABMR plateaued at 10 years after transplantation in the more recent 2010–2015 cohort versus 15 years in the 1995–2005 cohort, with cumulative incidence of ABMR of respectively 15% and 12% at 10 years post-transplantation (**Figure 3**). ABMR-free graft survival showed a remarkable comparable pattern in the elderly in the two different study cohorts (**Supplementary Figure S1**).

The Kaplan-Meier curves for ABMR-related graft loss for the cohorts 1995–2005 and 2010–2015 practically overlapped (**Figure 3**).

Graft Survival of ABMR Cases Is Poor, But Infrequently the Cause of Graft Loss in the Elderly

Graft survival of cases with ABMR was similarly poor for both cohorts (**Figure 4**) and indicated that >80 percent of cases with ABMR will eventually lose their graft because of ABMR. No significant differences were observed among the different age groups (**Figure 4B**).

In the elderly age category, death with functioning graft is a major competing risk, and about 30% of all cases with ABMR died with a functioning graft (**Figure 5**). This is in sharp contrast to the young recipients with ABMR who will eventually all lose their graft because of ABMR, as is evident from the 1995–2005 cohort with a follow up of at least 15 years.

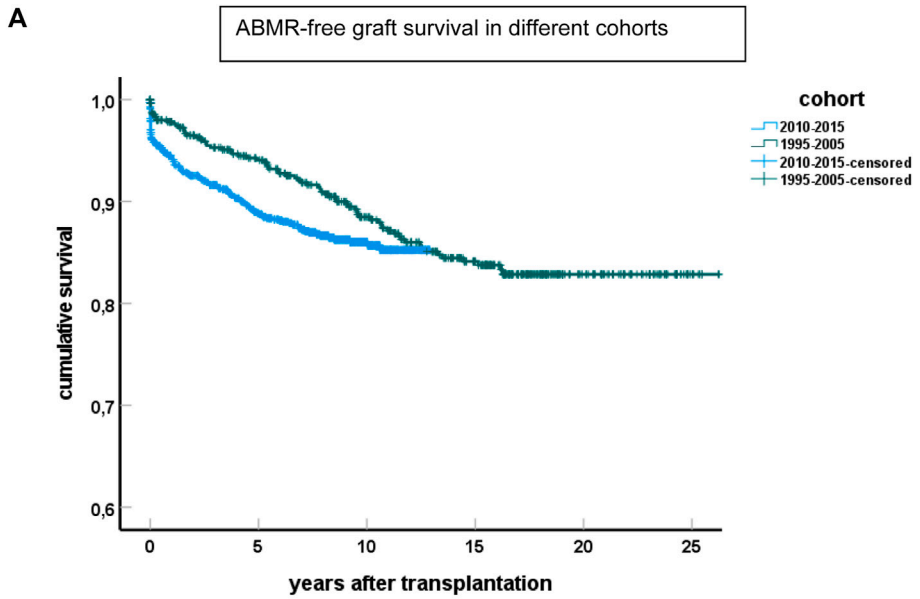
DISCUSSION

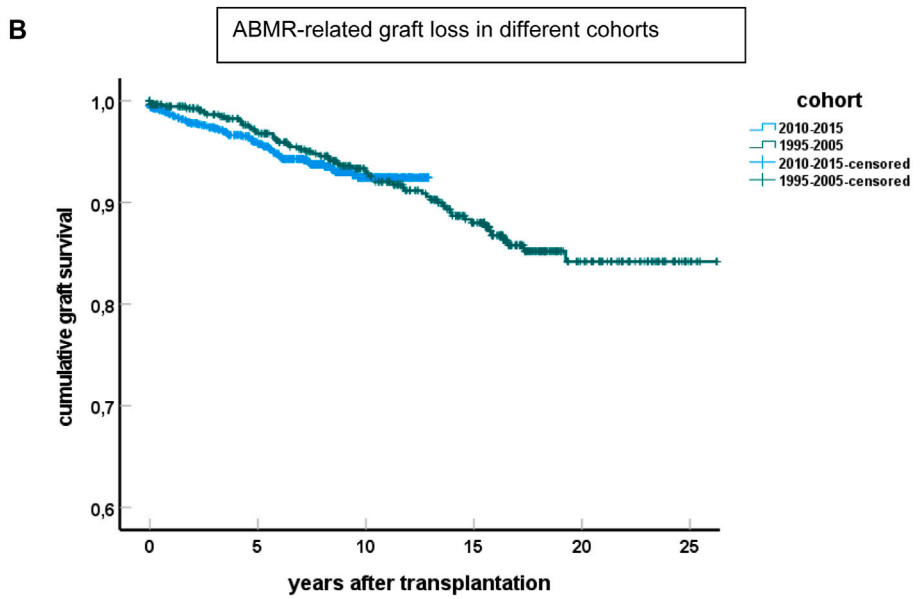
The major findings in this study are the relationship between the decreased risk for ABMR with increasing age of the recipient, the plateauing of the cumulative incidence of ABMR, and the overall small contribution of ABMR to overall graft loss in the older recipients.

The finding of the age-dependent risk of ABMR requires a large cohort of recipients followed over a long period of time after transplantation, given the relatively low yearly incidence of ABMR. Furthermore, regular follow-up of recipients and a rigorous kidney biopsy protocol should be in place to establish the reason for progressive kidney function decline. The finding of a decreased cumulative incidence of ABMR with climbing age is not unexpected, as immunological aging is related to a less vigorous immune response [24, 25]. ABMR is thought to be primarily caused by *de novo* antibody formation against donor antigens, and immunological aging leads to a decrease in antibody formation which may be due to both impaired T and B cell responsiveness [26, 27].

Given the constant exposure of allo-antigens to the immune system of the recipient, the risk of development of donor-specific antibodies seems to be continuously present. However, DSA formation shows plateauing of the cumulative incidence of serum DSA [28, 29]. This phenomenon parallels the observation in the current study that ABMR incidence also shows evidence of plateauing [28, 29]. Of interest is the observation that the curves of ABMR-free survival have shifted more to an early time of diagnosis in the recent cohort as compared to the earlier cohort from 1995 to 2005. In contrast, the curves for ABMR-related graft loss appear to be very similar between both cohorts. An explanation for these findings could be that in the last decade a kidney biopsy is being considered at an earlier stage of graft deterioration as the awareness of ABMR has increased. However, given the overlapping graft survival of cases with ABMR, it seems likely that the underlying dynamics of ABMR development for both cohorts is comparable. In addition, the graft survival of ABMR cases indicated that given time, most grafts with ABMR will fail because of ABMR after censoring for death.

In the elderly, ABMR contributed to all graft loss for only 4%. This low percentage is largely explained by death as a competitive risk factor, not only because elderly recipients die before they can develop ABMR, but also because in 30% of elderly patients with ABMR, death occurs before ABMR could have led to graft failure.





2010-2015	1098	832	271	0	0	0
1995-2005	573	458	357	259	75	9

FIGURE 3 | Kaplan-Meier analysis of ABMR and graft survival for different cohorts of recipients. **(A)** Shows ABMR-free graft survival and **(B)** the survival curves for ABMR-related graft loss censored for death and other causes of graft failure. The numbers of recipients in follow-up at different time points after transplantation are shown beneath the bottom figure.

Similar to other age categories, the cumulative incidence of ABMR in the elderly plateaus at about 8–10 years after transplantation. Similarly to TCMR, where the plateau is reached much earlier after transplantation [8], only a certain proportion of recipients will develop ABMR. Evidently, the presence of mismatches on HL-DR/DQ is an important risk factor for DSA development [30]. The mechanism by which most recipients do not develop ABMR, even after many years of exposing allogeneic HLA molecules to their immune system, has not been elucidated. Given the low contribution of ABMR-related graft loss to overall graft loss in the elderly, in combination with the current lack of precision tools to predict ABMR, it seems that modern immune suppressive drug regimens are sufficient in the majority of elderly patients to protect them from ABMR-related graft loss. However, this observation also

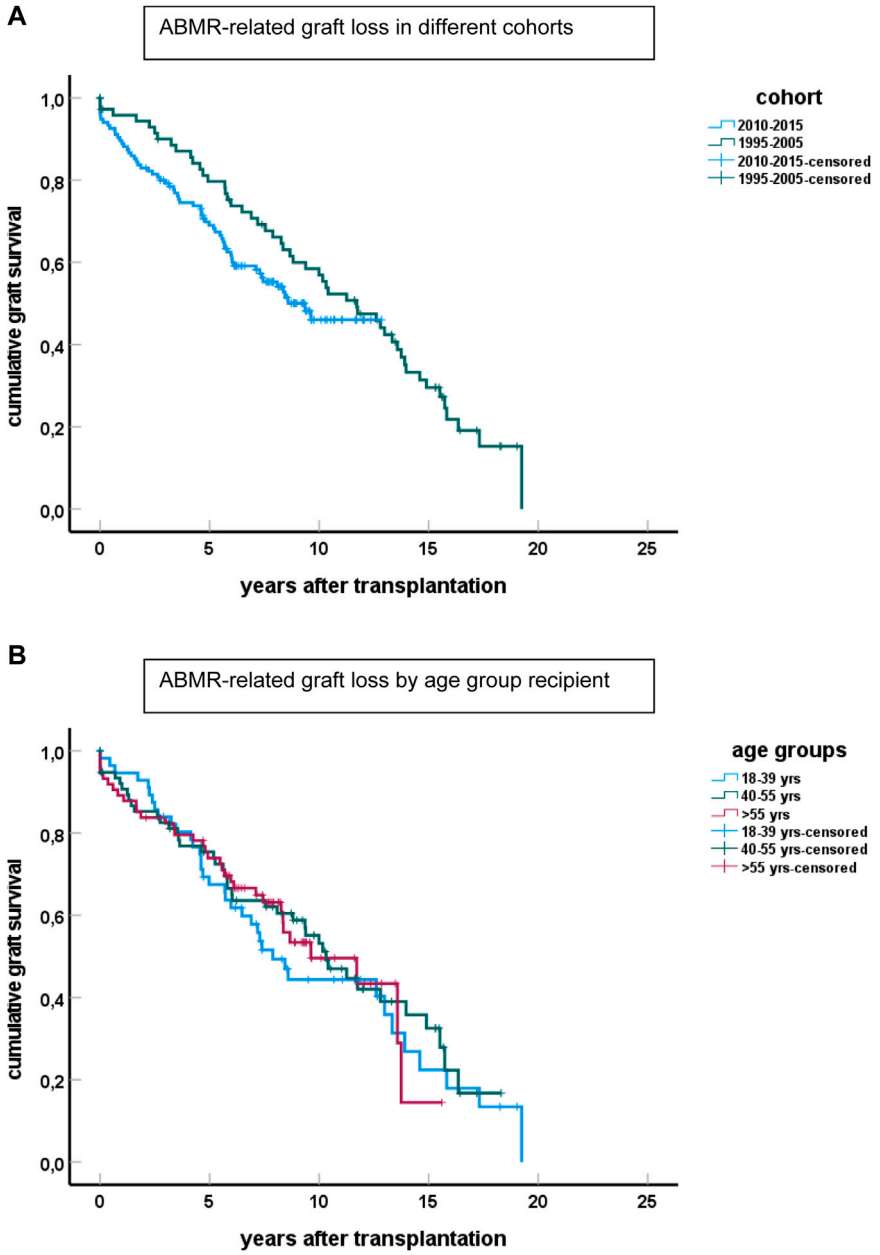


FIGURE 4 | Graft survival of cases diagnosed with ABMR censored for other causes of graft loss than ABMR for the different cohorts is shown in **(A)** ($p > 0.1$ for difference between groups). The number of cases with ABMR in the cohort 1995–2005 is 98 and in the 2010–2015 cohort 135. **(B)** Shows the graft loss because of ABMR for the cases in the different age groups, combining cases from both cohorts.

raises important clinical questions such as to what extent the intensity of the immune suppressive drugs could be lowered, specifically in the long term. Such a question is important in elderly recipients with an aged immune system, who are prone to infectious, metabolic, and cardiovascular side effects of immune suppressive medication [31–33].

In a recent study, immunological low-risk patients, of whom the majority were elderly, were randomized for continuing standard immune suppression vs. tacrolimus monotherapy at 1 year after transplantation [34]. This feasibility study showed that after discontinuation of MMF, DSA remained undetectable for at least 4 years, infections were reduced, and vaccination responses to SARS-CoV-19 were superior [35]. These data are at least encouraging as it appears feasible to lower immune suppression in selected groups of recipients without increasing the risk for ABMR. However, long term follow-up (until, at least, plateauing of ABMR has been reached) are not yet available.

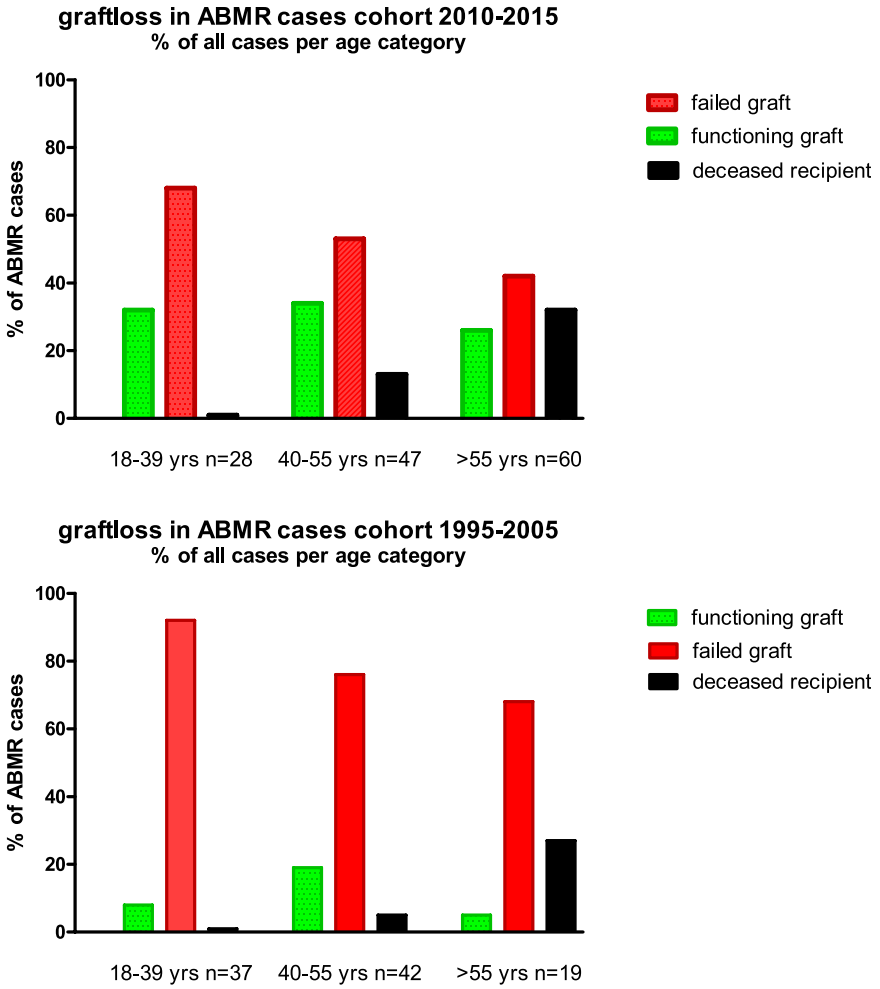


FIGURE 5 | The cause of graft failure of cases with ABMR within the different age groups and cohorts is given. The follow up for the 1995–2005 cohort is at least 15 years (maximum 24 years) and for the 2010–2015 cohort the minimum follow up is 7 years (maximum 13 years).

A potential limitation of the study is the generalizability, as treatment protocols may differ between centers and, for example, prednisone withdrawal at 3 months is not standard in all centers. In addition, the incidence of (subclinical) ABMR would likely have been higher when protocol biopsies would have been taken (for instance based on a DSA surveillance protocol). To what extent this would have changed our conclusions is unclear, especially as there is no proven effective treatment for late ABMR. Another major potential confounder is medication adherence, which may be worse in the younger recipients leading to a higher incidence of ABMR. Unfortunately, the database did not include regular measurements of immune suppressive drugs through levels or a validated medication adherence questionnaire (e.g., BAASIS) to account for this confounder. In a previous study, using the BAASIS questionnaire, no significant effect of recipient age was found on self-reported non-adherence [36].

In conclusion, the incidence of ABMR plateaus at around 10 years after kidney transplantation in an era using the modern immune suppressive medication regime. Increasing recipient age is independently associated with a lower risk for ABMR, and death is a major competitive risk factor for ABMR-related graft loss in the elderly. For these reasons, ABMR contributes relatively little to overall graft loss in the long-term in elderly recipients.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by the Erasmus Medical Center (MEC-2021-0357). 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

MB participated in research design, writing of the paper, performance of the research and in the data analysis. JK-vG participated in the performance of the research and in data analysis. JR participated in research design and in the writing of the paper. AdW participated in research design, writing of the paper, and the performance of the research.

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.2023.11751/full#supplementary-material

Supplementary Figure 1 | (A) The antibody-mediated rejection (ABMR) free graft survival in different cohorts of age at time of transplantation. The green line depicts the data from the cohort 1995-2005 and the blue line from the cohort 2010-2015. Graft loss is a censored event and shown by small crosses. **(B)** The cumulative incidence of antibody-mediated rejection (ABMR) over a period of 10 years in different cohorts of age at time of transplantation. Data derived from the 2010-2015 cohort are shown.

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ORIGINAL RESEARCH
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Morphologic and Molecular Features of Antibody-Mediated Transplant Rejection: Pivotal Role of Molecular Injury as an Independent Predictor of Renal Allograft Functional Decline

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Current knowledge about the factors correlating with functional decline and subsequent failure of kidney allografts in antibody-mediated rejection (ABMR) is limited. We conducted a cohort study involving 75 renal allograft recipients diagnosed with late ABMR occurring at least 6 months after transplantation. The study aimed to examine the correlation of molecular and histologic features with estimated glomerular filtration rate (eGFR) trajectories and death-censored graft survival. We focused on sum scores reflecting histologic ABMR activity versus chronicity and molecular scores of ABMR probability ($ABMR_{Prob}$), injury-repair response (IRRAT) and fibrosis (ciprob). In multivariable Cox analysis, a Banff lesion-based chronicity index (ci+ct+cg[x2]; hazard ratio per interquartile range [IQR]: 1.97 [95% confidence interval: 0.97 to 3.99]) and IRRAT (1.93 [0.96 to 3.89]) showed the strongest associations with graft failure. Among biopsy variables, IRRAT exhibited the highest relative variable importance and emerged as the sole independent predictor of eGFR slope (change per IQR: −4.2 [−7.8 to −0.6] mL/min/1.73 m^2/year). In contrast, morphologic chronicity associated with baseline eGFR only. We conclude that the extent of molecular injury is a robust predictor of renal function decline. Transcriptome analysis has the potential to improve outcome prediction and possibly identify modifiable injury, guiding targeted therapeutic interventions.

Keywords: antibody-mediated rejection, graft outcome, kidney transplantation, transcriptomics, transplant injury

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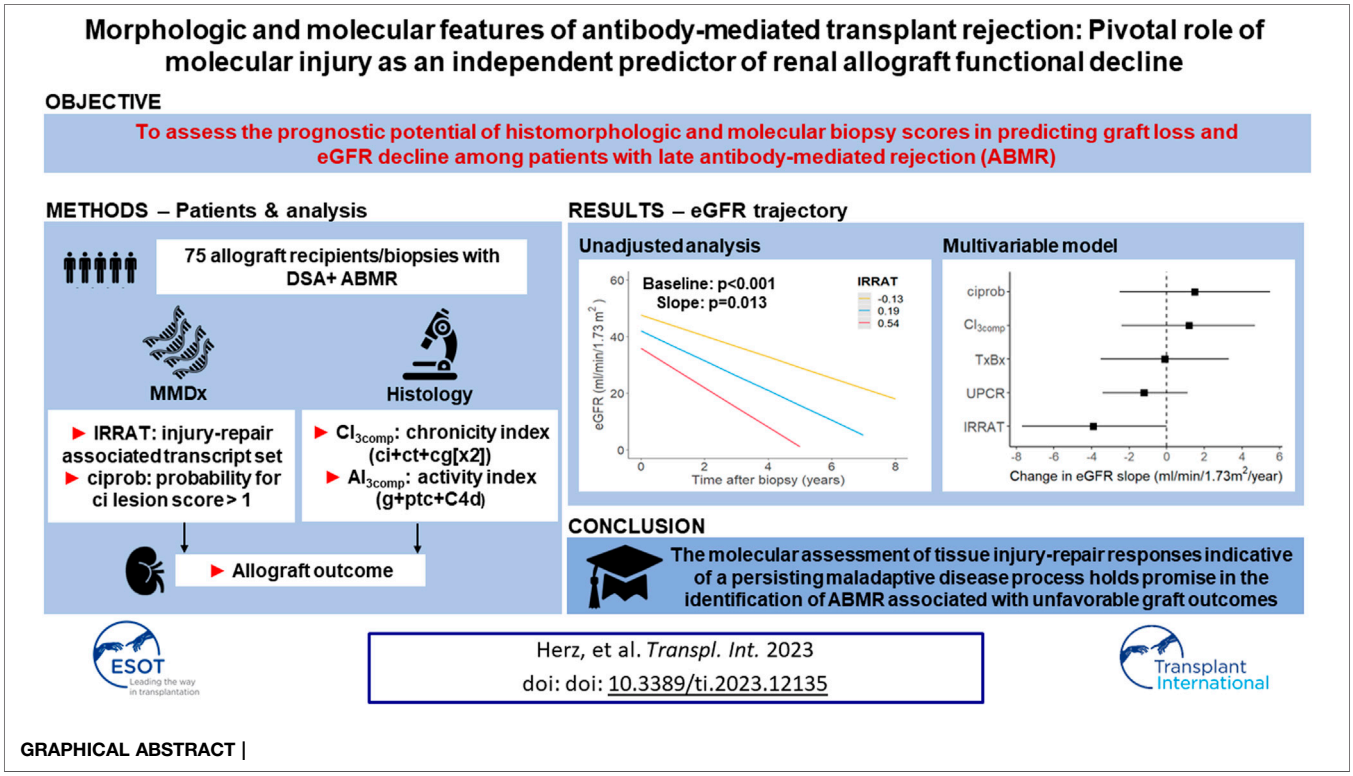
Abbreviations: ABMR, antibody-mediated rejection; $ABMR_{Prob}$, molecular classifier reflecting the probability of histologic ABMR diagnosis; AI, activity index (g+ptc+v+C4d); AI_{3comp}, simplified activity index (g+ptc+C4d); cg, glomerular double contours; CI, chronicity index (ci+ct+cv+cg[x2]); CI_{3comp}, simplified chronicity index (ci+ct+cg[x2]); ci, interstitial fibrosis; ciprob, molecular classifier reflecting the probability of histologic ci-lesion score >1; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; ct, tubular atrophy; cv, vascular fibrous intimal thickening; DCGF, death-censored graft failure; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; g, glomerulitis; IQR, interquartile range; IRRAT, injury-repair response-associated transcript; MFI, mean fluorescence intensity; MMDx, Molecular Microscope® Diagnostic System; PBT, pathogenesis-based transcript set; ptc, peritubular capillaritis; TCMR, T cell-mediated rejection; UPCR, urinary protein/creatinine ratio; v, intimal arteritis.



GRAPHICAL ABSTRACT |

INTRODUCTION

Antibody-mediated rejection (ABMR) is a cardinal cause of graft failure, characterized by a progressive decline in renal function and ultimately leading to accelerated graft loss [1–5]. Currently, there is only weak evidence supporting the effectiveness of any specific ABMR treatment [6]. According to the Banff scheme, the diagnosis of ABMR depends on certain combinations of distinct morphologic lesions, such as peritubular capillaritis (ptc), glomerulitis (g), glomerular double contours (cg), intimal arteritis (v) and/or vascular fibrous intimal thickening (cv), in conjunction with the detection of circulating donor-specific anti-HLA antibodies (DSA) and/or capillary C4d [7]. ABMR is, based on the presence or absence of features indicating rejection activity or chronic tissue injury, classified into different phenotypes, that are, active, chronic active, and chronic (inactive) ABMR [7].

For clinical practice, it would be highly beneficial to identify precise predictors of graft performance in the context of ABMR diagnosis, particularly features related to the dynamics of renal functional decline (rather than solely the estimated glomerular filtration rate [eGFR] at baseline), which could inform individualized clinical decisions and guide anti-rejection treatment. To date, however, only few studies have specifically addressed the predictive value of clinical and/or biopsy-based variables among ABMR patients. For instance, in a multicenter study involving 91 patients with chronic active ABMR, only a few factors such as study site, donor age, and HLA DSA class were found to be predictive of eGFR at the time of biopsy [4]. Surprisingly, none of the tested factors independently predicted eGFR slope, which itself emerged as a robust surrogate of graft survival [4]. Second, in a study of 70 ABMR patients conducted at our unit, graft survival was found to be associated with cg, while the only biopsy-based predictor of eGFR slope was the diagnosis of concurrent glomerulonephritis [8]. In a study of 278 patients with active ABMR, Viglietti et al. [9] established a dynamic composite prediction score integrating various factors, such as eGFR and interstitial fibrosis/tubular atrophy at the time of diagnosis, along with changes in eGFR, peritubular capillaritis, and DSA levels post-treatment. This score showed favorable calibration and discrimination, a finding that was validated in an independent cohort [9]. Finally, a recent study examining 147 ABMR cases, focusing on morphologic indices similar to those proposed for lupus nephritis [10], revealed that a chronicity index (CI) comprising cg, interstitial fibrosis (ci), tubular atrophy (ct), and cv was strongly predictive of graft survival, even independent of baseline eGFR [11]. However, a sum score incorporating a set of morphologic features reflecting ABMR activity (g, ptc, v, C4d) did not show the same impact [11]. Notably, analyses of eGFR trajectories were not included, which may be a crucial aspect to consider, as different (modifiable or non-modifiable) predictors of graft survival, may have differing impacts on eGFR intercept versus slope.

Incorporating gene expression analysis, e.g., using the Molecular Microscope® Diagnostic System (MMDx), alongside conventional histopathology, shows promising potential for enhancing outcome prediction [12]. In the INTERCOMEX

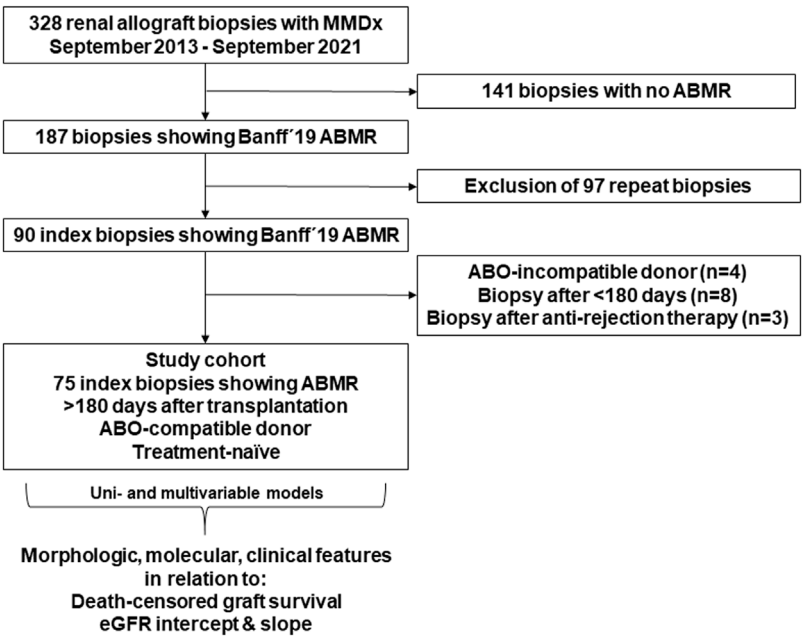


FIGURE 1 | Flowchart illustrating the selection process of the study cohort. Key inclusion criteria were the availability of complete gene expression analysis via the Molecular Microscope® Diagnostic System (MMDx), diagnosis of antibody-mediated rejection (ABMR) according to the Banff 2019 scheme, and a treatment-naïve index biopsy after >180 days post-transplantation. From a total of 328 biopsies, 75 index biopsies performed in 75 recipients were selected for the study. Morphologic, molecular, and clinical variables were analyzed in relation to death-censored graft survival and estimated glomerular filtration rate (eGFR) intercept and slope.

multicenter study, a distinct pathogenesis-based transcript (PBT) set reflecting injury-repair response (IRRAT score) demonstrated the highest predictive value for graft survival [13]. Notably, its impact was even independent of morphologic features [13]. However, rates of eGFR decline, which reflect the actual dynamics of graft deterioration, were not analyzed.

This retrospective single-center study, conducted on late DSA-positive ABMR cases using the Vienna MMDx biopsy database, aimed to analyze the relative importance and independent predictive value of biopsy features. In addition to studying graft failure as an endpoint, a distinct aspect of our present study was the examination of dynamic changes in eGFR over time to gain a deeper understanding of how individual predictors impact the progression of rejection and graft dysfunction. Specifically, the study examined features reflecting the extent of rejection activity and acute versus chronic injury, by integrating gene expression analysis with morphologic results.

MATERIALS AND METHODS

Study Design and Patients

This retrospective cohort study conducted at the transplant unit of the Medical University of Vienna included 75 recipients of an ABO-compatible renal allograft diagnosed with ABMR >180 days after transplantation. Study patients were selected from a cohort of 195 consecutive recipients who underwent at least one biopsy between September 2013 and September 2021, and for whom gene expression analysis via the MMDx platform was available (**Figure 1**). Baseline variables are provided in **Tables 1**, **2**. For survival analysis, patient records were reviewed until March 2023. In addition, eGFR trajectories were determined by analyzing every creatinine measurement between 30 days before the biopsy and either death-censored graft failure (DCGF) or loss to follow-up. The study was approved by the institutional review board of the Medical University Vienna (approval number: 1451/2023).

Biopsies

A total of 75 allograft biopsies were included in the study, performed for graft dysfunction, proteinuria and/or a positive DSA result. Morphologic analysis was performed on formalin-fixed paraffin-embedded sections. Single lesions and rejection phenotypes were scored and classified according to the Banff 2019 scheme [7]. We used published algorithms to calculate morphologic sum scores reflecting ABMR activity (activity index [AI]: g+ptc+v+C4d) and chronic injury (chronicity index [CI]: ci+ct+cv+[cgx2]) [11]. In 12 biopsies, the absence of arteries made it impossible to score vascular lesions. To ensure a larger sample size for statistical analysis, we simplified AI and CI (AI_{3comp}; CI_{3comp}) by excluding v and cv scores, respectively. However, for three biopsies, these indices could not be calculated due to an insufficient number of glomeruli for valid g and cg scoring. Notably, none of the biopsies showed significant v lesions, so v was not included individually in statistical models.

All index biopsies underwent gene expression analysis via the MMDx platform (ATAGC, University of Alberta, Edmonton, AB,

TABLE 1 | Baseline variables.

Variables	Study patients *n* = 75	Data available (n)
Variables recorded at the time of transplantation		
Recipient age, median (IQR)	45 (34.5–53.5)	75
Female sex, n (%)	37 (49.3)	75
Deceased donor, n (%)	63 (84.0)	75
Living donor, n (%)	12 (16.0)	75
Donor age (years), median (IQR)	46 (26–58)	73
Prior kidney transplant, n (%)	27 (37.0)	73
Current CDC panel reactivity ≥10%, n (%)	15 (21.4)	70
Preformed anti-HLA DSA, n (%)[a]	25 (62.5)	40
Cold ischemia time (hours), median (IQR)	12 (8.1–17.1)	71
HLA mismatch (A, B, DR), median (IQR)	3 (2–4)	73
HLA mismatch (A, B, C, DRB1, DQB1), median (IQR)	5 (4–6)	58
Initial immunosuppression		75
Induction with anti-thymocyte globulin, n (%)	31 (41.3)	75
Induction with IL-2 receptor antibody, n (%)	19 (25.3)	75
Tacrolimus-based immunosuppression, n (%)	44 (58.7)	75
Cyclosporine A-based immunosuppression, n (%)	29 (38.7)	75
mTOR inhibitor-based immunosuppression, n (%)	5 (6.7)	75
Peri-transplant immunoadsorption, n (%)	27 (36)	75
Variables recorded at the time of index biopsy		
Years after transplantation, median (IQR)	5.17 (2.41–13.21)	75
Renal parameters		
eGFR (mL/min/1.73 m^2), median (IQR)	39.9 (26.7–59.6)	75
UPCR (mg/g), median (IQR)	373 (134–1252)	75
UPCR >1,000 mg/g, n (%)	23 (30.7)	75
Immunosuppression at the time of index biopsy		
Triple immunosuppression (%)	60 (80.0)	75
Tacrolimus, n (%)	51 (68.0)	75
Cyclosporine A, n (%)	20 (26.7)	75
mTOR inhibitor, n (%)	3 (4.0)	75
Belatacept, n (%)	1 (1.3)	75
Anti-rejection therapy triggered by ABMR diagnosis, n (%)	42 (56.0)	75
Bortezomib	20 (26.7)	75
Clazakizumab	15 (20.0)	75
BIVV009	9 (12.0)	75
Tocilizumab	2 (2.7)	75
Imlifidase/IVIG/rituximab	1 (1.3)	75
Immunoadsorption	1 (1.3)	75

DSA, donor-specific antibody; CDC, complement-dependent cytotoxicity; eGFR, estimated glomerular filtration rate; IQR, interquartile range; mTOR, mammalian target of rapamycin; UPCR, urinary protein/creatinine ratio.

[a]*For recipients transplanted before 2009, solid-phase HLA antibody screening on the transplant wait list was not available.*

Canada) [12]. Approximately 3 mm portions of one core from each biopsy underwent microarrays. Molecular scores were generated based on lesion-based transcript sets associated with rejection types [ABMR, T cell-mediated rejection (TCMR), "all Rejection"] and transcript sets related to injury-repair response (IRRAT score) or the probability of a histologic ci-lesion score >1 (ciprob score). Rejection archetypes were generated as described previously. The algorithms utilized a reference set of 1529 biopsies [14].

HLA Antibody Detection

For HLA antibody detection, we utilized LABscreen Single Antigen assays (One Lambda, a brand of Thermo Fisher Scientific, Canoga Park, CA, USA). Serum samples were treated with ethylenediaminetetraacetic acid or subjected to heat inactivation to prevent complement interference [15]. The presence of DSA (mean fluorescence intensity [MFI] threshold >1,000) was determined according to serological and/or low- or high-resolution donor/recipient HLA typing (HLA-A, -B, -Cw, -DR, -DQ and/or DP).

Immunosuppression

Out of the 75 biopsies, 9 were performed during routine clinical assessments, while 66 were conducted as part of screening for interventional trials. These trials evaluated different treatments, including bortezomib vs. placebo (ClinicalTrials.gov: NCT01873157; n = 50) [16], anti-interleukin-6 antibody clazakizumab (NCT03444103; n = 12) [17], imlifidase together with intravenous immunoglobulin/rituximab (NCT03897205; n = 1) or anti-C1s antibody BIVV009 (NCT02502903; n = 3) [18]. Details regarding baseline immunosuppression and treatment administered after the diagnosis of ABMR are provided in **Table 1**. Following index biopsies, 42 (56%) received antirejection therapy, which included investigational

TABLE 2 | Serologic data and biopsy results.

Variables	Cohort	Data (n)
DSA characteristics		
DSA-positive, n (%)	75 (100)	75
HLA class I DSA	45 (63.4)	71
HLA class II DSA	60 (84.5)	71
HLA class I plus II DSA	34 (47.9)	71
DSA-MFI[a] >10,000	31 (42.5)	73
Morphologic biopsy results		
ABMR phenotypes, n (%)		
Active ABMR	15 (20)	75
Chronic active ABMR	47 (62.7)	75
Chronic (inactive) ABMR	13 (17.3)	75
Peritubular capillary C4d deposition	30 (40.0)	75
Single lesion scores, median (IQR)		
Capillary C4d (c4d)	0 (0 to 2)	75
Glomerulitis (g)	2 (1 to 2)	72
Peritubular capillaritis	1 (0 to 2)	75
Intimal arteritis (v)	0 (0 to 0)	62
Glomerular double contours (cg)	1 (0 to 2)	72
Interstitial fibrosis (ci)	2 (1 to 3)	75
Tubular atrophy (ct)	1 (1 to 2)	75
Vascular fibrous intimal thickening (cv)	1 (1 to 2)	62
Sum scores, median (IQR)		
AI (g+ptc+v+C4d)	4 (2 to 5)	61
AI_{3comp} (g+ptc+C4d)	4 (3 to 5)	72
CI (ci+ct+cv+[cgx2])	7 (4 to 10)	61
CI_{3comp} (ci+ct+[cgx2])	6 (3 to 8)	72
Banff borderline lesion, n (%)	4 (5.3)	75
Mixed rejection, n (%)	2 (2.7)	75
BK virus nephropathy, n (%)	1 (1.3)	75
Glomerulonephritis, n (%)[b]	3 (4.0)	75
Molecular biopsy results (MMDx)		
Rejection-associated scores, median (IQR)		
$ABMR_{Prob}$[c]	0.54 (0.32 to 0.73)	75
TCMR	0.03 (0.02 to 0.05)	75
"all Rejection" score	0.67 (0.44 to 0.82)	75
Injury-associated scores, median (IQR)		
IRRAT	0.19 (–0.13 to 0.54)	75
ciprob	0.58 (0.30 to 0.75)	75
Most probable archetype, n (%)		
No rejection	15 (20)	75
TCMR	1 (1.3)	75
Early-stage ABMR	20 (26.7)	75
Fully-developed ABMR	31 (41.3)	75
Late-stage ABMR	8 (10.7)	75

ABMR, antibody-mediated rejection; AI, activity index; CI, chronicity index; ciprob, molecular classifier reflecting the probability of histologic ci lesion score >1; DSA, donor-specific antibody; IQR, interquartile range; IRRAT, transcript set associated with injury-repair response; MFI, mean fluorescence intensity; TCMR, T cell-mediated rejection.

[a]*MFI of the immunodominant DSA.*

[b]*Cases of glomerulonephritis included two cases of IgA nephropathy and one case of unspecified immune complex-mediated glomerulonephritis.*

[c]*Sixty-three recipients (84%) had an $ABMR_{Prob}$ score >0.2.*

drugs or center-specific standard-of-care treatment, such as immunoadsorption (**Table 1**).

Statistical Analysis

For descriptive analysis, continuous variables were reported as median (interquartile range [IQR]) and categorical variables as absolute counts and relative frequencies. For Kaplan-Meier survival analysis, variables were dichotomized based on their respective medians. Differences between groups were assessed using the log-rank test. Cox regression was used for univariable and multivariable survival analysis. Hazard ratios (HR) were reported per IQR increases of the tested variables. The proportional hazards assumption was assessed visually by plotting Schoenfeld residuals against time. To evaluate the functional form of the independent variables, they were fitted with restricted cubic splines with three knots. Then log hazards were plotted against the respective independent variables and deviations from linearity were visually assessed. Urinary protein/creatinine ratio (UPCR) was subsequently log-transformed. For eGFR slope analysis, we retrieved every serum creatinine measurement from 30 days before index biopsies until December 2022 from our database. Estimated GFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [19]. Overall, 3885 measurements (in median 49 per patient (IQR: 38–64) were recorded. To examine associations between predictor variables and eGFR trajectories, we employed linear mixed models with eGFR as outcome variable and random slopes as well as random intercepts for the association between time and eGFR for each patient (random effect) using an unstructured variance-covariance matrix. Each predictor variable was included as main effect and in an interaction term with time. We used random forest analysis to calculate the relative importance of variables in relation to eGFR slope and graft loss, employing the permutation method. Statistical differences were tested at a two-sided significance level of 5%. All analyses were performed using R version 4.2.3 (R Foundation for Statistical Computing, Vienna, Austria). Utilized packages are provided as Supplemental Material.

RESULTS

Patient and Biopsy Cohort

The study included 75 renal allograft recipients diagnosed with ABMR >180 days after transplantation. As detailed in **Table 1**, the cohort included 37 (49%) female patients, 27 (37%) recipients of a re-transplant, and 12 (16%) recipients of a living donor allograft. At the time of transplantation, the median recipient age was 45 years. Fifteen subjects (21.4%) had a cytotoxic panel reactivity ≥10% and 63% of the patients had preformed DSA. Twenty-seven (36%) subjects had been subjected to immunoadsorption-based desensitization [20]. Index biopsies were performed after a median of 5.17 years post-transplantation. Most patients (80%) were on triple immunosuppression, primarily tacrolimus-based therapy (68%). The median eGFR was 39.9 mL/min/1.73 m^2, and median UPCR levels were 373 mg/g. After ABMR diagnosis, 56% of the patients received anti-rejection treatment, mostly in the context of interventional trials (**Table 1**). As shown in **Table 2**, all patients were DSA-positive at biopsy, with 60 patients (85%) having HLA class II DSA. The MFI of the immunodominant DSA was >10,000 in 43% of the patients. Biopsy results are provided in **Table 2**. Morphologic ABMR phenotypes

TABLE 3 | Biopsy results and DCGF - Unadjusted Cox proportional hazards analysis.

Variables[a]	Hazard ratio (95% confidence interval)	*p*-value	Data (n)
Biopsy variables			
Morphologic single lesion scores			
C4d (c4d)	1.58 (0.91–2.74)	0.102	75
Glomerulitis (g)	0.88 (0.62–1.24)	0.46	72
Peritubular capillaritis (ptc)	1.17 (0.57–2.41)	0.67	75
Glomerular double contours (cg)	2.72 (1.39–5.33)	0.004	73
Interstitial fibrosis (ci)	1.75 (0.88–3.50)	0.11	75
Tubular atrophy (ct)	1.82 (1.19–2.78)	0.006	75
Vascular fibrous intimal thickening (cv)	1.00 (0.67–1.49)	>0.99	63
Morphologic indices			
AI (g+ptc+v+C4d)	1.39 (0.67–2.87)	0.38	61
AI_{3comp} (g+ptc+C4d)	1.20 (0.76–1.89)	0.43	73
CI (ci+ct+cv+[cgx2])	2.83 (1.24–6.43)	0.013	61
CI_{3comp} (ci+ct+[cgx2])	2.90 (1.51–5.57)	0.001	73
Molecular scores			
$ABMR_{Prob}$	0.94 (0.52–1.68)	0.83	75
IRRAT	2.66 (1.56–4.55)	<0.001	75
ciprob	2.71 (1.32–5.54)	0.006	75
Clinical/immunological variables			
Variables recorded at transplantation			
Recipient age (years)	0.50 (0.31–0.81)	0.005	75
Male sex	0.87 (0.43–1.77)	0.71	75
Deceased donor	0.55 (0.24–1.23)	0.14	75
Donor age (years)	0.98 (0.50–1.95)	0.96	75
Prior kidney transplant	1.15 (0.54–2.45)	0.72	73
HLA mismatch (A, B, DR)	0.79 (0.42–1.49)	0.47	73
HLA mismatch (A, B, C, DRB1, DQB1)	0.73 (0.44–1.21)	0.22	58
Variables recorded at index biopsy			
Time to biopsy (years)	1.54 (0.93–2.55)	0.095	75
eGFR (ml/min/1.73m^2)	0.23 (0.11–0.48)	<0.001	75
UPCR at biopsy (mg/g)	2.47 (1.33–4.60)	0.004	75
DSA MFI ≥10000	1.93 (0.93–3.98)	0.076	73
Tacrolimus-based immunosuppression	1.21 (0.56–2.64)	0.63	75
Anti-rejection treatment	0.83 (0.41–1.67)	0.60	75

ABMR, antibody-mediated rejection; AI, activity index; cg, glomerular double contours; CI, chronicity index; ci, interstitial fibrosis; ciprob, molecular classifier reflecting the probability of histologic ci lesion score >1; ct, tubular atrophy; cv, intimal fibrous thickening; DCGF, death-censored graft survival; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; g, glomerulitis; IRRAT, transcript set associated with injury-repair response; MFI, mean fluorescence intensity; ptc, peritubular capillaritis; UPCR, urinary protein/creatinine ratio; v, intimal arteritis.

[a]*For continuous and ordinal categorical variables, hazard ratios were calculated per increase from the first to the third quartile.*

included active ABMR (20%), chronic active ABMR (63%), and chronic (inactive) ABMR (17%), respectively. Thirty index biopsies (40%) were positive for C4d. In MMDx analysis, 63 (84%) specimens were classified as ABMR with an $ABMR_{Prob}$ score ≥0.2, and 59 (78.7%) biopsies were grouped into one of three distinct morphological ABMR archetypes (**Table 2**). Among the 13 patients with morphologic chronic (inactive) ABMR, 8 recipients displayed an ABMR score equal to or above a threshold of 0.2. The most probable corresponding molecular archetypes for these cases were no rejection (n = 8), early stage ABMR (n = 1), fully-developed ABMR (n = 3) and late-stage ABMR (n = 1), respectively. Differences between morphologic ABMR phenotypes regarding morphologic and molecular indices/scores are detailed in **Supplementary Table S1**.

Biopsy Results and Graft Survival

During follow-up, 32 episodes of DCGF were recorded, resulting in a median graft survival of 7.1 years. In a first step, we evaluated associations of biopsy features and clinical variables with DCGF applying unadjusted Cox proportional hazards analysis (**Table 3**). Among Banff single lesion scores, only cg and ct turned out to be associated with survival. When assessing morphologic indices that reflect either ABMR activity or chronic tissue injury, we observed strong associations for CI and CI_{3comp}, but not for AI and AI_{3comp}, respectively. Regarding molecular scores, we found IRRAT and ciprob scores, but not $ABMR_{Prob}$, to be significant (**Table 3**). Among clinical variables, eGFR and UPCR recorded at the time of biopsy, showed a strong association with survival in the unadjusted analysis. Moreover, we found a trend for DSA-MFI >10,000, but no significant effects were observed for time to biopsy, HLA mismatch and donor age. Finally, the use of anti-rejection treatment did not show a significant association with improved survival (**Table 3**). **Figure 2** depicts Kaplan-Meier graft survival curves for DSA-MFI and selected biopsy scores (AI_{3comp}, CI_{3comp}, $ABMR_{Prob}$, IRRAT, ciprob) dichotomized by their median.

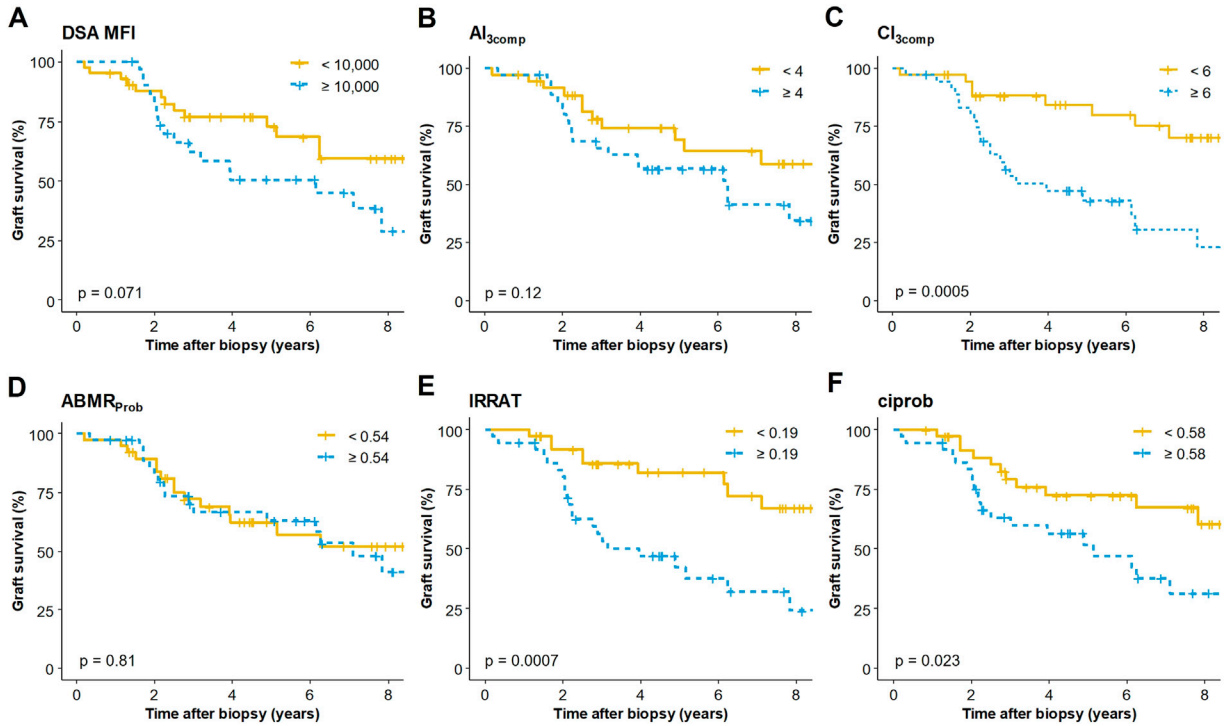


FIGURE 2 | Kaplan Meier death-censored graft survival in relation to **(A)** the mean fluorescence intensity (MFI) of the immunodominant donor-specific antibody (DSA), **(B)** a simplified activity index (AI_{3comp}), **(C)** a simplified chronicity index (CI_{3comp}), **(D)** a classifier reflecting the probability of ABMR diagnosis ($ABMR_{Prob}$), **(E)** an injury-repair response–associated transcript set (IRRAT) and **(F)** a classifier reflecting fibrosis (ciprob). Variables were dichotomized by their medians. The Mantel Cox log-rank test was used to compare survival rates between groups.

TABLE 4 | Adjusted Cox proportional hazards analysis for the prediction of DCGF[a].

Variables[b]	Hazard ratio (95% confidence interval)	*p*-value	Data (n)
Model 1 (biopsy variables)			72
IRRAT	1.93 (0.96–3.89)	0.067	
CI_{3comp} (ci+ct+[cgx2])	1.97 (0.97–3.99)	0.059	
ciprob	1.24 (0.54–2.83)	0.61	
Model 2 (biopsy and clinical variables)			72
IRRAT	1.44 (0.66–3.14)	0.36	
CI_{3comp} (ci+ct+[cgx2])	1.36 (0.64–2.86)	0.42	
ciprob	0.96 (0.42–2.19)	0.92	
Recipient age (years)	0.54 (0.31–0.95)	0.033	
eGFR (mL/min/1.73 m^2)	0.32 (0.15–0.70)	0.005	
UPCR at Bx (mg/g)	1.87 (0.87–4.03)	0.11	

cg, glomerular double contours; ci, interstitial fibrosis; ciprob, molecular classifier reflecting the probability of histologic ci lesion score >1; ct, tubular atrophy; cv, intimal fibrous thickening; DCGF, death-censored graft survival; eGFR, estimated glomerular filtration rate; IRRAT, transcript set associated with injury-repair response; UPCR, urinary protein/creatinine ratio.

[a]*Adjusted models (model 1: biopsy variables; model 2: biopsy plus clinical variables) included variables (morphologic indices, molecular scores and/or clinical parameters) associated with DCGF, in univariable analysis (see **Table 3**).*

[b]*For continuous variables and ordinal categorical variables, hazard ratios were calculated per increase from the first to the third quartile.*

In adjusted Cox proportional hazards analysis that included biopsy variables showing associations ($p < 0.05$) in univariable analysis, IRRAT score (HR per IQR: 1.93 [95% CI: 0.96 to 3.89], $p = 0.067$) and CI_{3comp} (1.97 [0.97 to 3.99], $p = 0.059$) exhibited the strongest associations with DCGF (**Table 4**). In a second model that also included clinical variables, the associations of biopsy variables with DCGF were no longer significant. Only eGFR at biopsy, and to a lesser extent recipient age, remained as the only variables associated with DCGF (**Table 4**). As shown in **Supplementary Table S2** and **Supplementary Figure S1**, similar

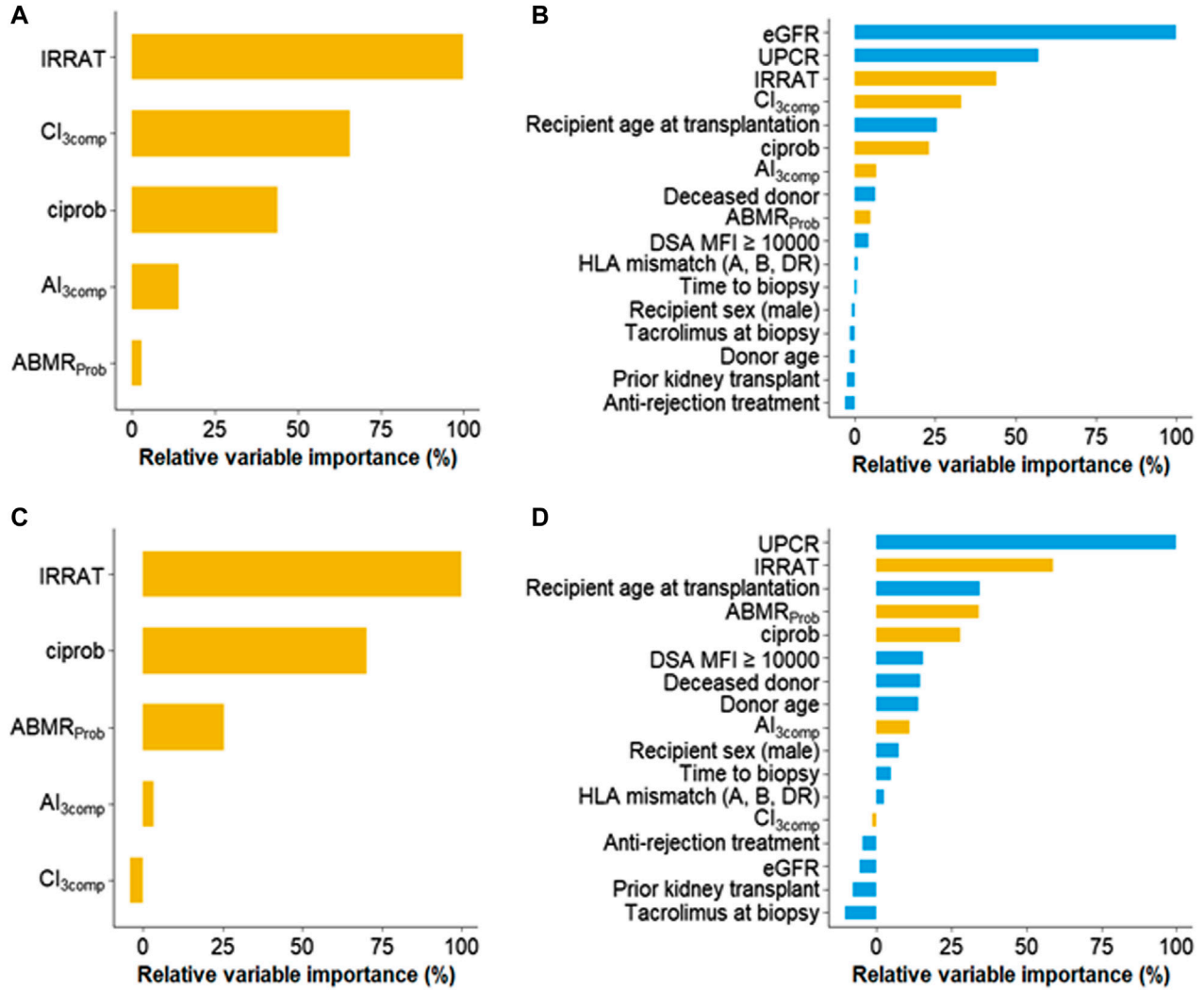


FIGURE 3 | Random forest models to examine the impact of clinical, histologic, and molecular features on death censored graft loss **(A,B)** and estimated glomerular filtration rate (eGFR) slope **(C,D).** The prediction models comprised either biopsy-related features only **(A, C)** or a combination of both clinical and biopsy-related features **(B, D)**. Within each set, individual variables were sorted based on their importance. Abbreviations: $ABMR_{Prob}$, molecular classifier reflecting the probability of histologic diagnosis of antibody-mediated rejection; AI_{3comp}, simplified activity index (g+ptc+C4d); CI_{3comp}, simplified chronicity index (ci+ct+cg[x2]); ciprob, molecular classifier reflecting the probability of histologic ci-lesion score >1; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; IRRAT, injury-repair response-associated transcript; MFI, mean fluorescence intensity; UPCR, urinary protein/creatinine ratio.

results were obtained in models including CI instead of CI_{3comp} (61 instead of 72 included cases), even though, a multivariable model including biopsy variables only revealed a significant impact of the IRRAT score.

In another approach, we applied random forest analysis to determine the relative importance of biopsy-based and/or clinical variables in predicting death-censored graft loss (**Figure 3**). In a first model evaluating biopsy parameters alone, IRRAT emerged as the most important variable, followed by features of chronic injury (CI_{3comp}, ciprob). Morphologic or histologic ABMR activity had the least importance. In a second model including clinical variables, eGFR emerged as the most important variable, followed by UPCR, IRRAT, CI_{3comp} and recipient age (**Figure 3**). Similar results were obtained for unmodified CI and AI (**Supplementary Figure S2**).

Biopsy Results in Relation to eGFR Slope

In a linear mixed model, which included in median 49 eGFR values per subject (from 30 days before biopsy to DCGF or loss of follow-up), mean eGFR at baseline, the intercept, was 41.4 (95% confidence interval: 37.6–45.2) mL/min/1.73 m [2] and the mean slope was −5.4 (−7.0 to 3.7) mL/min/1.73 m [2] per year (data not shown). In unadjusted models, the IRRAT score was associated with lower eGFR at baseline and, as the only variable, with a steeper eGFR slope, while chronicity indices (CI, CI_{3comp}) and ciprob were only associated with lower baseline eGFR values.

TABLE 5 | Linear mixed models for the prediction of eGFR trajectories after index biopsy.

	Variables[a,b]	Baseline association (time = 0)	*p*-value	Change in slope (interaction term)	*p*-value	n
Unadjusted analysis	**Biopsy variables**					
	AI (g+ptc+v+C4d)	−1.8 (−8.1–4.6)	0.59	−0.2 (−2.9–2.5)	0.88	61
	AI_{3comp} (g+ptc+C4d)	−1.9 (−7.1–3.3)	0.47	0.4 (−1.9–2.8)	0.71	72
	CI (ci+ct+cv+[cgx2])	−14.6 (−20.6–−8.5)	<0.001	−0.5 (−3.4–2.3)	0.72	61
	CI_{3comp} (ci+ct+[cgx2])	−15.9 (−22.1–−9.6)	<0.001	−1.4 (−4.5–1.8)	0.40	72
	$ABMR_{Prob}$	1.9 (−4.6–8.3)	0.58	1.5 (−1.3–4.3)	0.29	75
	IRRAT	−13 (−19–−7.1)	<0.001	−3.6 (−6.4–−0.9)	0.013	75
	ciprob	−14.1 (−20.4–−7.9)	<0.001	−1.8 (−4.9–1.3)	0.26	75
	Clinical/immunological variables at transplantation					
	Recipient age	2.5 (−2.6–7.6)	0.34	2.6 (0.5–4.7)	0.020	75
	Male sex	2.9 (−4.7–10.5)	0.46	−1.9 (−1.3–5.2)	0.25	75
	Deceased donor	0.4 (−10–−3.8)	0.95	2.9 (−1.5–7.4)	0.20	75
	Donor age	0.7 (−5.9–7.3)	0.83	1.3 (−1.5–4.2)	0.38	73
	Prior kidney transplant	−0.7 (−8.8–7.3)	0.86	−0.4 (−3.9–3)	0.81	73
	HLA mismatch (A, B, DR)	2.8 (−4.5–10.1)	0.45	0.7 (−2.4–3.8)	0.67	73
	HLA mismatch (A, B, C, DRB1, DQB1)	4.58 (−0.8–10)	0.10	0.8 (−1.8–3.4)	0.55	58
	at index biopsy					
	Time to biopsy (years)	−9.3 (−15.1–−3.6)	0.002	−1.1 (−3.7–1.5)	0.40	75
	UPCR at biopsy (mg/g)	−7.2 (−11.5–-3.0)	0.001	−1.8 (−3.8–0.1)	0.065	75
	DSA MFI ≥10000	1.9 (−6–9.8)	0.63	−2.7 (−6–0.6)	0.11	73
	Tacrolimus-based immunosuppression	5.6 (−2.5–13.6)	0.18	−1.3 (−4.8–2.2)	0.46	75
	Anti-rejection treatment	3 (−4.7–10.7)	0.44	−0.2 (−3.5–3.2)	0.93	75
Model 1 (biopsy variables)						72
	CI_{3comp} (ci+ct+[cgx2])	−11.2 (−17.5–−4.9)	<0.001	−0.1 (−3.4–3.2)	0.96	
	IRRAT	−5.8 (−12.7–1)	0.10	−4.2 (−7.8–−0.6)	0.029	
	ciprob	−7 (−14.1–0.2)	0.066	1.2 (−2.6–5)	0.55	
Model 2 (biopsy and clinical variables)						72
	CI_{3comp} (ci+ct+[cgx2])	−8.8 (−15.6–−2.1)	0.016	1.2 (−2.4–4.7)	0.54	
	IRRAT	−8.4 (−15.7–−1.1)	0.034	−3.9 (−7.7–0)	0.066	
	ciprob	−4.2 (−11.8–3.4)	0.30	1.5 (−2.5–5.5)	0.49	
	Recipient age	−2.5 (−7.1–2.2)	0.31	2 (−0.5–4.5)	0.14	
	Time to biopsy	−6.9 (−13.1–−0.6)	0.043	−0.1 (−3.5–3.3)	0.95	
	UPCR at biopsy	−1.1 (−5.3–3.1)	0.63	−1.2 (−3.4–1.1)	0.32	

AI, activity index; cg, glomerular double contours; CI, chronicity index; ci, interstitial fibrosis; ciprob, molecular classifier reflecting the probability of histologic ci lesion score >1; ct, tubular atrophy; cv, intimal fibrous thickening; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; g, glomerulitis; IRRAT, transcript set associated with injury-repair response; MFI, mean fluorescence intensity; ptc, peritubular capillaritis; UPCR, urinary protein/creatinine ratio; v, intimal arteritis.

[a]*Each predictor is included as main effect and in an interaction term with time.*

[b]*For continuous and ordinal categorical independent variables, the estimates are shown for an increase by one interquartile range of the respective variable.*

Among clinical variables, time to biopsy and UPCR were associated with eGFR at baseline, the latter with a trend towards an association with eGFR slope (**Table 5**; **Figure 4**). In a multivariable model including biopsy variables showing associations ($p < 0.05$) in univariable analysis, IRRAT remained associated with eGFR slope. Conversely, CI_{3comp} remained associated with baseline eGFR (**Table 5**). Similar results were observed in a second model that adjusted also for clinical variables, even though the effects of IRRAT on eGFR slope were no longer significant ($p = 0.066$). Among clinical variables only time to biopsy exhibited a significant association with eGFR at baseline (**Table 5**). As shown in **Supplementary Table S3** and **Supplementary Figure S1**, multivariable models including CI instead of CI_{3comp} revealed comparable results (61 instead of 72 included cases).

In random forest models, irrespective whether clinical variables were included or not, the IRRAT score turned out to be the most important biopsy variable in predicting eGFR slope. Other biopsy features demonstrated lesser importance (ciprob) or showed negligible impact (features of ABMR activity). Among clinical variables, UPCR displayed the highest relative importance (**Figure 3**). IRRAT demonstrated a high level of variable importance also in models that incorporated CI and AI instead of CI_{3comp} and AI_{3comp} (**Supplementary Figure S2**).

DISCUSSION

A major finding of this study, which aimed to identify predictors of graft performance in late ABMR, was that among a selection of various morphologic and molecular biopsy features the MMDx-generated IRRAT score emerged as the sole independent predictor of dynamic eGFR decline. In a model adjusted for clinical variables this association lost statistical significance ($p = 0.066$), although the point estimate of the effect size remained consistent between the models, suggesting the absence of relevant

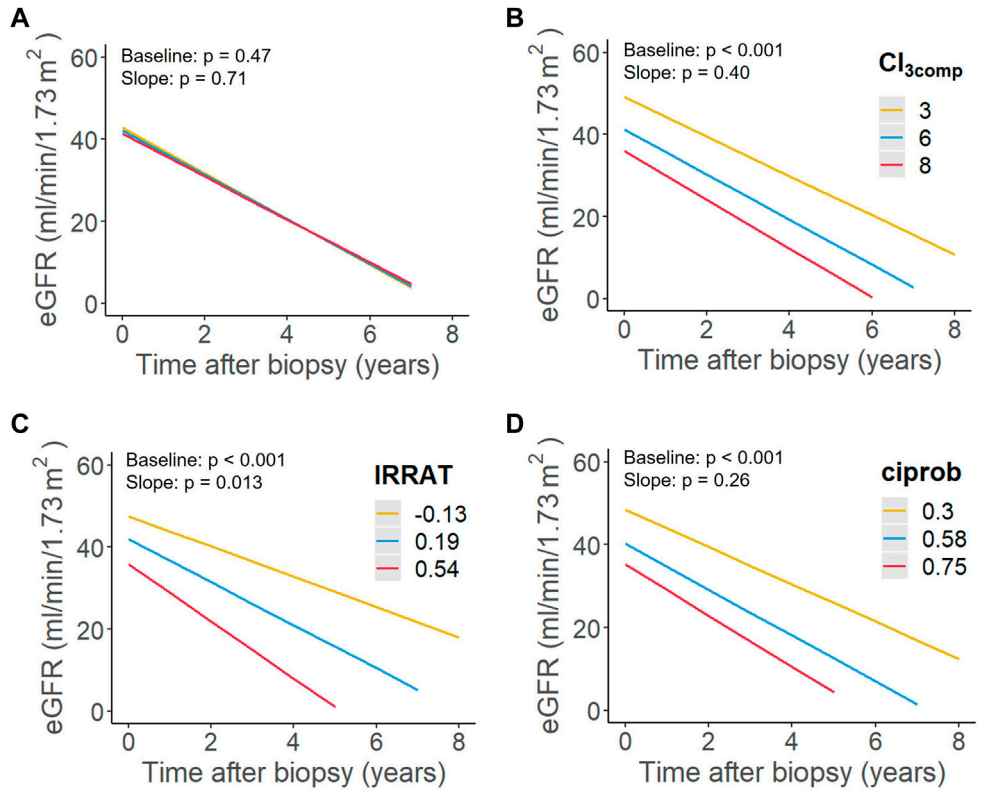


FIGURE 4 | Predictive performance of **(A)** a simplified activity index (AI_{3comp}), **(B)** a simplified chronicity index (CI_{3comp}), **(C)** an injury-repair response–associated transcript set (IRRAT) as well as **(D)** a classifier reflecting fibrosis (ciprob) in relation to estimated glomerular filtration rate (eGFR) trajectory. Shown are the estimated association between each variable and eGFR slope in unadjusted linear mixed models. Lines were drawn for the first, second, and third quartile of each predictor.

effect modification by the included clinical variables. A considerable predictive power was supported by the fact that the IRRAT score exhibited the highest variable importance in random forest models. In contrast, Banff lesion-based or molecular scores reflecting chronic injury solely influenced baseline eGFR without affecting eGFR trajectories [11, 12]. Morphologic and molecular scores indicating ABMR activity or probability had no effect.

Our approach, which involved the use of linear mixed models incorporating a substantial number of creatinine measurements (a median of 49 measurements per patient), allowed us to examine associations for both baseline eGFR and eGFR slopes. In line with previous research [4, 8], we observed a significant decline in renal functional following ABMR diagnosis, with an average eGFR slope of −5.4 mL/min/1.73 m [2] per year. Through this detailed examination of the eGFR course, we were able to distinguish between processes contributing to the dynamic progression of graft dysfunction, which might be amenable to intervention, and processes related to the irreversible loss of nephrons. Both types of processes can associate with a shortened period of graft survival.

Among tested variables, we found that the IRRAT PBT set was the most powerful biopsy-derived predictor of eGFR decline. This finding remained significant even after adjusting for clinical variables such as recipient age, time to biopsy, and proteinuria, each of which individually showed significant associations. The changes in eGFR slope observed were substantial, with approximately −4 mL/min/1.73 m^2/year decrease for each IQR increase in IRRAT. Our findings underscore the significance of integrating molecular gene expression analysis for predicting the risk of graft dysfunction and loss.

Injury-repair response–associated transcripts (IRRATs) were initially identified from early rejection-free post-transplant biopsies obtained within the first 6 weeks after transplantation by comparing biopsies with dysfunction to pristine protocol biopsies [21]. Unlike acute tubular injury based on morphological analysis, a pathogenesis-based transcript set generated from the 30 top IRRATs (IRRAT score) was found to correlate with eGFR at the time of biopsy and subsequent eGFR decline [21]. IRRATs comprise transcripts that are increased in acute kidney injury, such as kidney injury molecule 1 [22], and they were found to overlap substantially with injury and repair-induced transcripts triggered by the transplantation process in mouse kidney isografts [21]. In light of these results, our finding of IRRAT as a biopsy-based predictor of eGFR slope implies that repair responses, as evidenced by distinct transcriptional changes, may be maladaptive and insufficient to effectively counter ongoing parenchymal injury.

In a large multicenter trial (INTERCOMEX), the IRRAT score emerged as one of the strongest predictors of graft loss, in

both patients with pure ABMR (n = 321) and those with any diagnostic category (n = 1,120), while rejection-related scores did not demonstrate relevant predictive value [13]. However, the impact of IRRAT on the course of eGFR during follow-up was not analyzed. Our present study aimed to address this gap and provide additional insights into the relationship between IRRAT score and both the baseline eGFR and its slope. Previous studies have demonstrated a close association between the eGFR slope in ABMR, serving as a potentially valuable surrogate endpoint, and long-term graft survival [4, 8]. As expected, our mixed model analysis revealed associations between IRRAT score and baseline eGFR, and univariable Cox regression demonstrated a strong association between IRRAT score and graft loss (2.7-fold risk; $p < 0.001$). However, in a multivariable Cox model that considered clinical variables such as eGFR, recipient age, and UPCR, the survival effect of the IRRAT score was no longer significant, with baseline eGFR emerging as the dominant predictor. These findings align with the major findings of INTERCOMEX, where random forest survival analysis identified baseline eGFR as one of the most important predictors of outcome [13]. Additionally, a recent multicenter study that focused on late DSA-positive ABMR found that eGFR at the time of biopsy was the sole predictor of graft survival [4].

Remarkably, established histomorphologic lesion scores reflecting ABMR activity, such as scores of single lesions reflecting inflammation in the microcirculation (g and ptc), did not exhibit predictive value for clinical outcomes in our cohort. Even when combining different single lesion scores (g, ptc, c4d, and/or v) to calculate activity indices, they still failed to demonstrate significant predictive capability. These findings align with a recent study by Haas et al. [11], further supporting the limited predictive value of these histomorphologic scores for clinical outcomes in the context of ABMR.

In the study by Haas et al. [11], however, a Banff-based histologic chronicity index incorporating ci, ct, cv, and cg, demonstrated predictive value for DCGF, even after adjusting for eGFR. In our cohort, the chronicity index (CI) or a simplified version excluding cv lesion scores, showed a significant effect in predicting DCGF in unadjusted analysis, but this association was no longer observed in multivariable analysis once clinical variables were considered. Several potential explanations could account for the differences between the two studies. One factor may be the smaller sample size in our cohort, which could have limited the statistical power for more complex analyses. Moreover, differences in selection criteria between the two studies could have contributed to the variations observed. Our cohort focused specifically on late ABMR cases, whereas the study by Haas et al. [11] included a significant number of early ABMR cases. Including only late ABMR cases, our study population exhibited significantly higher levels of chronic injury, as indicated by a median CI of 7 (IQR: 4–10). This contrasted with lower CI values observed in cases recruited from Cedars Sinai Medical Center, Los Angeles (3 [1–7]) and Necker Hospital, Paris (2 [0–4]) [23]. The timing of ABMR diagnosis may have major implications for outcome effects. In a recent analysis of the ANZDATA registry, which included 510 patients with early ABMR and 396 patients with late ABMR (defined as occurring >180 days after transplantation), late ABMR was associated with a twofold increased risk of graft loss, despite the utilization of various treatment approaches [24]. Underscoring treatment resistance of late ABMR, the use of different types of treatment in our cohort, both within and outside interventional trials, failed to improve eGFR slope or graft survival rates.

There are several inherent limitations of our study that should be acknowledged. Firstly, it is important to note that our study is a retrospective single-center evaluation with a partially confirmatory nature. While the multicenter INTERCOMEX trial has previously demonstrated a robust predictive value of IRRAT in relation to graft outcome, the strength of our present study, however, lies in its high granularity, encompassing detailed analyses of both biopsy-based and clinical endpoints, including comprehensive assessments of eGFR trajectories. Moreover, it is noteworthy that a significant proportion of our patients had preformed DSA and underwent desensitization, factors known to potentially influence outcome results. This could limit the generalizability of our findings to cohorts primarily consisting of patients with *de novo* DSA [25]. Another limitation to generalizability may arise from a heterogeneity in biopsy indications, including those performed in the context of interventional trials. In addition, our sample size was limited, resulting in insufficient statistical power to detect small effect sizes. Due to the risk of overfitting, we were unable to construct larger multivariable models. Another limitation was the lack of adequate arterial sections in 12 of the 75 index biopsies. This prevented us from calculating the original CI described in the study by Haas et al. [11] for all patients. To circumvent this caveat and thus to increase the sample size for statistical analysis, we decided to simplify the activity and chronicity indices to three variables each. This approach was supported by our observation that, unlike the findings in the study by Haas et al. [11], arterial intimal fibrous thickening indicated by the cv score was not associated with DCGF. Additionally, a recent study proposing an algorithm for clustering kidney biopsies based on their chronic Banff lesion scores found that ci, ct, and cg were the most informative lesions for outcome prediction, while the other including cv were less important [26]. Nonetheless, even when using the original indices and reducing the sample size to 61 subjects, the results remained largely unchanged. Our study highlights the common issue of sampling error in clinical practice and supports the use of molecular analysis, which may be less susceptible to sampling bias [27]. It is important to note that we specifically focused on a cohort selected for late ABMR. Hence, it remains unclear whether the IRRAT score is also useful for predicting eGFR slope in cases of early ABMR, where gene expression patterns related to injury could be confounded by transient perturbations such as ischemia reperfusion injury. Lastly, a potential limitation is the heterogeneity of therapeutic approaches in our cohort. However, the lack of any long-term treatment effect implies that this heterogeneity may not have had a significant impact on our outcome results, particularly regarding predictors that showed significance in univariable analysis. In this context, it is noteworthy that treatment in our

patients was not guided by molecular features reflecting injury, such as IRRAT or ciprob.

In conclusion, our study provides evidence that a PBT set associated with injury-repair response (IRRAT) may have particular value in predicting eGFR decline in patients with late ABMR (diagnosed after >180 days after transplantation). Unlike morphologic and molecular features of chronic injury, which may indicate irreversible nephron loss and not necessarily correlate with accelerated functional decline after biopsy, injury-repair-associated transcripts reflect a potentially modifiable state of ongoing graft damage that is not visible with conventional morphology. Future trials, which may also include earlier types of ABMR, are needed to investigate whether changes in IRRAT score can be observed in response to effective ABMR therapy, potentially serving as a guide for targeted anti-rejection treatment. Additionally, it remains to be investigated whether patients with higher baseline IRRAT scores exhibit greater treatment responses compared to those with predominant chronic injury patterns.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: non-anonymized data. Requests to access these datasets should be directed to the corresponding author (GB).

ETHICS STATEMENT

The studies involving humans were approved by Ethics committee of the Medical University of Vienna. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because this study was a retrospective analysis of data.

AUTHOR CONTRIBUTIONS

CH, MD, PH, and GB participated in research design, performance of the research, data analysis and writing of the paper. AK, KM, KD, and NK participated in data analysis and writing of the paper. All authors contributed to the article and approved the submitted version.

CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.12135/full#supplementary-material

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ORIGINAL RESEARCH
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Oscillometry in Stable Single and Double Lung Allograft Recipients Transplanted for Interstitial Lung Disease: Results of a Multi-Center Australian Study

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Peak spirometry after single lung transplantation (SLTx) for interstitial lung disease (ILD) is lower than after double lung transplantation (DLTx), however the pathophysiologic mechanisms are unclear. We aim to assess respiratory mechanics in SLTx and DLTx for ILD using oscillometry. Spirometry and oscillometry (tremoflo® C-100) were performed in stable SLTx and DLTx recipients in a multi-center study. Resistance (R_5, $R_{5–19}$) and reactance (X_5) were compared between LTx recipient groups, matched by age and gender. A model of respiratory impedance using ILD and DLTx data was performed. In total, 45 stable LTx recipients were recruited (SLTx $n = 23$, DLTx $n = 22$; males: 87.0% vs. 77.3%; median age 63.0 vs. 63.0 years). Spirometry was significantly lower after SLTx compared with DLTx: %-predicted mean (SD) FEV_1 [70.0 (14.5) vs. 93.5 (26.0)%]; FVC [70.5 (16.8) vs. 90.7 (12.8)%], $p < 0.01$. R_5 and $R_{5–19}$ were similar between groups ($p = 0.94$ and $p = 0.11$, respectively) yet X_5 was significantly worse after SLTx: median (IQR) X_5 [−1.88 (−2.89 to −1.39) vs. −1.22 (−1.87 to −0.86)] $cmH_2O.s/L$], $p < 0.01$. R_5 and X_5 measurements from the model were congruent with measurements in SLTx recipients. The similarities in resistance, yet differences in spirometry and reactance between both transplant groups suggest the important contribution of elastic properties to the pathophysiology. Oscillometry may provide further insight into the physiological changes occurring post-LTx.

Keywords: interstitial lung disease, resistance, oscillometry, single and double lung transplantation, reactance

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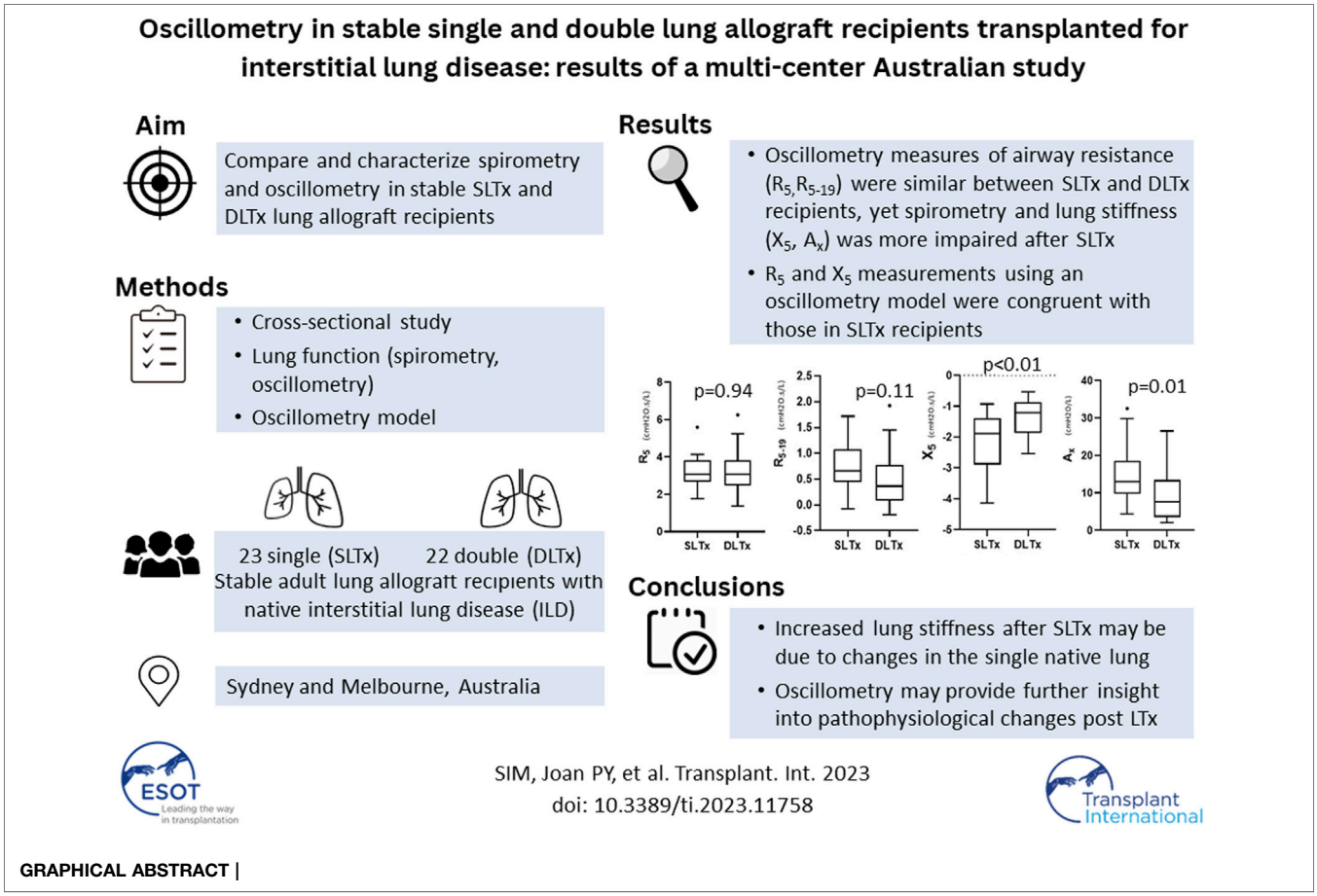
Abbreviations: A_X, Reactance Area; BO, Bronchiolitis obliterans; DLTx, Double Lung Transplantation; FEV_1, Forced Expiratory Volume in one second; FVC, Forced Vital Capacity; ILD, Interstitial Lung Disease; R_5, Resistance at 5 Hz; $R_{5–19}$, Resistance between 5 Hz and 19 Hz; SLTx, Single Lung Transplantation; TLC, Total Lung Capacity; X_5, Reactance at 5 Hz.



GRAPHICAL ABSTRACT |

INTRODUCTION

Lung transplantation (LTx) is an established intervention for patients with advanced interstitial lung disease (ILD) refractory to medical therapy [1]. LTx improves survival in patients with ILD [2] and outcomes depend on donor and recipient factors, choice of procedure and post-operative progress [3]. Single lung transplantation (SLTx) has been the predominant procedure used in patients with ILD, however, double lung transplantation (DLTx) is increasingly used [4]. Survival after LTx is limited by acute and chronic allograft dysfunction and subsequent failure, however there is conflicting data comparing outcomes post-SLTx versus DLTx [1,5,6].

Chronic allograft dysfunction is usually detected on spirometric surveillance [7] and defined as a persistent decline in the forced expiratory volume in one second (FEV_1), from the best achieved post-operative FEV_1 [8]. Studies have consistently demonstrated that FEV_1 and forced vital capacity (FVC) are significantly lower in patients post-SLTx compared to post-DLTx during both short- and long-term follow up [9–11]. Lower spirometry post-SLTx may be attributed to disease progression in the contralateral native lung [9]. However, spirometry alone provides limited insight into the mechanisms contributing to the complex physiological differences between SLTx and DLTx. Furthermore, spirometry may be confounded and therefore produce variable results in SLTx recipients due to possible allograft compression during the forced breathing maneuver [12].

Oscillometry is a non-invasive lung function test performed during quiet tidal breathing that measures the respiratory mechanics of the chest wall, lung and airways [13]. During oscillometry measurement, pressure oscillations, usually of frequencies between 5 and 19 Hertz (Hz), are superimposed at the mouth [14]. The measured pressure and airflow changes are used to calculate impedance—comprised of resistance (R_{rs}), a measure of airway calibre; and reactance (X_{rs}) representing the elastic (compliance) components. Oscillometry has predominantly been used in obstructive respiratory diseases with a paucity of studies in patients with ILD. Studies have demonstrated increased R_{rs} and decreased X_{rs} in those with ILD [15, 16] compared to healthy controls [17] and people with mild-moderate COPD [18]. Conversely, other studies have demonstrated that R_{rs} in ILD, specifically interstitial pulmonary fibrosis, is normal yet X_{rs} is decreased [19, 20], likely reflecting reduced lung compliance from lung fibrosis [19]. Despite its increasing use in tertiary centers, including six in Sydney thus far, studies assessing oscillometry measurements post-LTx remain limited. One study identified physiological changes, increased R_{5-19} and reactance area (A_x)

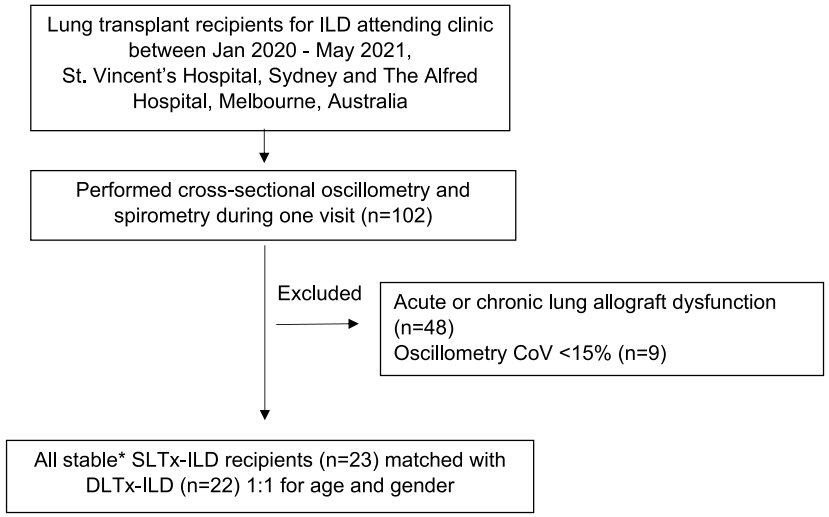


FIGURE 1 | Flow diagram of inclusion and exclusion criteria for participant selection. Definition of abbreviations: ILD, Interstitial Lung Disease; CoV, Coefficient of variation; SLTx-ILD, Single lung transplant for ILD; DLTx, Double lung transplant for ILD. *Defined as concurrent/baseline $FEV_1 \geq 90\%$.

and decreased X_5, in biopsy-proven acute cellular rejection post-DLTx that were undetectable by spirometry [21]. Mathematical models have also been used to calculate impedance using various airway and lung tissue models to describe respiratory mechanics in different disease states [22]. However, none has examined oscillometry measurements in patients with ILD following LTx. Thus, combining our existing knowledge of oscillometry in other disease states and the lack of understanding in our study's patient population, oscillometry may provide further useful pathophysiological insights in patients with ILD following LTx.

We hypothesized that in patients with ILD who have undergone SLTx, resistance (R_{rs}) would be increased, reactance (X_{rs}) decreased, and A_x increased compared to those post-DLTx. Thus, the aim of this study was to characterize resistance (R_5 and R_{5-19}) and reactance (X_5) and A_x in stable recipients and evaluate the relationship between spirometry and oscillometry results following SLTx and DLTx for ILD.

MATERIALS AND METHODS

A cross-sectional study of adult LTx recipients performed for patients with ILD was undertaken at two Australia centers (Sydney and Melbourne), between January-2020 and May-2021. Patients attending routine clinic appointments were approached and consented to participate in the study. The study was initiated just prior to the COVID-19 pandemic which limited data collection. ILD was defined by a consensus clinical, physiological and radiological diagnosis. Donor and recipient matching and surgical techniques were performed as per standard clinical practice [23, 24]. Patients underwent unilateral or bilateral thoracotomy for SLTx and DLTx, respectively. For ILD recipients, lung donors for DLTx are selected based on the predicted total lung capacity (TLC), usually being between the recipients actual measured TLC and their predicted TLC. Lung donors for SLTx are typically larger than that of the recipients (i.e., oversized).

LTx recipients with stable allograft function, defined as concurrent/baseline $FEV_1 \geq 90\%$, were eligible for study enrolment [25]. Baseline FEV1 was defined as the best FEV_1 measurement achieved post LTx. Recipients with acute or chronic lung allograft dysfunction were excluded [25] therefore bronchoscopy and transbronchial biopsy data were not included. Selected patient data were also used in Darley et al.'s recent study "Airway oscillometry parameters in baseline lung allograft dysfunction: Associations from a multicenter study," whose results have no implications on this study [26]. Study participants performed oscillometry followed by spirometry during a single visit (**Figure 1**). Participants were classified into two groups (SLTx and DLTx) and were matched 1:1 for age and gender. Chest radiographs performed as part of standard clinical care within at least 6 months of the study visit were used as a surrogate measure of lung volumes in the SLTx group.

Lung Function

Oscillometry measurements were performed using the tremoflo device (THORASYS® tremoflo® C-100 Airway Oscillometry System) according to European Respiratory taskforce recommendations [27]. Artefacts and tests that did not meet quality control (three measurements per patient with a R_5 coefficient variation of <15%) were excluded [28]. Spirometry (Vmax Software, BreezeSuite) was performed as per American Thoracic Society/European Respiratory Society task force recommendations [29]. Standard oscillometry (R_5, R_{5-19}, X_5, A_X) and spirometry (FEV_1, FVC, FEV_1/FVC) parameters were reported. Z-scores for oscillometry and %-predicted values for spirometry measurements were

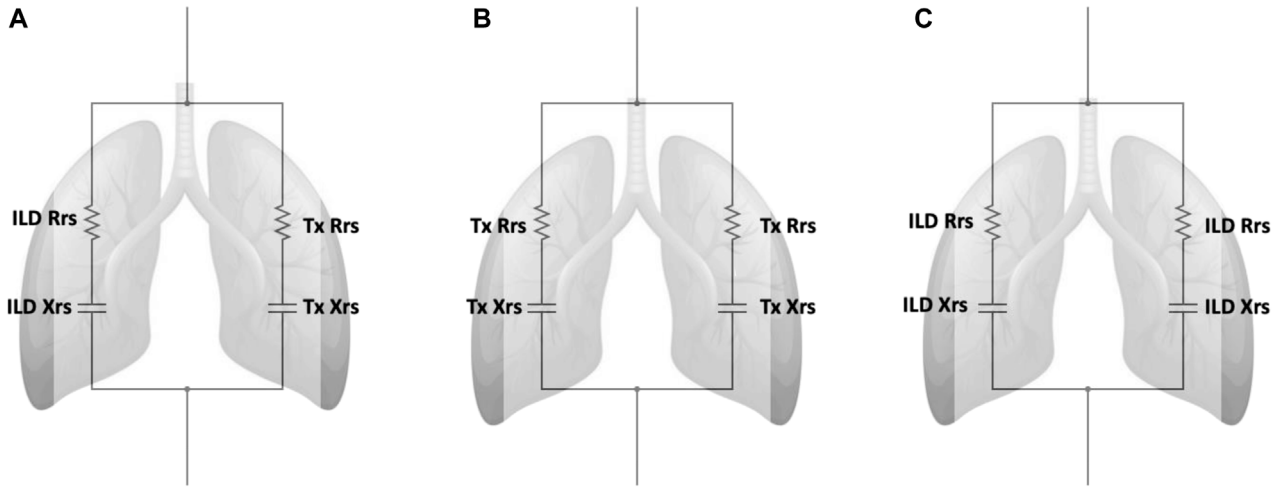


FIGURE 2 | Inhomogeneous models with separate parallel pathways for each lung. Definition of abbreviations: ILD, Interstitial lung disease; Tx, Transplant; Rrs, Respiratory Resistance; Xrs, Respiratory Reactance. **(A)** Model of the single lung transplant group. This model contains separate parallel pathways with a single ILD lung and a single transplanted lung. **(B)** Model of the double lung transplant group. This model contains two parallel pathways of a single transplanted lungs. **(C)** Model of ILD group. This model contains two parallel pathways of a single ILD lungs. ILD Rrs and Tx Rrs are the mean resistance from a single lung from the ILD and Double lung transplant groups respectively. ILD Xrs and Tx Xrs are the mean reactance from a single lung from the ILD and Double lung transplant groups respectively.

calculated using published predictive equations [14, 30]. A normal Z-score was determined by ±one standard deviation from the mean (Z-score of ±1.64).

Chest Radiographs

Digital chest radiograph (CXR) measurements [lung height and width (cm)]) were obtained from the allograft and native lung in the SLTx recipients. CXR measurements were performed using in-software Cerner Enterprise Web Viewer 3.0 calipers. Lung height was measured from the mid-diaphragm to the lung apex and width was measured from the inside of the chest wall across the mid-height of the two diaphragms [31].

Modelling

Oscillometry measurements from patients with ILD and from the DLTx group were used in a standard model of respiratory impedance. ILD patients with an FVC measurement of <80% to match spirometry of the LTx groups were included. Patients with ILD (n = 25, male = 19) had a mean ± SD age of 72.2 ± 6.5 years and %-predicted FVC of 63.9% ± 10.6%.

In brief, the standard model obtained from oscillometry is typically expressed with separate resistive (R) and reactive (X) components (**Figure 2**). This model can be advanced to an inhomogeneous airway model with two parallel pathways (one for each lung) to examine resistance (R_{rs}) and reactance (X_{rs}) from each lung independently [32]. The model was used to determine the R_{rs} and X_{rs} contribution from a single lung in both the DLTx and ILD groups by using the median R_5 and X_5 from each group (**Supplementary Equations S1, S2**). Modelling of R_5 and X_5 for a SLTx recipient was derived by combining the results from a single lung from each of the DLTx and ILD groups (**Figure 2**). Further details are outlined in the **Supplementary Material**.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 8.4.2 and IBM SPSS Statistics 26. Descriptive statistics were summarized using mean with standard deviation or median with interquartile range for continuous variables for parametric and non-parametrically distributed data, respectively; and frequency (%) for categorical variables. Results were compared using the two-sample t-test for continuous variables and the chi-square test for categorical variables. Relationships between oscillometry and spirometry were assessed using Spearman's correlation. Statistical significance was set at a 2-sided level of 0.05.

GUIDELINES

The study was approved by the St Vincent's Hospital Human Research Ethics Committee (2019/ETH12765) and the Alfred Health Human Research Ethics Committee (HREC 50035).

RESULTS

A total of 45 stable recipients after LTx for ILD were recruited (23 SLTx and 22 DLTx recipients). Baseline demographics (**Table 1**) between the SLTx and DLTx groups were similar with regards to recipient gender (87.0% versus 77.3% males) and recipient and donor age [median (IQR) age for recipients: 63.0 (57.0–67.0) versus 63.0 (58.0–66.3) years and mean ± SD age for donors: 44.6 ± 12.9 versus 49.9 ± 18.1 years, for SLTx and DLTx, respectively]. Recipient height, weight and BMI, donor-recipient height difference and donor smoking history were similar between SLTx and DLTx groups. Concurrent FEV_1/

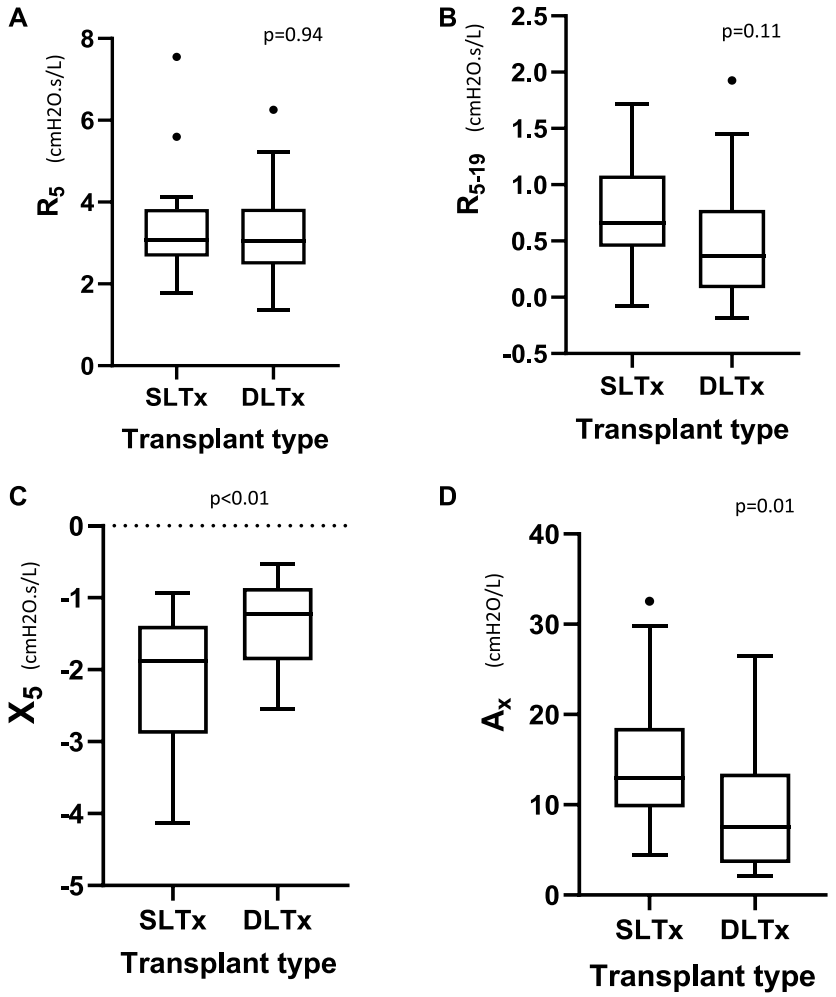


FIGURE 3 | Tukey boxplot comparing the oscillometry indices of **(A)** R_5, **(B)** $R_{5\text{-}19}$, **(C)** X_5, **(D)** A_x in 23 SLTx and 22 DLTx patients. Outliers are marked with dots outside the boxplots. Definition of abbreviations: SLTx, Single Lung Transplant; DLTx, Double Lung Transplant; R_5, resistance at 5Hz; $R_{5\text{-}19}$, Resistance between 5Hz and 19Hz; X_5, Reactance at 5Hz; A_x, Reactance Area.

baseline FEV_1% were also similar between the two groups [median (IQR) 96.0 (92.5–101.0)% versus 98.3 (94.5–100.0)% for SLTx and DLTx, respectively], indicating lung function stability and no evidence of chronic allograft dysfunction. The duration post-LTx was significantly shorter in the SLTx compared to the DLTx group [median (IQR) 1.0 (0.7–1.9) versus 1.6 (1.0–2.7) years ($p < 0.05$), for SLTx and DLTx, respectively]. Donor height was significantly taller in the SLTx compared to the DLTx group (mean ± SD 176.0 ± 6.7 versus 167.0 ± 11.0 cm) ($p < 0.01$). CXR measurements in the SLTx group demonstrated smaller height (169.2 ± 26.9 cm) and width (89.3 ± 13.0 cm) in the native lung compared to the allograft (207.0 ± 31.4 cm and 127.0 ± 22.0 cm, for height and width, respectively) ($p < 0.01$). Most CXRs (18/23 patients) were performed on the same day or within a month of lung function measurements. Three patients in the SLTx group had bronchial complications—two with left bronchial stenoses requiring stent insertion at four and 6 months prior to lung function measurements. One patient had a left anastomotic stricture.

Lung Function

FEV_1 and FVC were significantly lower in the SLTx group compared to the DLTx group (**Table 1**). Mean ± SD FEV_1-% predicted was 70.0 ± 14.5 versus 93.5% ± 26.0% ($p < 0.01$) and FVC-% predicted was 70.5 ± 16.8 versus 90.7% ± 12.8% ($p < 0.01$), in SLTx and DLTx groups, respectively. Oscillometry demonstrated that R_5 in both SLTx and DLTx groups were within normal limits (median Z-score <1.64). However, X_5 and A_x were abnormal in the SLTx group (median Z-scores of −2.26 and 2.22 for X_5 and A_x, respectively) and within normal limits in the DLTx group (**Table 2**).

Oscillometry showed similar measurements in resistance (R_5 and R_{5-19}) between both groups. Median (IQR) R_5 was 3.06 (2.67–3.83)

TABLE 1 | Baseline recipient and donor demographics of single and double lung transplant groups.

Patient characteristics	SLTx (*n* = 23)	DLTx (*n* = 22)	*p*-value
Recipient age (years)	63.0 (57.0–67.0)*	63.0 (58.0–66.3)*	0.78
Recipient height (cm)	172.0 (10.6)	171.0 (8.2)	0.60
Recipient weight (kg)	80.3 (12.7)	77.0 (15.7)	0.44
Recipient BMI (kg/m^2)	26.9 (3.9)	26.5 (5.1)	0.61
Gender (*n*, % total)			
Males	20 (87.0%)	17 (77.3%)	0.46
Females	3 (13.0%)	5 (22.7%)	
Duration Post-transplant (years)	1.0 (0.7–1.9)*	1.6 (1.0–2.7)*	<0.05
Allograft side			
Left	8	22	-
Right	14	22	-
Types of ILD			
Idiopathic pulmonary fibrosis	14	18	-
Hypersensitivity pneumonitis	5	1	-
Connective tissue disease-ILD	1	0	-
Combined pulmonary fibrosis emphysema	1	1	-
Nonspecific interstitial pneumonia	1	1	-
Lymphoid interstitial pneumonia	1	0	-
Niemann-pick type B	0	1	-
Donor age (years)	44.6 (12.9)	49.9 (18.1)	0.28
Donor height (cm)	176.0 (6.7)	167.0 (11.0)	<0.01
Donor-recipient height difference (cm)	5.9 (4.0)	8.0 (5.4)	0.17
Donor smoking history (*n*, % total)			
No	9 (39.1%)	14 (63.6%)	0.20
Yes	11 (47.8%)	6 (27.3%)	
Not reported	3 (13.0%)	2 (9.1%)	
Spirometry post-LTx			
Concurrent FEV_1/baseline FEV_1 (%)	96.0 (92.5–101.0)*	98.3 (94.5–100.0)*	0.45
FEV_1-% predicted	70.0 (14.5)	93.5 (26.0)	<0.01
FVC-% predicted	70.5 (16.8)	90.7 (12.8)	<0.01
Concurrent FEV_1/FVC	0.80 (0.098)	0.80 (0.080)	0.81

All data are reported as mean (SD) or median (IQR). Definition of abbreviations: SLTx, Single Lung Transplant; DLTx, Double Lung Transplant; BMI, body mass index; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity.*

TABLE 2 | Oscillometry data in the single and double lung transplant groups and ILD group.

	SLTx *n* = 23	DLTx *n* = 22	*p*-value	ILD *n* = 25	Model data (single lung)
R_5 ($cmH_2O.s/L$)	3.06 (2.67–3.83)	3.06 (2.48–3.84)	0.94	3.41 (2.85–3.69)	3.23
Z-score	0.61 (–0.18 to 1.29)	0.11 (–0.79 to 1.27)	0.54	0.002 (–0.60 to 1.29)	—
Z-score >1.64, *n*	3	4	—	4	—
$R_{5\text{-}19}$ ($cmH_2O.s/L$)	0.66 (0.45–1.08)	0.36 (0.08–0.78)	0.11	0.81 (0.63–1.20)	—
X_5 ($cmH_2O.s/L$)	–1.88 (–2.89 to –1.39)	–1.22 (–1.87 to –0.86)	<0.01	–2.24 (–2.74 to –1.97)	–1.73
Z-score	–2.26 (–3.76 to –0.83)	–0.36 (–1.44 to 0.37)	<0.01	–2.52 (–3.53 to –1.42)	—
Z-score <–1.64, *n*	14	4	—	16	—
A_x (cmH_2O/L)	13.00 (9.73–18.50)	7.58 (3.55–13.50)	0.01	17.0 (13.65–22.22)	—
Z-score	2.22 (1.52–2.68)	1.17 (0.44–2.25)	0.01	2.21 (1.62–2.68)	—
Z-score >1.64, *n*	17	9	—	19	—

All data are reported as median (IQR) Definition of abbreviations: SLTx, Single Lung Transplant; DLTx, Double Lung Transplant; ILD, interstitial lung disease; R_5, resistance at 5 Hz; R_{5-19}, Resistance between 5 and 19 Hz; X_5, Reactance at 5 Hz; A_x, Reactance Area.*

versus 3.06 (2.48–3.84) $cmH_2O.s/L$ ($p = 0.94$) and $R_{5\text{-}19}$ was 0.66 (0.45–1.08) versus 0.36 (0.08–0.74) $cmH_2O.s/L$ ($p = 0.11$) in the SLTx and DLTx groups, respectively. Reactance (X_5) was significantly lower and A_x significantly higher (i.e., more abnormal) in the SLTx group compared to the DLTx group. Median (IQR) X_5 was –1.88 (–2.89 to –1.39) versus –1.22

(−1.87 to −0.86) $cmH_2O.s/L$ ($p < 0.01$) and A_x was 13.00 (9.73–18.50) versus 7.58 (3.55–13.50) cmH_2O/L ($p = 0.01$) in the SLTx and DLTx groups, respectively (**Table 2**; **Figure 3**). Z-score comparisons of oscillometry measurements between SLTx and DLTx groups were similar to that observed with raw values (**Table 2**).

There were significant associations between oscillometry parameters (R_5, $R_{5–19}$, X_5 and Ax) and FVC in the SLTx group [R_5 (rs = −0.47, $p = 0.02$), R_{5-19} (rs = −0.45, $p = 0.03$), X_5 (rs = 0.72, $p < 0.01$) and Ax (rs = −0.70, $p < 0.01$)]. In the DLTx group, significant correlations with FVC were only demonstrated between X_5 (rs = 0.65, $p < 0.01$) and Ax (rs = −0.52, $p = 0.01$). Similar correlations were observed when comparing FEV_1 with oscillometry indices for both SLTx and DLTx groups.

Modelling

The derived single lung values of R_5 and X_5 for DLTx and ILD groups are displayed in **Table 2**. There was close agreement between the inhomogeneous oscillometry model predicted R_5 (3.23 $cmH_2O.s/L$) and X_5 (−1.73 $cmH_2O.s/L$) with the measured R_5 (3.06 $cmH_2O.s/L$) and X_5 (−1.88 $cmH_2O.s/L$) in the SLTx group.

DISCUSSION

Our multicenter cross-sectional study is the first study, to our knowledge, to report oscillometry measurements in stable single (SLTx) and double (DLTx) lung transplantation recipients, exclusively in patients with ILD as their native lung disease. Our novel findings demonstrate that resistance (R_5 and $R_{5–19}$) measured by oscillometry was similar between SLTx and DTLx recipients despite FEV_1 and FVC being significantly lower in the SLTx group. Furthermore, reactance at 5 Hz (X_5) and A_x were significantly worse in the SLTx recipients compared to the DLTx recipients. These findings were replicated using a simple mathematical model based on real-life data obtained from DLTx recipients and patients with ILD. Our data, suggests that the differences in respiratory mechanics after SLTx and DLTx may be predominantly attributed to changes in the elastic properties rather than airway caliber.

Resistance (R_5 and $R_{5–19}$) was not increased (i.e., not more abnormal) in the SLTx compared to DLTx recipients. This may be due to patients in our study having stable disease as indicated by the preserved spirometric ratio and concurrent/baseline FEV_1 being greater than 90% [25] and thus suggesting the absence of spirometric obstruction and acute or chronic lung allograft dysfunction. Chronic allograft dysfunction is commonly due to bronchiolitis obliterans (BO) [33] with the underlying pathology being fibroproliferative airway plugging [34]. Airway plugging may lead to a reduction in airway caliber and an increase in airway resistance. As resistance was similar between SLTx and DLTx recipients, allograft dysfunction due to BO seems unlikely. This is supported by our cohort being spirometrically-stable. The underlying pathology in the native single ILD lung typically affects the lung parenchyma rather than the airways. However, airway epithelial cell proliferation and expansion in a number of bronchioles can also occur in the distal airways of those with ILD [35]. We speculate that changes in the distal airways may increase airway caliber in the native single ILD lung and thus explain the similarities in resistance between the SLTx and DLTx recipients. Our results are consistent with recent oscillometry studies demonstrating normal resistance in ILD [19, 20]. However, data is conflicting as other studies report resistance to be increased or impaired in patients with ILD in those with more severe lung restriction and lung function impairment [17]. Comparatively, in our study, spirometry demonstrated that lung function impairment was worse in our SLTx recipients compared to DLTx recipients, yet resistance derived from oscillometry was not. Comparisons with other studies are limited because previous oscillometry studies examined ILD patients that did not include LTx recipients.

In contrast to resistance, reactance (X_5) was significantly lower, and A_x was significantly higher (i.e., X_5 and A_x were more impaired) in the SLTx compared to the DLTx recipients. These findings are consistent with previous studies showing more abnormal reactance in patients with ILD compared to healthy controls [17, 20] and in those with ILD and more severe lung restriction [15]. Reduced lung volume due to the diseased native ILD lung could account for X_5 and A_x being more abnormal as these parameters are dependent on lung volume [36]. In the SLTx recipients the native ILD lung was significantly smaller compared to the allograft, which we confirmed using chest radiograph measurements. The allograft side may have contributed to lung volume differences in the SLTx group because left-sided allografts are typically smaller because of the position of the heart. However, a majority of our SLTx recipients underwent a right-sided LTx thus unlikely to contribute to our results (**Table 1**). Differences in lung volumes between the native lung and allograft in SLTx recipients may lead to asynchrony and altered lung mechanics during respiration. This phenomenon has not been demonstrated in SLTx recipients with ILD, but asynchrony can occur in SLTx recipients with emphysema. The native emphysematous lung and allograft can inflate and empty at different rates and subsequently lead to chest wall asymmetry and mediastinal shift during respiration [12]. The reduced lung volumes may therefore explain a more abnormal reactance. The forced maneuver during spirometry versus tidal breathing during oscillometry measurement needs to be taken into consideration, however the impact on the resulting physiological measurements remains elusive. Additionally, asynchrony in muscle forces, which may result from diaphragm dysfunction, can develop between the two sides of the chest after SLTx [37] and may exacerbate chest wall asymmetry and alter chest wall and lung mechanics. Studies assessing reactance measured via oscillometry in patients with SLTx, respiratory muscle dysfunction and/or chest wall deformities are lacking therefore we can only speculate these mechanisms.

Our study included a simple model that incorporated measurements from real-life ILD and LTx patients to support our *in vivo* findings in SLTx recipients. The inhomogeneous

model shows that in the SLTx group, the single transplanted lung has low reactance while the non-transplanted lung has high reactance (i.e., an increased X_5) which corroborates our novel findings. Agreement between the predicted X_5 from the model and the measured X_5 in the actual SLTx group further ascertains that the increased X_5 measured in the SLTx group is indeed attributed to the increased reactance in the native ILD lung. While there is close agreement between the predicted and measured median X_5, the measured X_5 was slightly more abnormal (−1.73 versus −1.88 $cmH_2O.s/L$, respectively). The more negative X_5 may be a reflection of more advanced disease in the SLTx group before transplantation. Using the ILD group's single lung reactance in the SLTx model, we may have underestimated the reactance in the single native ILD lung. The results derived from this model replicates and provides further evidence to support our *in vivo* findings in a small number of ILD patients after single and double lung transplantation.

Spirometry was significantly lower or more impaired in our SLTx recipients compared to DLTx recipients as demonstrated in other studies [9, 10]. Anthropometrics in the SLTx and DLTx recipients were similar and thus unlikely to contribute to differences in spirometry. Donor height was significantly taller in the SLTx recipients however is unlikely to be relevant because there was no difference in donor-recipient height matching between the two groups, suggesting appropriate lung size matching. The maximal spirometry measurement achieved is typically lower in SLTx compared to DLTx recipients [10, 38] and thought to be related to the remaining single diseased native lung. The disease pathology in the native lung is also reflected in the normal FEV_1/FVC ratio in SLTx, consistent with that of restrictive lung disease.

Limitations that must be acknowledged include the small sample size in our study. The patient cohort was small, as we only included patients with ILD as their native disease. Only one other study has measured oscillometry in SLTx and DLTx recipients however these authors assessed LTx recipients with various forms of native lung diseases, with COPD comprising the majority of their patient cohort [39]. Limiting our study participants to one native disease, ILD, avoids confounding factors from including various diseases. Furthermore, our study groups were matched for age and gender and there were no significant differences between recipient baseline characteristics to confound our results. Differences in lung volume likely contribute to our findings and additionally we did not report lung volume measurements. As a surrogate we showed that there was a significant difference in lung size between the native and allograft lung in the SLTx group using a standardized technique of chest radiograph measurements [31]. The effect of significant differences between donor and recipient height must also be acknowledged however, optimum size matching was performed in accordance with local guidelines. There was no significant difference in smoking history between the two groups and the effect of donor smoking is not known but donor smoking history must also be acknowledged.

The time post-LTx was statistically significantly shorter in the SLTx compared to the DLTx group, however it is clinically insignificant since both groups should have achieved and maintained their maximal spirometry at the time of measurement during the study [9]. The specific effect of relevant clinical parameters such as bronchial stenosis and/or other bronchial or pleural complications were not examined in this cross-sectional study and require further evaluation. Furthermore, the trajectory of oscillometry measurements is not established and will likely alter over time. Spirometry declines more rapidly in SLTx than in DLTx recipients [9, 40] and whether this also occurs in oscillometry is yet to be determined.

CONCLUSION

In summary, in SLTx recipients, oscillometry measurement of resistance is similar to that observed in DLTx recipients. However, similarly to spirometry, reactance is more impaired in SLTx compared to DLTx recipients. This is likely attributed to changes in the elastance due to reduced alveolar volume in the native ILD lung in SLTx recipients and may lead to asynchrony in respiratory mechanics. Whether the breathing maneuver performing during lung function testing impacts respiratory mechanics is yet to be elucidated but "quiet" tidal breathing may be a more attractive measurement compared to the forced maneuver used in spirometry.

These cross-sectional findings highlight the physiological complexities of LTx that are not completely understood. The significance of normal resistance, yet abnormal spirometry and abnormal reactance as a predictor of clinical outcomes, requires reliable reference values and further longitudinal investigation. Further study in LTx recipients with obstructive lung disease would also improve our understanding. A better understanding of the physiological changes after SLTx and DLTx is vital for developing novel diagnostic and therapeutic approaches to improve LTx outcomes.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving humans were approved by the St Vincent's Hospital Human Research Ethics Committee (2019/ETH12765) and the Alfred Health Human Research Ethics Committee

(HREC 50035). 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

KN, DD, GS, MP, and KT contributed to the conception and design of the work; JS, KN, DD, BB, BL, JV, SE, and KT contributed to data acquisition and 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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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11758/full#supplementary-material

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