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The boomerang of glomerular disease

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FORUM
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Estimation of Donor Renal Function After Living Donor Nephrectomy: The Value of the Toulouse-Rangueil Predictive Model

Thomas Prudhomme[1,2]*, *Mathieu Roumiguie*[1] *and Marc Olivier Timsit*[3]

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Keywords: living donor nephrectomy, renal function, external validation, predictive model, concordance

A Forum discussing:

External Validation of the Toulouse-Rangueil Predictive Model to Estimate Donor Renal Function After Living Donor Nephrectomy
by Almeida M, Calheiros Cruz G, Sousa C, Figueiredo C, Ventura S, Silvano J, Pedroso S, Martins LS, Ramos M and Malheiro J (2023). Transpl Int 36:11151. doi: 10.3389/ti.2023.11151

Assessment of renal function after living donor nephrectomy represents a current hot topic in kidney transplantation. It is well known that living donor kidney transplantation (LDKT) is the best treatment for end-stage renal disease (ESRD) patients eligible for transplantations [1]. However, it has been demonstrated that living donors are at an increased risk of chronic kidney disease (CKD) and ESRD comparing to healthy non-donors [2, 3], exposing them to cardiovascular and global morbidity and mortality of CKD [4]. For these reasons, personalized instruments to evaluate post-operative renal function are necessary in order to offer the benefit of LDKT to recipients while controlling the immediate and long-term negative effects of reduced nephron mass for the donor.

Almeida et al. [5], have just published in Transplant International, an external validation of the predictive model to estimate the donor renal function after living donor nephrectomy, developed by our team at the Rangueil University Hospital in Toulouse. They reported a significant correlation (Pearson $r = 0.67$; $p < 0.001$) and concordance (Bland-Altman plot with 95% limits of agreement −21.41 to 26.47 mL/min/1.73 m^2; $p < 0.001$) between predicted and observed 1-year estimated glomerular filtration rate (eGFR). The area under the receiver operating characteristic curve (AUROC) showed a good discriminative ability of the formula to predict CKD [AUC: 0.83 (CI 95%: 0.78–0.88; $p < 0.001$)].

It is very rewarding to see a confirmation of our previous report regarding this predictive model to estimate the 1-year post-donation eGFR and risk of CKD. First, Benoit et al. [6] retrospectively evaluated our living donor cohort and demonstrated that age and preoperative eGFR were strong independent predictors of postoperative eGFR. A formula, using multiple linear regression model, was designed: postoperative eGFR (CKD-EPI, mL/min/1.73 m^2) = 31.71 + (0.521 × preoperative eGFR) − (0.314 × age). The internal validation reported an optimal statistical performance with a significant correlation ($r = 0.65$) and an AUROC of the model of 0.83 (CI 95%: 0.72–0.93; $p < 0.001$). Then, this model was externally validated using the Necker Hospital living donor cohort [7]. A significant correlation (Pearson $r = 0.66$; $p < 0.001$) and concordance (Bradley-Blackwood F = 49.189; $p < 0.001$) were reported between predicted and observed 1-year eGFR. The AUROC in this external population confirmed discriminative ability

Forum

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of this model to predict CKD (AUC: 0.86 (CI 95%: 0.82–0.89; $p < 0.01$)). Kullik et al. [8] also externally validated the model and reported a good correlation to the observed 1-year post-donation eGFR.

External validation is necessary to determine predictive model reproducibility and generalizability to different patients [9]. Correlation and concordance are usually degraded by external validations [10], the outcomes reported by Almeida et al. [5] confirmed the effectiveness of the formula to estimate the 1-year post-donation eGFR and risk of CKD. The strength of their study is the evaluation of model performance according to the sex of the donor; they reported similar performance between females and males.

Along with an extension of marginal deceased donors (extended criteria donor, donation after circulatory death donors) [11, 12], extending the age limit for living donors to expand the pool has been considered [13, 14]. However, donor age was to be a strong predictor of CKD after living donor nephrectomy which may defer the wish to extend the age limit of donors [6, 15]. Preoperative eGFR was also an independent predictor of postoperative eGFR [6]. Thus, a particular attention should be paid to preoperative eGFR and the risk of postoperative CKD with potential an increasing pool of donor over 60 years. However, considering the performance of the predictive model, the use of this tool at the living donor evaluation consultation, combined with a global donor assessment (i.e., comorbidities assessment, donor age and expected lifespan), could contraindicated some potential donors with predicted lower 1-year eGFR.

The prediction of the postoperative renal function is the key point of donor's candidate evaluation. This predictive model, represents a simple, low cost, non-invasive tool that can be easily joined to the living donor evaluation routine consultation, paving the way for an improved decision-making process.

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

TP: Manuscript writing, MR: Manuscript editing, MT: Manuscript editing.

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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Living Donor Kidney Transplant in Recipients With Glomerulonephritis: Donor Recipient Biologic Relationship and Allograft Outcomes

Rasha El-Rifai[1], *Adam Bregman*[1], *Nattawat Klomjit*[1], *Richard Spong*[1], *Scott Jackson*[2], *Patrick H. Nachman*[1] *and Samy Riad*[1,3]*

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Using the Scientific Registry of Transplant Recipients, we examined the association between donor-recipient biologic relationship and long-term recipient and allograft survival among glomerulonephritis (GN) patients. Four GN types were studied: membranous nephropathy, IgA, lupus-associated nephritis, and focal segmental glomerulosclerosis (FSGS). We identified all adult primary living-donor recipients between 2000 and 2018 (n = 19,668): related (n = 10,437); unrelated (n = 9,231). Kaplan-Meier curves were generated for the recipient, death-censored graft survival and death with functioning graft through ten years post-transplant. Multivariable Cox proportional hazard models were used to examine the association between the donor-recipient relationship and outcomes of interest. There was an increased risk for acute rejection by 12 months post-transplant among the unrelated compared to the related group in IgA (10.1% vs. 6.5%, p<0.001), FSGS (12.1% vs. 10%, p-0.016), and lupus nephritis (11.8% vs. 9.2%; p-0.049). The biological donor-recipient relationship was not associated with a worse recipient or graft survival or death with functioning graft in the multivariable models. These findings are consistent with the known benefits of living-related-donor kidney transplants and counter the reports of the potential adverse impact of the donor-recipient biologic relationship on allograft outcomes.

Keywords: graft survival, long term outcomes, disease recurrence, living related donor, glomerulonephritis

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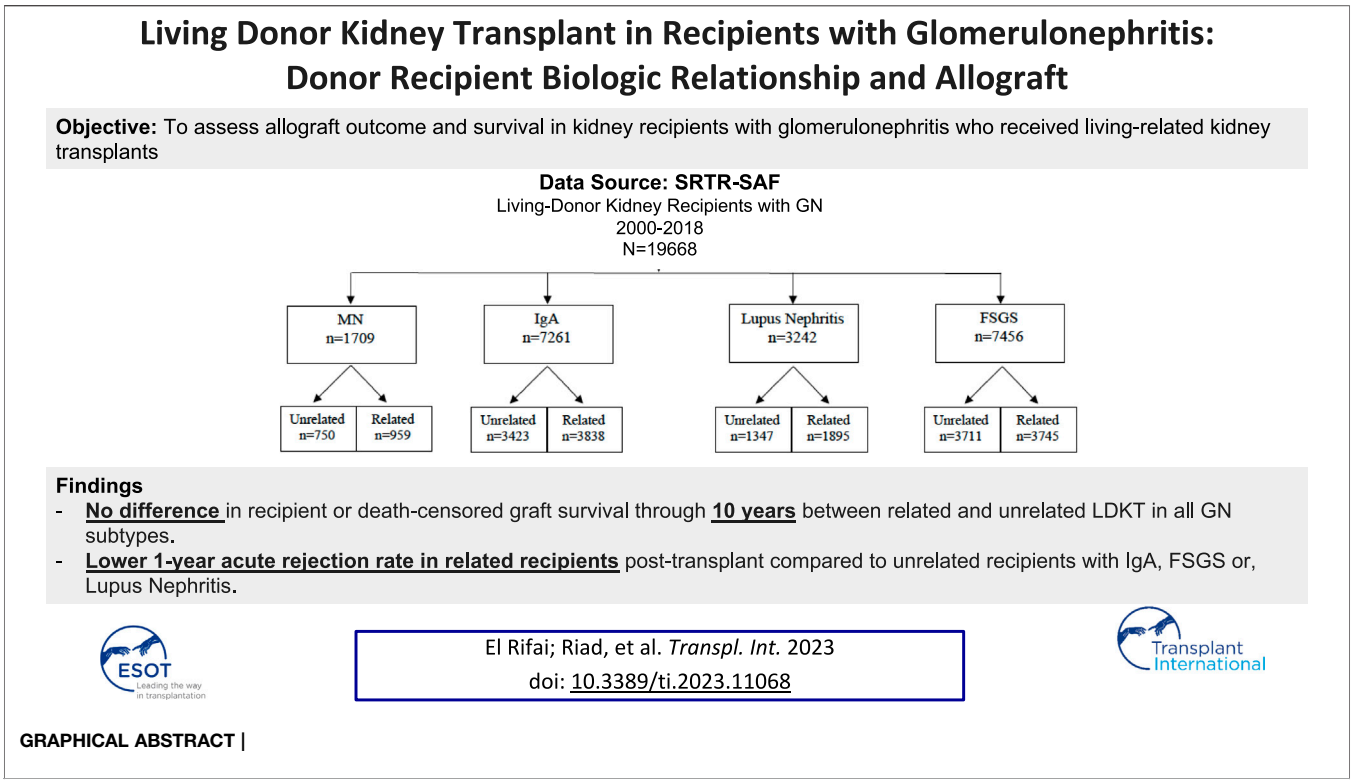
Abbreviations: CI, confidence interval; DCGS, death censored graft survival; ESKD, end stage kidney disease; FSGS, focal segmental glomerulosclerosis; GN, glomerulonephritis; HLA, human leukocyte antigen; HRSA, the health resources and services administration; KDIGO, kidney disease improving global outcomes; KTA, kidney alone transplant; LDKTx, living donor kidney transplant; LRKTx, living related donor kidney transplant; LUKTx, living unrelated donor kidney transplant; MN, membranous nephropathy; OPTN, organ procurement and transplantation network; SPK, simultaneous pancreas-kidney; SRTR, scientific registry of transplant recipients; US, United States; UNOS, united network for organ sharing.



GRAPHICAL ABSTRACT |

INTRODUCTION

Glomerulonephritis (GN) is among the major causes of ESKD accounting for about 30% of kidney transplants in the United States (USRDS report 2015) (1). Common glomerulonephritides such as membranous nephropathy (MN), IgA nephropathy, and focal segmental glomerulosclerosis (FSGS) can recur at variable rates ranging between 35% and 50% at 5 years post-transplant (2). Recurrent disease is the third most common cause of allograft loss after chronic rejection and death with a functioning graft (3). About 15% of death censored graft failures are attributed to recurrent GN's though this may be an underestimate of the true incidence especially with the challenges distinguishing between *de novo* and recurrent GN (2).

Controversies exist about the association of donor recipient biologic relationship with allograft outcomes for recipients with ESKD due to glomerulonephritis. An earlier study by Choy et al in 2006 (4) suggested that biologic relationship between donor and recipient was not associated with an increased risk of GN recurrence after kidney transplant. However, a more recent study by Kennard et al in 2017 (5) including 7,236 patients from ANZDATA transplant registry data showed a significantly higher 10-year risk of disease recurrence among living related compared to living unrelated donor kidney transplant recipients (16.2% vs. 10.3%, respectively). This effect was observed among recipients with IgA nephropathy and FSGS.

A more recent study by Husain et al examined the association between donor-recipient biologic relationship and allograft survival after living donor kidney transplant (1). The study included more than 72,980 living donor kidney transplants in the US between 2000 and 2014, 59% (43,147) were biologically related. After adjustment for multiple donor and recipient characteristics including HLA matching, donor recipient biologic relationship was associated with a slightly higher risk for death censored graft failure. However, the study did not focus on recipients with ESKD due to GN.

Data on strong familial clustering of certain GNs (6) are accumulating. Accordingly, we sought to focus our analysis on the association between donor-recipient relationship in GN recipients. We studied the four most common GN disorders: membranous nephropathy (MN), IgA nephropathy, lupus nephritis (LN), and focal segmental glomerulosclerosis (FSGS).

MATERIALS AND METHODS

Data Source

This study used data from the Scientific Registry of Transplant Recipients (SRTR). The SRTR data system includes data on all donor, wait-listed candidates, and transplant recipients in the US, submitted by the members of the Organ Procurement and Transplantation Network (OPTN). The Health Resources and Services Administration (HRSA), U.S. Department of Health and Human Services provides oversight to the activities of the OPTN and SRTR contractors.

Study Population

We analyzed the scientific registry of transplant recipients (SRTR) standard analysis file for all primary kidney transplants in recipients with GN between 1 January 2000 and 30 June 2018. The 4 GN subtypes we studied included membranous nephropathy (MN), IgA nephropathy, lupus nephritis (LN), and FSGS. We excluded recipients younger than 18 years or those who received a previous kidney transplant. Our final cohort consisted of 19,668 kidney transplant recipients (**Figure 1**). Donor recipient pairs were classified as related if any biological relationship was reported: parent, child, sibling, or twin (n = 10,437) or unrelated otherwise (n = 9,231). Recipients were categorized according to the type of GN: MN (n = 1709), IgA (n = 7,261), LN (n = 3,242), and FSGS (n = 7,456).

Outcomes of Interest

The primary outcomes were 10-year recipient survival and death censored kidney graft survival (DCGS). Secondary outcomes were rejection and recipient eGFR by CKD-EPI 2021 (7) at 1 year post transplant. We also assessed the cause of graft loss among the two recipient groups across the different GN types. However, the high degree of missingness did not allow for meaningful statistical analysis.

Statistical Analysis

Univariate comparisons for recipient and death-censored graft survival outcomes were performed using Kaplan-Meier curves with log-rank tests. All survival analyses were censored at 10 years post-transplant. Multivariable Cox proportional hazard model was used to examine the association between donor-recipient relation and outcomes of interest. Each GN subtype was analyzed separately. The recipient survival, death censored graft survival (DCGS) and death with functioning graft models were adjusted for pertinent recipient and donor factors. Recipient factors included: age, sex, ethnicity, years on dialysis, preemptive status, HLA mismatch, cross match, induction, maintenance immunosuppression, and transplant year. Donor factors included donor age, sex, ethnicity, BMI, HTN, and eGFR. Covariates were included based on clinical relevance to avoid unnecessary investigator biases. Proportional hazards assumption was assessed using Schoenfeld residuals (cox.zph in the R survival package). All analyses were performed in R (ver. 4.0.2).

RESULTS

Baseline Characteristics

The study included 19,668 adult primary living donor kidney transplants in recipients with GNs between 1 January 2000 and 30 June 2018; 53% (n = 10,437) included related donor-recipient pairs, and 47% (n = 9,231) unrelated pairs. Four GN groups were included in the study cohort: membranous nephropathy (MN) of which n = 959 recipients had a living related donor kidney transplant (LRKT) and n = 750 recipients had living unrelated donor kidney transplant (LUKT); IgA nephropathy recipients of LRDKT were n = 3,838 and LURDKT n = 3,423. Additionally, lupus nephritis (LN) recipients of LRDKT were n = 1895 and LURDKT n = 1,347, and FSGS recipients of LRDKT were n = 3,745 and LURDKT n = 3,711 (**Figure 1**). Detailed description of the cohort is included in the baseline characteristics **Tables 1**, **2** and can be summarized as follows: across the study population, LRDKT recipient-donor pairs tended to be slightly younger, had fewer HLA mismatches and shorter dialysis vintage.

Primary and Secondary Outcomes by GN Type

Membranous

In the Kaplan-Meier analysis for recipient survival in those with membranous nephropathy, donor-recipient biologic relationship was not a predictor of recipient survival (log-rank, p = 0.81). Similarly, in the Kaplan-Meier analyses for death-censored graft survival and death with functioning graft in recipients with membranous nephropathy, donor-recipient biologic relationship was not associated with worse graft survival (log-rank p = 0.82) or death with functioning graft (log-rank p = 0.28) (**Figures 2A–C**). In the multivariable models, donor recipient relationship was not associated with neither recipient [HR 1.04, 95%C.I. (0.73, 1.49), p = 0.82] nor death-censored graft survival [HR 1.18, 95%C.I. (0.89, 1.57), p = 0.24] or death with functioning graft [HR 1.12, 95%C.I. (0.72, 1.73), p = 0.62] (**Table 3**). The 1-year rejection rate and eGFR were similar among recipients with membranous nephropathy irrespective of donor type (**Table 4**).

IgA Nephropathy

In the Kaplan-Meier analysis for recipient survival in those with IgA nephropathy, donor-recipient biologic relationship was not a predictor of recipient survival (log-rank, p = 0.31). Similarly, in the Kaplan-Meier survival for death-censored graft survival and death with functioning graft in recipients with IgA nephropathy, donor-recipient biologic relationship was not associated with worse graft survival (Log-rank, p = 0.83) or death with functioning graft (log-rank p = 0.13) (**Figures 3A–C**). In the multivariable models, donor recipient relationship was not associated with neither recipient [HR 1.02, 95%C.I. (0.77, 1.36), p = 0.88], death-censored graft survival [HR 1.01, 95%C.I. (0.84, 1.21), p = 0.96] or death with functioning graft [HR 0.97, 95%C.I. (0.69, 1.37), p = 0.86] (**Table 3**). The 1-year rejection rate was 3.6% lower (p < 0.01) in LRDKT recipients with IgA nephropathy. The 1-year eGFR was similar among both groups of recipients with IgA nephropathy (**Table 4**).

Lupus Nephritis

In the Kaplan-Meier analyses for recipient survival and death with functioning graft in those with lupus nephritis, donor-recipient biologic relationship was associated with slightly better recipient survival (log-rank, p = 0.03) and death with functioning graft (log-rank p = 0.05). However, in the Kaplan-Meier survival for death-censored graft survival in recipients with lupus nephritis, donor-recipient biologic relationship was not associated with worse graft survival (Log-rank, p = 0.14)

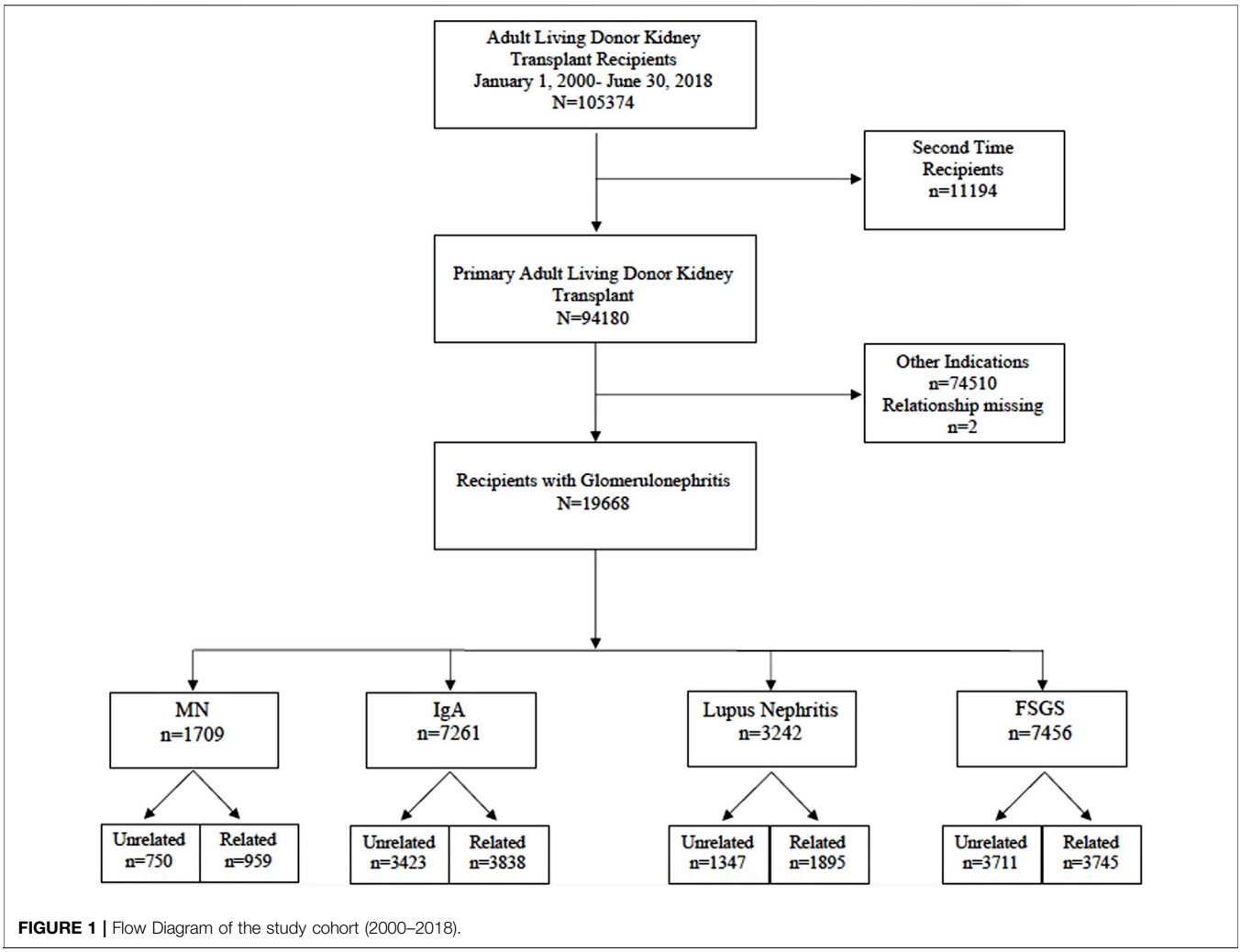


FIGURE 1 | Flow Diagram of the study cohort (2000–2018).

(**Figure 4**). In the multivariable models, donor recipient relationship was not associated with recipient [HR 0.87, 95% C.I. (0.66, 1.16), $p = 0.34$], death with functioning graft [HR 0.84, 95%C.I. (0.58, 1.23), $p = 0.38$] or death-censored graft survival [HR 0.87, 95%C.I. (0.70, 1.07), $p = 0.18$] (**Table 3**). The 1-year rejection rate was 2.6% lower ($p < 0.05$) in LRDKT recipients with lupus nephritis. The 1-year eGFR was slightly better in LRDKT recipients with lupus nephritis (**Table 4**).

FSGS

In the Kaplan-Meier analysis for recipient survival in those with FSGS, donor-recipient biologic relationship was not a predictor of recipient survival (log-rank, $p = 0.11$). Similarly, in the Kaplan-Meier survival for death-censored graft survival in recipients with FSGS, donor-recipient biologic relationship was not associated with worse graft survival (Log-rank, $p = 0.19$), however death with functioning graft was slightly worse in the FSGS recipients of unrelated living donors (log-rank $p = 0.04$) (**Figure 5**). In the multivariable models, donor recipient relationship was not associated with recipient [HR 1.06, 95%C.I. (0.87, 1.29), $p = 0.57$], death with functioning graft [HR 0.92, 95%C.I. (0.73, 1.17), $p = 0.52$], or death-censored graft survival [HR 1.09, 95%C.I. (0.93, 1.28), $p = 0.29$] (**Table 3**). The 1-year rejection rate was 2.1% lower ($p < 0.01$) in LRDKT recipients with FSGS. The 1-year eGFR was slightly better in LRDKT recipients with FSGS (**Table 4**).

Causes of Graft Loss

The causes of graft loss are detailed in **Table 5**. Unfortunately, due to the large proportion of missing graft loss causes, we were not able to perform a statistical analysis. Seemingly, graft loss due to rejection was less frequently observed in related vs. unrelated recipients in those with membranous, IgA nephropathy and FSGS, while graft loss due to disease recurrence was more frequently observed in the same groups. Recipients with lupus nephritis appear to have similar rates of graft loss and disease recurrence irrespective of the donor-recipient biologic relationship.

Rejection Rate Among Recipients With Greater Than One HLA Mismatches

In a subgroup analysis of the entire cohort including those with HLA mismatches>1, rejection rate at six- and 12-months were

TABLE 1 | Baseline Recipient and Donor characteristics in Membranous and IgA N (%) or Mean [SD].

	Membranous		IgA	
	Unrelated *n* = 750	**Related *n* = 959**	**Unrelated *n* = 3,423**	**Related *n* = 3,838**
Recipient Characteristics				
Recipient Age	46.72 [13.8]	43.64 [15.4]**	42.99 [11.6]	40.89 [12.8]
Recipient Male	497 (66.3)	600 (62.6)	2,430 (71.0)	2,667 (69.5)
Race				
White	623 (83.1)	803 (83.7)	2,824 (82.5)	3,155 (82.2)
Black	77 (10.3)	108 (11.3)	113 (3.3)	138 (3.6)
Other	50 (6.7)	48 (5.0)	486 (14.2)	545 (14.2)
BMI(Kg/m^2)	27.07 [5.3]	26.49 [5.6]*	27.31 [5.2]	26.74 [5.2]
HLA-MM	4.37 [1.3]	2.36 [1.4]**	4.35 [1.25]	2.22 [1.49]
PRA				
0%–20%	686 (91.8)	888 (93.9)	3,055 (90.1)	3,478 (92.2)
20%–80%	51 (6.8)	45 (4.8)	272 (8.0)	250 (6.6)
80%–100%	10 (1.3)	13 (1.4)	63 (1.9)	46 (1.2)
XM Positive	41 (5.7)	34 (3.6)	70 (2.1)	92 (2.5)
Induction	Overall $p < 0.001$		Overall $p < 0.001$	
Depletional	350 (53.4)	358 (43.8)	1864 (61.0)	1,580 (48.3)
Non-Depletional	217 (33.1)	298 (36.4)	863 (28.2)	1,159 (35.5)
Mixed	24 (3.7)	11 (1.3)	79 (2.6)	64 (2.0)
None	65 (9.9)	151 (18.5)	252 (8.2)	465 (14.2)
CNI Maintenance			Overall $p < 0.001$	
Cyclosporine	98 (13.3)	164 (17.4)	317 (9.4)	520 (13.8)
None	40 (5.4)	50 (5.3)	143 (4.2)	190 (5.0)
Tacrolimus	600 (81.3)	731 (77.4)	2,915 (86.4)	3,068 (81.2)
Steroid	522 (70.7)	683 (72.2)	2,280 (67.4)	2,619 (69.2)
Mycophenolate	661 (89.6)	831 (87.8)	3,140 (92.9)	3,421 (90.4)**
mTOR	15 (2.0)	20 (2.1)	49 (1.4)	71 (1.9)
Pre-emptive Transplant	245 (31.8)	354 (34.4)	1,191 (34.3)	1,474 (37.9)**
Dialysis Vintage (months)	21.9[22.6]	19.2[20.7]*	18.5[19.6]	15.3 [18.7]**
Donor Characteristics				
Age	42.73 [11.5]	40.36 [11.5]**	41.55 [10.8]	40.86 [11.6]*
Male	285 (38.0)	429 (44.7)*	1,309 (38.2)	1703 (44.4)**
Race	Overall $p = 0.006$		Overall $p < 0.001$	
White	664 (88.5)	803 (83.7)	3,004 (87.8)	3,171 (82.6)
Black	50 (6.7)	106 (11.1)	118 (3.4)	132 (3.4)
Other	36 (4.8)	50 (5.2)	301 (8.8)	535 (13.9)
BMI (Kg/m^2)	26.45 [4.15]	26.65 [4.59]	26.52 [4.77]	29.35 [101.8]
Hypertension	20 (3.6)	14 (2.4)	67 (2.3)	71 (2.5)
CKD-EPI 2021 eGFR (mL/min/1.73m^2)	96.7 [16.2]	99.0 [17.4]*	97.6 [16.45]	98.06 [16.72]

** Significance level <0.05; ** Significance level <0.001; BMI: body mass index; HLA-MM: human leukocytic antigen mismatch; PRA: panel reactive antibody; XM: crossmatch; CNI: calcineurin; mTOR: mammalian target of rapamycin; CKD-EPI 2021: chronic kidney disease epidemiology collaboration; eGFR: estimated glomerular filtration rate.*

not statistically different in related and unrelated recipients (**Supplementary Table S1**).

DISCUSSION

To date our study is the largest analysis with extended follow up of donor-recipient relationship and survival outcomes in adult recipients with different types of glomerular disorders. Our results can be summarized as follows: 1. Donor-recipient biologic relationship was not associated with worse long-term recipient or graft survival in those with membranous, IgA, lupus nephritis or FSGS. 2. Rejection rates were significantly lower in most of the GN recipients of LRDKT. 3. Graft loss due to recurrence was more frequently documented in recipients of related compared to unrelated donor kidney transplants.

There is concern about earlier onset as well as increased risk for GN recurrence among living related kidney transplant recipients (5, 8–11). Multiple studies have shown significant genetic predisposition for GNs including IgA nephropathy (10), FSGS (8), lupus nephritis (9, 11), and membranous (12) nephropathy. The evidence for increased GN recurrence risk among biologically related donor-recipient pair has led many investigators to speculate this effect could be attributed to inherited genetic predisposition for kidney disease (5). Recently, Husain et al. analyzed more than 70,000 living donor kidney transplants (1), of which 22% had GN as the primary cause of ESKD. They found that the donor-recipient biologic relationship was associated with a 5% worse renal allograft survival. Interestingly, the observed association was predominantly noted in kidney transplants from live African American donors, which may be attributed to the higher rates of APOL1 risk variants. Kidneys from donors with high-risk

TABLE 2 | Baseline recipient and donor characteristics in SLE and FSGS N (%) or Mean [SD].

	SLE		FSGS	
	Unrelated *n* = 1,347	**Related *n* = 1,895**	**Unrelated *n* = 3,711**	**Related *n* = 3,745**
Recipient Age	39.3 [11.1]	37.2 [12.0]**	45.1 [13.5]	42.1 [15.0]**
Recipient Male	282 (20.9)	337 (17.8)*	2,421 (65.2)	2,389 (63.8)
Race				
White	823 (61.1)	1,176 (62.1)	2,693 (72.6)	2,805 (74.9)
Black	398 (29.5)	535 (28.2)	851 (22.9)	795 (21.2)
Other	126 (9.4)	184 (9.7)	167 (4.5)	145 (3.9)
BMI (Kg/m^2)	25.1 [5.2]	24.6 [5.5]*	28.4 [5.6]	27.5 [5.8]**
HLA-MM	4.4 [1.28]	2.2 [1.48]**	4.4 [1.22]	2.3 [1.42]**
PRA	Overall $p = 0.004$		Overall $p < 0.001$	
0%–20%	1,087 (81.5)	1,611 (85.7)	3,269 (88.9)	3,391 (92.1)
20%–80%	169 (12.7)	194 (10.3)	338 (9.2)	235 (6.4)
80%–100%	77 (5.8)	74 (3.9)	69 (1.9)	56 (1.5)
XM Positive	115 (9.0)	176 (9.6)	103 (2.9)	109 (3.0)
Induction	Overall $p < 0.001$		Overall $p < 0.001$	
Depletional	738 (62.6)	761 (47.8)	1935 (60.8)	1,517 (48.8)
Non-Depletional	313 (26.5)	550 (34.5)	933 (29.3)	1,083 (34.8)
Mixed	41 (3.5)	55 (3.5)	73 (2.3)	69 (2.2)
None	87 (7.4)	227 (14.2)	242 (7.6)	441 (14.2)
CNI Maintenance	Overall $p < 0.001$		Overall $p < 0.001$	
Cyclosporine	109 (8.3)	234 (12.6)	374 (10.3)	519 (14.2)
None	57 (4.3)	90 (4.8)	131 (3.6)	176 (4.8)
Tacrolimus	1,155 (87.4)	1,533 (82.6)	3,125 (86.1)	2,970 (81.0)
Steroid	1,054 (79.6)	1,485 (79.5)	2,452 (67.3)	2,558 (69.6)*
Mycophenolate	1,194 (90.2)	1,678 (89.8)	3,347 (91.8)	3,262 (88.8)**
mTOR	35 (2.6)	37 (2.0)	68 (1.9)	65 (1.8)
Pre-emptive Transplant	285 (20.4)	426 (28.3)	1,331 (35.3)	1,435 (37.6)*
Dialysis Vintage (months)	28.8 [28.2]	23.04 [22.2]**	22.2 [23.6]	17.8 [22.8]**
Donor Characteristics				
Age	40.11 (10.82)	40.03 (11.57)	42.32 (11.56)	40.30 (11.52) **
Male	593 (44.0)	838 (44.2)	1,404 (37.8)	1,614 (43.1) **
Race	Overall $p < 0.001$		Overall $p < 0.001$	
White	1,013 (75.2)	1,183 (62.4)	3,050 (82.2)	2,804 (74.9)
Black	272 (20.2)	526 (27.8)	533 (14.4)	788 (21.0)
Other	62 (4.6)	186 (9.8)	128 (3.4)	153 (4.1)
BMI (Kg/m^2)	208.6 (6490.7)	27.10 (5.2)	27.06 (9.3)	29.61 (100.2)
Hypertension	29 (2.7)	36 (2.7)	87 (2.8)	95 (3.5)
CKD-EPI 2021 eGFR (mL/min/1.73 m^2)	99.75 (17.4)	100.87 (17.5)	98.26 (17)	99.54 (17.5)*

** Significance level <0.05; ** Significance level <0.001; BMI, body mass index; HLA-MM, human leukocytic antigen mismatch; PRA, panel reactive antibody; XM, crossmatch; CNI, calcineurin; mTOR, mammalian target of rapamycin; CKD-EPI 2021, chronic kidney disease epidemiology collaboration; eGFR, estimated glomerular filtration rate.*

APOL1 variants have been associated (13, 14) with worse allograft survival in the African American population. Therefore, their analysis may not have fully accounted for residual confounding. Nonetheless, due to the large sample size, Husain could detect statistical differences, albeit very small.

Earlier studies in living donor kidney transplant recipients with IgA nephropathy and FSGS had conflicting results. McDonald et al analyzed a small cohort from the ANZDATA registry (15) and reported an 8.5-fold increased risk for IgA recurrence among zero HLA mismatched LDKTx. Nonetheless, graft survival was not different. Similarly, Han et al. in 2010 (16) studied the outcomes of 221 recipients in Korea with IgA nephropathy and noted significantly higher rates of recurrence among living related donor kidney transplant. Interestingly, living related donor was associated with better 10-year graft survival. Taken together, it leads us to reevaluate what constitute the most relevant outcomes of interest and avoid unnecessary impedance to live related donations.

Previous results of studies in recipients with FSGS (17–19) are also inconclusive. A couple of studies showed increased risk for FSGS recurrence and worse graft survival among living related kidney transplant recipients. However, a subsequent study by Kennard et al showed (5) no difference in graft survival despite increased risk for FSGS recurrence among the related donor recipient pair, which is concordant with our graft survival results in our FSGS population.

Kennard et al reported (5) on the outcomes of 2,280 living donor kidney transplants in recipients with primary GN in the ANZDATA registry including IgA, FSGS, MN, and mesangiocapillary GN (MCGN). An increased risk for GN recurrence was noted in living related donor kidney transplant (16.2% LRKTx vs. 10.3% LURTx); especially in recipients with IgA nephropathy (4). However, the 10-year death censored graft survival was similar among living related and unrelated kidney transplants and superior to deceased donor kidney transplant (4).

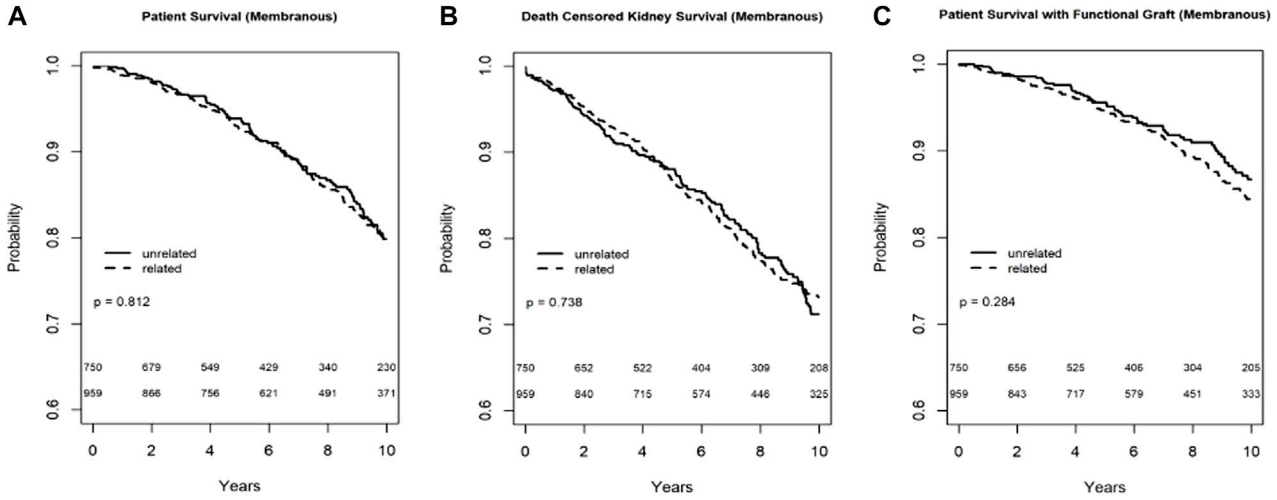


FIGURE 2 | Recipient survival **(A)**, death censored graft survival **(B)** and death with functioning graft **(C)** in MN patients by biologic relationship.

TABLE 3 | Multivariable Cox Proportional Hazard Model for Recipient and Death Censored Graft Survival by GN type.

	Recipient survival HR, 95% C.I., *p*-value	Death censored graft survival HR, 95% C.I., *p*-value	Death with functioning graft HR, 95% C.I., *p*-value
Membranous	1.04, (0.73, 1.48), $p = 0.81$	1.18, (0.89, 1.57), $p = 0.31$	1.12, (0.72, 1.73), $p = 0.62$
IgA Nephropathy	1.02, (0.77, 1.36), $p = 0.88$	1.01, (0.84, 1.21), $p = 0.96$	0.97, (0.69, 1.37), $p = 0.86$
Lupus Nephritis	0.87, (0.66, 1.16), $p = 0.34$	0.87, (0.70, 1.07), $p = -0.18$	0.84, (0.58, 1.23), $p = 0.38$
FSGS	1.06, (0.87, 1.29), $p = 0.57$	1.09, (0.93, 1.28), $p = 0.29$	0.92, (0.73, 1.17), $p = 0.30$
All	1.00, (0.88, 1.14), $p = 0.97$	1.02, (0.93, 1.13), $p = 0.62$	0.94, (0.80, 1.11), $p = 0.46$

C.I., confidence interval; FSGS, focal segmental glomerulosclerosis. Models are adjusted for recipient and donor age, gender, race, recipient years on dialysis, preemptive status, HLA, cross match, transplant year; donor HTN, eGFR, BMI. HRs are for biologically related donor-recipient pair (reference group, unrelated donor-recipient pair).

TABLE 4 | Secondary outcomes among Related vs. Unrelated kidney transplant recipients with different GN types N (%), Mean [SD].

	Membranous		IgA	
	Unrelated *n* = 750	**Related *n* = 959**	**Unrelated *n* = 3,423**	**Related *n* = 3,838**
6-Month Rejection	46 (8.3)	35 (5.7)	228 (7.8)	129 (4.5)**
12-Month Rejection	59 (10.9)	50 (8.5)	286 (10.1)	181 (6.5)**
12-Month CKD-EPI 2021 eGFR (mL/min/1.73 m^2)	58.3 [19.2]	59.5 [18.9]	60.8 [17.3]	61.2 [17.7]
	SLE		**FSGS**	
	Unrelated *n* = 1,347	**Related *n* = 1,895**	**Unrelated *n* = 3,711**	**Related *n* = 3,745**
6-Month Rejection	86 (8.1)	84 (6.1)	264 (8.8)	200 (7.3)
12-Month Rejection	122 (11.8)	122 (9.2)*	353 (12.1)	263 (10.0)*
12-Month CKD-EPI 2021 eGFR (mL/min/1.73 m^2)	63.9 [21.4]	65.7 [21.7]*	59.2 [18.94]	60.1 [18.9]*

** Significance level <0.05; ** Significance level <0.001.*

Outcome data on donor-recipient relationship in lupus nephritis is limited to only two very small studies (20–22) with short-term follow up and conflicting results. Our results indicate significantly lower 1 year rejection rates in recipients of a related donor kidney transplant and comparable 10-year death censored graft survival rate.

An analysis of the European Renal Association-European Dialysis and Transplant Association Registry (23), including over 14,000 primary kidney transplants, suggested similar outcomes with living related vs. unrelated donor kidney transplants in IgA, membranous nephropathy and FSGS. Moreover, organs from living donors outperformed organs

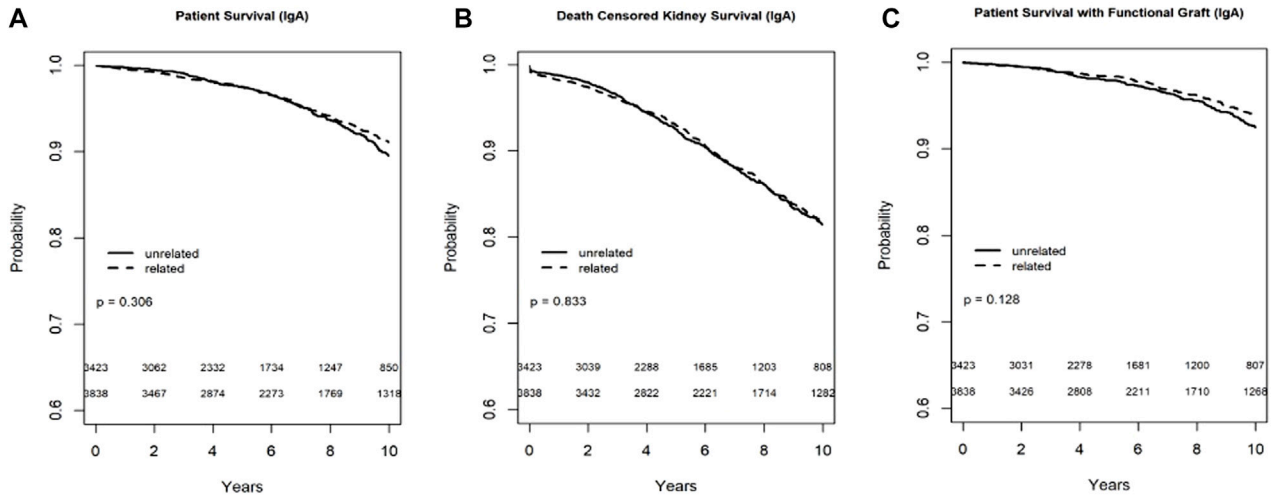


FIGURE 3 | Recipient survival **(A)**, death censored graft survival **(B)** and death with functioning graft **(C)** in IgA patients by biologic relationship.

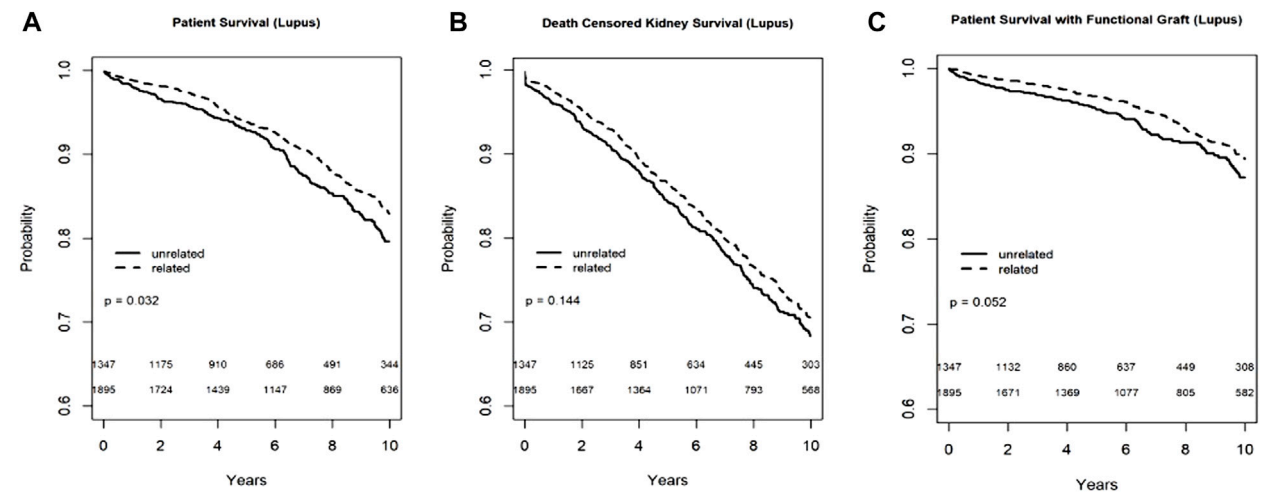


FIGURE 4 | Recipient survival **(A)**, death censored graft survival **(B)** and death with functioning graft **(C)** in patients with lupus nephritis by biologic relationship.

from deceased donors except in membranoproliferative glomerulonephritis. Our study complements the above analysis as we included over 19,000 live donor transplants with over 50% first degree related donors compared to only 21% of the entire cohort from the European registry. Reassuringly, the results of the two studies are consistent and both refute the notion of questioning the performance of living related donor transplants in recipients with the studied glomerulonephritis groups. One exception is membranoproliferative, which was not covered in our study due to extensive heterogeneity of this disorder.

Our study results are validated by findings of Kennard (5), Han (16), and others (4, 23). We complement their reports by expanding our study to include other types of GN, the largest number of living donor recipients with a long follow up. Taken together, the data support the value of living related donor kidney transplant in recipients with GN despite the perceived increased risk for recurrence.

Strengths and Limitations

Our study has several strengths and limitations. This study is the largest cohort of living donor transplant in recipients with GN including 19,668 recipient-donor pairs. Biologic relationship between donor recipient pairs was clearly defined in SRTR and restricted to parent, sibling, twin, or child. One limitation is that some GN disorders, more than others, can be primary or secondary in nature, which is not clearly defined in the SRTR standard analysis file. Another limitation is that a cause specific graft loss was either listed as missing or "other" in a large portion of our cohort. This

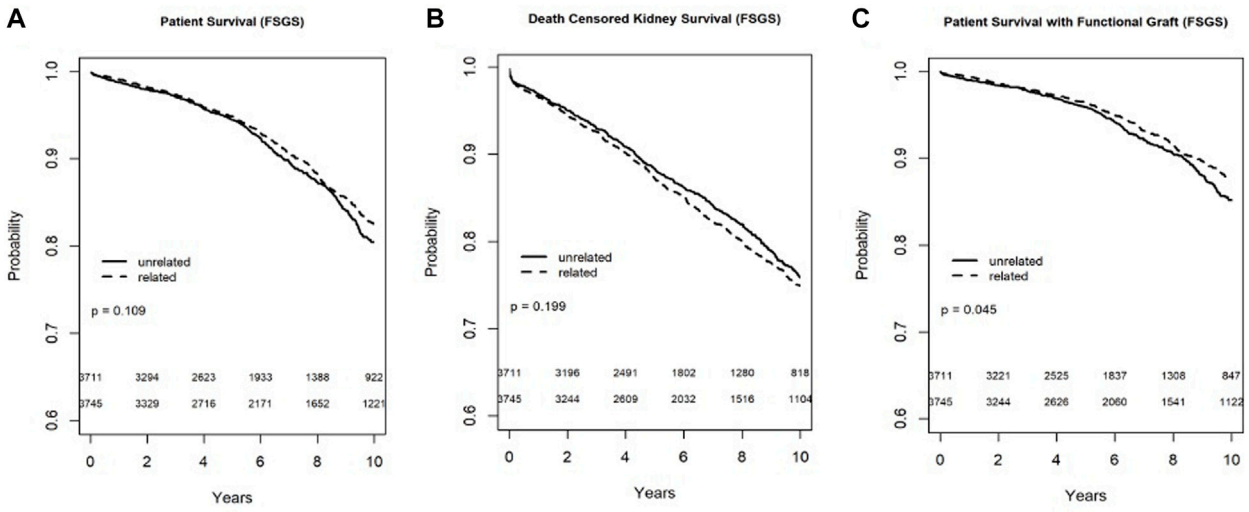


FIGURE 5 | Recipient survival **(A)**, death censored graft survival **(B)**, and death with a functioning graft **(C)** in patients with FSGS by biologic relationship.

TABLE 5 | Causes of Graft loss in related vs. Unrelated kidney transplant recipients with different GN types N (%).

	Membranous		IgA	
	Unrelated *n* = 750	**Related *n* = 959**	**Unrelated *n* = 3,423**	**Related *n* = 3,838**
Total Graft Loss	197 (26.3)	251 (26.2)	508 (14.8)	714 (18.6)
Acute or Chronic Rejection	81 (41.1)	89 (35.5)	225 (44.3)	269 (37.7)
Recurrent Disease	37 (18.8)	57 (22.7)	64 (12.6)	118 (16.5)
Missing/other	79 (40.1)	105 (41.8)	219 (43.1)	327 (45.8)
	Lupus Nephritis		**FSGS**	
	Unrelated *n* = 1,347	**Related *n* = 1,895**	**Unrelated *n* = 3,711**	**Related *n* = 3,745**
Total Graft Loss N (%)	325 (24.1)	512 (27.0)	728 (19.6)	862 (23.0)
Acute or Chronic Rejection	167 (51.4)	264 (51.6)	297 (40.8)	320 (37.1)
Recurrent Disease	17 (5.2)	32 (6.2)	128 (17.6)	173 (20.1)
Missing/other	141 (43.4)	216 (42.3)	303 (41.7)	369 (42.9)

Statistical analysis was not possible due to missingness; Total graft loss percentage represents graft loss out of the total number transplant in each GN category; Cause specific graft loss percentage represents cause specific count out of total graft loss count in each GN category.

limitation precluded formal analyses of the association between donor-recipient biologic relationship and disease recurrence. The lack of specific data on the cause of graft loss in a substantial proportion of kidney transplants could be due to different center practices and different thresholds to perform kidney transplant biopsies. Nonetheless, death and graft loss are strictly tracked in the SRTR allowing us to complete a robust analysis to help settle this important question.

Conclusion

In this large cohort study, biologic relationship was associated with lower rejection rates in IgA, lupus nephritis, and FSGS. Additionally, it was not associated with worse recipient or graft survival in any of the studied GN groups: MN, IgA, lupus nephritis, or FSGS. These findings are consistent with the known benefits of living related donor kidney transplant and counters reports about the adverse impact of donor recipient biologic relationship on allograft 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.

AUTHOR CONTRIBUTIONS

RE-R: concept/design, drafting article, data analysis/interpretation, critical revision of article, and approval of article. AB and RS: drafting article, critical revision of article, and approval of article. NK: data interpretation, critical revision of article, article approval and submission. SJ: data analysis/interpretation, critical revision of article, and approval of article. PN: concept/design, data analysis/interpretation, critical revision of article, and approval of article. SR: concept/design, data analysis/interpretation, drafting article, critical revision of article, and approval of article.

AUTHOR DISCLAIMER

The interpretation and reporting of these data are the responsibility of the authors and in no way should be seen as an official policy of or interpretation by the SRTR or the U.S. Government.

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.2023.11068/full#supplementary-material

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SYSTEMATIC REVIEW AND META-ANALYSIS
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Kidney Transplantation From Donors With Acute Kidney Injury: Are the Concerns Justified? A Systematic Review and Meta-Analysis

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Renal transplantation improves quality of life and prolongs survival in patients with end-stage kidney disease, although challenges exist due to the paucity of suitable donor organs. This has been addressed by expanding the donor pool to include AKI kidneys. We aimed to establish whether transplanting such kidneys had a detrimental effect on graft outcome. The primary aim was to define early outcomes: delayed graft function (DGF) and primary non-function (PNF). The secondary aims were to define the relationship to acute rejection, allograft survival, eGFR and length of hospital stay (LOS). A systematic literature review and meta-analysis was conducted on the studies reporting the above outcomes from PubMed, Embase, and Cochrane Library databases. This analysis included 30 studies. There is a higher risk of DGF in the AKI group (OR = 2.20, $p < 0.00001$). There is no difference in the risk for PNF (OR 0.99, $p = 0.98$), acute rejection (OR 1.29, $p = 0.08$), eGFR decline ($p = 0.05$) and prolonged LOS ($p = 0.11$). The odds of allograft survival are similar (OR 0.95, $p = 0.54$). Transplanting kidneys from donors with AKI can lead to satisfactory outcomes. This is an underutilised resource which can address organ demand.

Keywords: delayed graft function, acute kidney injury, primary non-function, donors and donation, graft outcome

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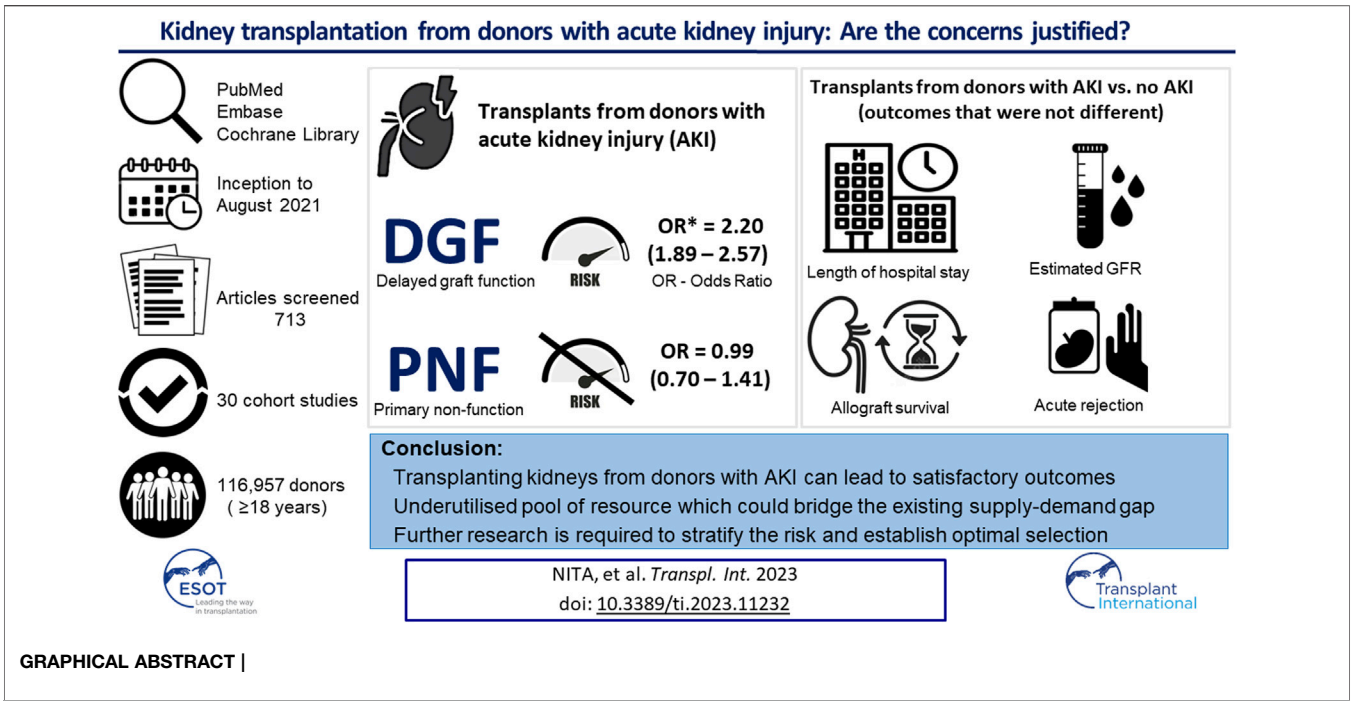
INTRODUCTION

Globally, the prevalence of chronic kidney disease (CKD) is approximately 13% [1]. Renal transplantation is a well-established safe procedure, shown to improve the quality of life (QoL) and prolong the life expectancy of CKD patients requiring renal replacement therapy (RRT) compared to dialysis [2–4]. There is an increasing demand for organs available for transplantation. The field of organ transplantation is constantly evolving and strategies such as expansion of the donor pool to include organs from donors after circulatory death (DCD) and the introduction of extended criteria donors (ECD) were adopted globally to address the disparity between organ supply and demand [5–7]. An additional strategy to overcome organ shortage is the utilisation of AKI donor kidneys. Despite this, the supply-demand mismatch remains significant. In 2019–2020 over 4,000 patients were active on the UK renal transplantation waiting list and less than 2,500 kidney transplants from deceased donors were performed nationally [8]. The waiting list mortality remains significant with 1-year and 3-year mortality reaching 2% and 4% in the UK. In the USA, the mortality rate has increased to 5.7 deaths per 100 waitlist years, the highest since 2012 [9, 10].



GRAPHICAL ABSTRACT |

The growing gap between supply and demand is exacerbated by discarding potentially usable organs. In the US, approximately 20% of the deceased donor kidneys are discarded annually [11, 12]. The discard rate for AKI donors remains high, reaching approximately 10% in the UK and 25% in the US [13]. Despite the introduction of the Kidney Donor Profile Index (KDPI) as a new allocation system in the US, the discard rate remains high [14].

This study aims to establish whether transplanting AKI donor kidneys has a detrimental effect on graft outcome, and subsequently to determine if AKI kidneys are a reasonable and safe option to address the organ shortage.

MATERIALS AND METHODS

A meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines [15]. Three databases were selected for literature search: PubMed (Medline), Embase (Ovid) and the Cochrane Library. To assess the quality of the studies included in this meta-analysis, the Newcastle-Ottawa Scale (NOS) and the Oxford Centre for Evidence-Based Medicine (OCEBM) hierarchy were utilised [16, 17].

Search Strategy

The search strategy included the terms "renal" or "kidney transplantation," "donor" or "donors" and "acute kidney injury" or "AKI." Databases were searched from inception to 1 May 2022. Two independent reviewers (GN and JG) performed a full-text screening of the studies. A third reviewer (DVD) resolved any conflicts.

- Medline (PubMed): ("renal transplantation"[tiab] OR "kidney transplantation[tiab]") AND ("acute kidney injury"[tiab] OR 'AKI'[tiab]) AND ("donor"[tiab] OR "donors"[tiab])
- Embase (Ovid): ((renal transplantation or kidney transplantation) and (acute kidney injury or AKI) and (donor or donors)) ab,ti.
- Cochrane Library: (renal transplantation OR kidney transplantation) AND (acute kidney injury OR AKI) AND (donor OR donors) in Title Abstract Keyword - (Word variations have been searched)

Inclusion and Exclusion Criteria

The aim was to identify all prospective studies (cohort studies or randomised control trials) performed in the adult population (≥18 years), reporting renal graft function, acute rejection, and graft survival, and comparing the outcome between donors with AKI versus non-AKI donors, from inception to May 2022 across PubMed (Medline), Embase (Ovid), and the Cochrane Library databases.

The inclusion criteria were defined as:

- studies reporting on adult patients (≥18 years of age)
- studies referring to patients receiving a renal transplantation as the primary and single transplant procedure AND
- comparing and reporting outcomes in the AKI and non-AKI donor groups
- articles fully accessible AND
- written in English

The exclusion criteria were defined as:

- studies reporting outcomes in the paediatric population (<18 years)

- studies comparing donors after brain death (DBD) with donors after circulatory death (DCD)
- studies reporting on simultaneous kidney pancreas (SPK) transplants or kidney re-transplantation/secondary transplant procedure
- studies on animal models
- studies lacking a control group
- case-series or low number studies (<50)
- abstracts-only available; letters or reviews
- full text not accessible or not available in English

Data Extraction and Quality Assessment

Data extracted from each study included: the first author name and publication year, country of origin, the study period and study design, the number of donors included, criteria utilised to define and classify AKI, mean donor age, gender, follow up period, and the reported endpoints (delayed graft function (DGF), primary non-function (PNF)), acute rejection, graft survival, eGFR at 1 year and duration of hospital stay). The Oxford Centre for Evidence-Based Medicine (OCEBM) 2011 Levels of Evidence hierarchy [16] and the 9-point Newcastle-Ottawa scale (NOS) were utilised to assess the level of evidence and quality of the studies included in the meta-analysis [17].

The initial search across the three databases returned 712 records (PubMed—160; Embase—343; Cochrane Library—164). 1 additional record was manually added (total n = 713). After the initial screening, 185 duplicate records were removed and 117 records were excluded. 68 records were further screened and 14 were further excluded (reviews and letters). 54 full-text articles were assessed for eligibility. Articles which could not be fully accessed, not written in English, reporting outcomes in the paediatric population, case series, reporting on different outcomes or lacking a control group (i.e., 24 records) were excluded. Finally, 30 studies were included comprising of 116,957 donors. This is illustrated in the PRISMA flowchart (**Figure 1**).

Five studies reported outcomes in two different groups: extended versus standard criteria donors—Kayler et.al. [18], Jacobi et al. [19], Heilman et al. [20], Ko et al. [21], and low versus high KDPI (Kidney Donor Profile Index)—Park et al. [22]. For these studies data was analysed separately comparing the outcomes for each subgroup of patients. The acceptable follow up period was established as 12 months post-transplantation for the study endpoints. Adequacy of follow-up was scored only where the follow up was complete and all the subjects were accounted for. No points were allocated for adequacy if the follow up rate was <80%, there was no description for lost to follow up patients or no statement with regards to follow up was made by the authors.

The primary outcome of this systematic review and meta-analysis was to determine the effect of transplanting AKI kidneys on the early graft function: delayed graft function (DGF) and primary non-function (PNF). The secondary aims were to determine the relationship between transplanting AKI donor kidneys and: acute rejection (AR), allograft survival, eGFR at 1-year post-transplantation, and length of hospital stay (LOS).

Data Analysis and Statistical Tests

The data was collated and analysed using Review Manager (RevMan) [Computer program]. Version 5.4. The Cochrane Collaboration, 2020.

Odds ratios (ORs) of every outcome and the 95% confidence intervals (CIs) were calculated for the dichotomous data (DGF, PNF, acute rejection and allograft survival). For the continuous data (eGFR and length of hospital stay), the weighted mean difference (WMD) and 95% confidence intervals (CI) were calculated. An estimate of the between-study variance was reported using the tau-squared (τ^2/Tau^2) and the Chi-squared (Chi^2) tests to assess whether the differences were due to chance. Accompanying p values were calculated for the heterogeneity tests. To quantify the percentage of variation due to heterogeneity the I^2 test was used. Thresholds for the interpretation of I^2 were established as per the Cochrane Handbook for Systematic Reviews of Interventions, Version 6.2, 2021 ("0%–40%: might not be important; 30%–60%: may represent moderate heterogeneity; 50%–90%: may represent substantial heterogeneity; 75%–100%: considerable heterogeneity") [23]. The random-effects model (Mantel-Haenszel method and the inverse variance methods) was chosen for this meta-analysis. The Z test was used for the pooled overall effect.

RESULTS

Study Characteristics & Quality Assessment

All the studies included in this meta-analysis were cohort studies (single centre, multi-centre, and National Transplant Registry analyses) from Europe, North America, Australia, and Asia. The study periods ranged between 1995 and 2017 with a follow-up period ranging between 12 months and 132 months. The main study characteristics are illustrated in **Table 1**.

The included studies correspond to Level 2 on the Oxford CEBM 2011 hierarchy [16]. The studies were assessed for quality according to the 9-point NOS (**Table 2**). Studies scoring 7 or greater on the NOS scale were regarded as good quality studies.

Primary Outcomes

Delayed Graft Function (DGF)

29 studies included in this meta-analysis reported on the incidence of DGF in the donor AKI versus the non-AKI groups [13, 14, 18–22, 24–41, 43–46]. The pooled odds of DGF are higher in the AKI group vs. the non-AKI group (OR = 2.20, 95% CI = 1.89–2.57, I^2 = 87%, Z = 10.05, p < 0.00001 (**Figure 2**).

Primary Non-Function (PNF)

5 studies: Farney et al. [26], Jacobi et al [19], Boffa et al. [13], Bauer et al [37] and Liu et al. [45], reported the incidence of PNF. The pooled result demonstrates no significant difference in the odds of developing PNF in AKI versus the non-AKI groups (OR 0.99, 95% CI = 0.70–1.41, I^2 = 43%, Z = 0.03, p = 0.98) (**Figure 3**).

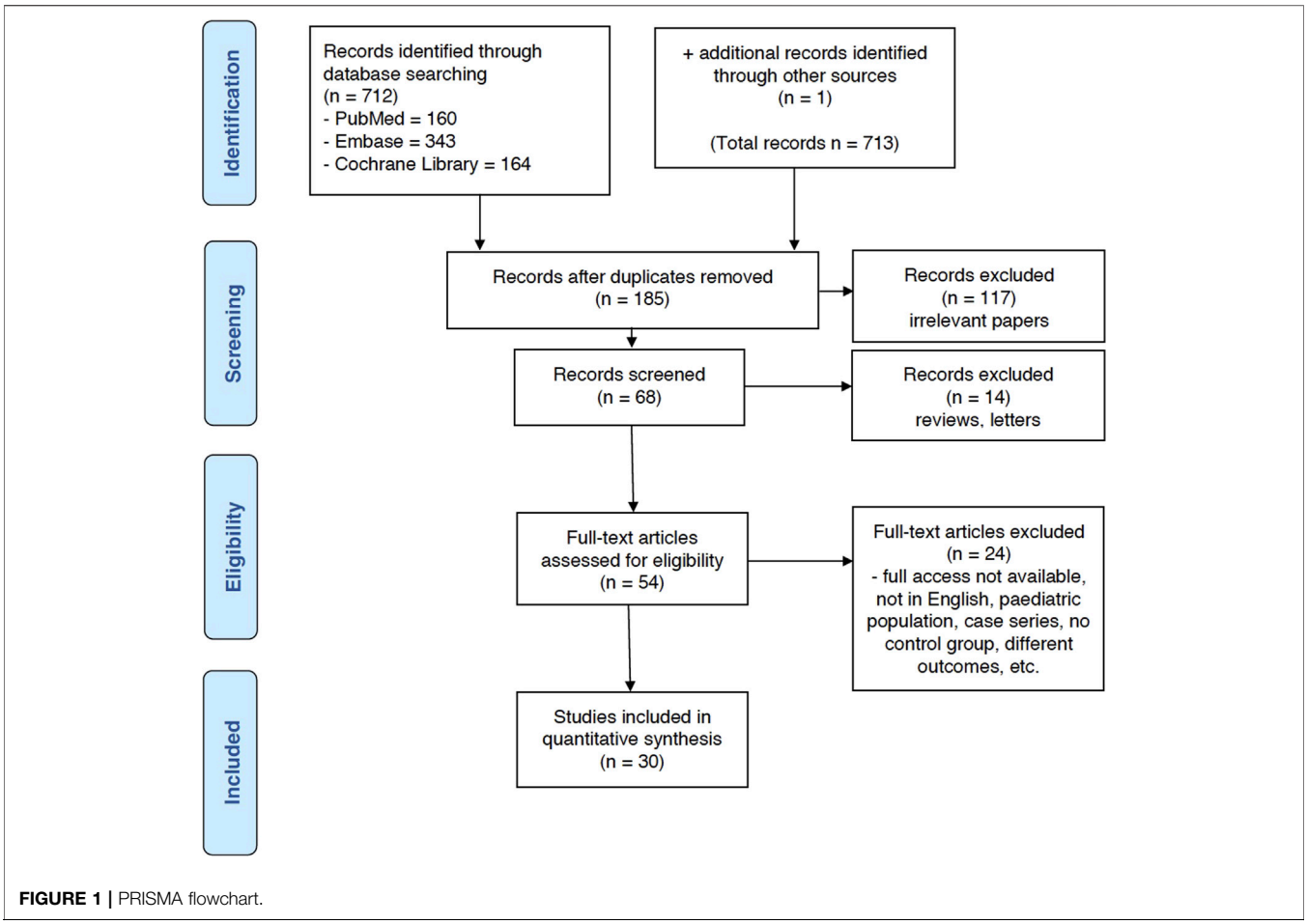


FIGURE 1 | PRISMA flowchart.

Secondary Outcomes

Acute Rejection

Data from 17 studies [14, 20–22, 24, 29–33, 35, 37, 38, 40, 41, 44, 46] reporting acute rejection was pooled. The results show no significant difference in the odds of acute rejection between donor AKI vs. non-AKI kidneys groups (OR 1.29, 95% CI = 0.97–1.71, $I^2 = 76\%$, Z = 1.75, $p = 0.08$). (**Figure 4**).

Allograft Survival

27 studies reported on allograft survival [13, 14, 18–22, 24–26, 28, 31–46]. The Forrest plot demonstrates similar odds of allograft survival between the two groups (OR 0.95, 95% CI = 0.81–1.12, $I^2 = 75\%$, Z = 0.61, $p = 0.54$). (**Figure 5**).

Estimated Glomerular Filtration Rate (eGFR)

14 studies [14, 19–22, 28–30, 35, 37, 39, 43–45] reported the eGFR at 12 or more months post-renal transplantation. The pooled results show similar eGFR levels between the AKI and non-AKI populations (WMD = −2.09, 95% CI = −3.56 to 0.62, $I^2 = 41\%$, Z = 2.79, $p = 0.05$) (**Figure 6**).

Length of Hospital Stay

4 studies [19, 32, 35, 37] reported the duration of hospitalisation in the 2 groups. These results demonstrate similar hospital stay length between the 2 populations (WMD = 1.52, 95% CI = −0.35 to 3.38, $I^2 = 18\%$, Z = 1.59, $p = 0.11$) (**Figure 7**).

DISCUSSION

AKI is highly common in the ITU population with over 35% of patients in ITU will developing AKI at some stage during their admission [24, 47]. Overall, the evidence from the single-centre, multi-centre and national registry studies included in this systematic review and meta-analysis supports transplanting these kidneys, potentially providing a significant boost to the prospective donor pool and reducing waitlist mortality. The UK transplant registry analysis found that 17% of the potential kidney donors had AKI. During a 10-year period (2003–2013) over 1,600 recipients received a kidney from a donor with AKI and had a functioning graft at 1-year post-transplant [13].

Kayler et al. [18] is the first large US transplant registry analysis investigating AKI donor kidneys. Their cohort of over 80,000 kidney transplant recipients was stratified based on the terminal serum creatinine levels (tSCr). Of note, high risk kidneys (deemed as those with tSCr >2 mg/dL) only represented 22% of the total pool of donors. This study demonstrated higher DGF rates in the AKI donor population, particularly in the ECD

TABLE 1 | Main study characteristics.

	Author	Year	Country	Study design	Study period	AKI criteria	Total no. of donors (n)	Mean donor age	Donor gender (M:F ratio)	Follow up (months)	Endpoints
1	Kayler et al.* (SCD) [18]	2009	USA	Cohort study[a]	1995–2007	tSCr	48,558	37	-	120	DGF allograft survival
	Kayler et al.* (ECD) [18]	2009	USA	Cohort study[a]	1995–2007	tSCr	17,051	37	-	120	DGF allograft survival
2	Rodrigo et al. [24]	2010	Spain	Cohort study[b]	1994–2006	RIFLE	176	AKI: 46.3 ± 13.2 non-AKI: 45.8 ± 16.7	AKI: 1.7: 1 non-AKI: 1.2:1	-	DGF acute rejection allograft survival
3	Kolonko et al. [25]	2011	Poland	Cohort study[b]	1996–2006	RIFLE	61	AKI: 50 non-AKI: 43	AKI: 1.5: 1 non-AKI: 2.4:1	49 ± 18	DGF allograft survival
4	Farney et al. [26]	2013	USA	Cohort study[b]	2007–2012	tSCr	367	AKI: 36 ± 13 non-AKI: 35 ± 15	AKI: 3.2: 1 non-AKI: 1.2:1	35 (6–70)	DGF PNF allograft survival
5	Jung et al. [27]	2013	Korea	Cohort study[b]	2009–2012	RIFLE	54	AKI: 45.67 ± 14.27 non-AKI: 50.39 ± 25.18	AKI: 8: 1 non-AKI: 1.6:1	23.2 ± 10.4	DGF
6	Jacobi et al.* (SCD) [19]	2014	Germany	Cohort study[b]	2008–2014	RIFLE	208	AKI: 42.5 ± 12.6 non-AKI: 39.5 ± 11.8	AKI: 2.7: 1 non-AKI: 0.9:1	12	DGF PNF allograft survival + eGFR + hospital stay
	Jacobi et al.* (ECD) [19]	2014	Germany	Cohort study[b]	2008–2014	RIFLE	174	AKI: 66.9 ± 9.5 non-AKI: 67.7 ± 6.9	AKI: 1.6: 1 non-AKI: 0.8:1	12	DGF PNF allograft survival + eGFR + hospital stay
7	Lee et al. [28]	2014	Korea	Cohort study[b]	1996–2012	AKIN	156	AKI: 43.3 ± 13.8 non-AKI: 41.1 ± 14.6	AKI: 0.3: 1 non-AKI: 2.3:1	12	DGF allograft survival + eGFR
8	Yu et al. [29]	2014	China	Cohort study[b]	2005–2011	RIFLE	57	AKI: 40 ± 9.8 non-AKI: 35 ± 12.2	AKI: 2.8: 1 non-AKI: 2.5:1	12	DGF acute rejection + eGFR
9	Yuan et al. [30]	2014	China	Cohort study[b]	2011–2013	RIFLE	89	AKI: 37 ± 15.2 non-AKI: 37.5 ± 13.5	AKI: 2.3: 1 non-AKI: 4:1	18 (7–26)	DGF acute rejection + eGFR
10	Molina et al. [31]	2015	Spain	Cohort study[b]	1976–2013	tSCr	118	AKI: 52 ± 13 non-AKI: 50 ± 13	AKI: 1.1: 1 non-AKI: 1.1:1	AKI: 101 mo ± 67 Non-AKI: 99 mo ± 70	DGF allograft survival
11	Ali et al. [32]	2015	Saudi Arabia	Cohort study[b]	2000–2012	AKIN	261	AKI: 36.7 ± 11.0 non-AKI: 35.0 ± 13.0	AKI: 4.6: 1 non-AKI: 10:1	120	DGF acute rejection allograft survival + eGFR
12	Benck et al. [33]	2015	Germany	Cohort study[b]	-	RIFLE	98	AKI: 53 ± 13 non-AKI: 54.8 ± 15.5	AKI: 3.1:1 (25/8) Non-AKI: 0.8:1 (28/37)	-	DGF allograft survival
13	Hall et al. [34]	2015	USA	Cohort study[c]	2010–2013	AKIN	1,369	AKI: 39 non-AKI: 41	AKI: 1.7: 1 non-AKI: 1.5:1	20 (11.5–28.5)	DGF
14	Heilman et al.* (SCD) [20]	2015	USA	Cohort study[b]	2004–2013	AKIN	621	AKI: 32.3 ± 13.2 non-AKI: 34.5 ± 15.4	AKI: 3.5: 1 non-AKI: 1.6:1	19.6–41.4	DGF acute rejection allograft survival + eGFR + hospital stay
	Heilman et al.* (ECD) [20]	2015	USA	Cohort study[b]	2004–2013	AKIN	160	AKI: 56.6 ± 9.1 non-AKI: 61.6 ± 9.2	AKI: 2.8: 1 non-AKI: 1:1	12.3–23.8	DGF acute rejection allograft survival eGFR hospital stay
15	Wiwattanathum et al. [35]	2016	Thailand	Cohort study[b]	2012–2013	AKIN	111	AKI: 43.9 ± 12.0 non-AKI: 42.9 ± 19.9	AKI: 2.2: 1 non-AKI: 1.1:1	48	DGF

(Continued on following page)

TABLE 1 | (*Continued*) Main study characteristics.

	Author	Year	Country	Study design	Study period	AKI criteria	Total no. of donors (n)	Mean donor age	Donor gender (M:F ratio)	Follow up (months)	Endpoints
16	Boffa et al. [13]	2017	UK	Cohort study[a]	2003–2013	AKIN	11,219	-	AKI: 1.8: 1 non-AKI: 1:1	12	DGF PNF allograft survival
17	Kim et al. [36]	2017	Korea	Cohort study[b]	1996–2014	KDIGO AKIN	285	AKI: 49.1 ± 11.3 non-AKI: 46.5 ± 8.0	AKI: 1: 1 non-AKI: 1.3:1	-	DGF allograft survival eGFR
18	Bauer et al. [37]	2018	Germany	Cohort study[b]	2005–2016	pSCr	642	AKI: 49.31 ± 16.34 non-AKI: 55.28 ± 16.08	AKI: 3.7: 1 non-AKI: 0.6:1	55.82 ± 34.97	DGF PNF acute rejection allograft survival eGFR
19	Yeon et al. [38]	2018	Korea	Cohort study[b]	2005–2014	KDIGO	413	AKI: 45 [35–56] non-AKI: 48 [35–55]	AKI: 1.7: 1 non-AKI: 1.9:1	52.8	DGF acute rejection allograft survival
20	Ko et al.* (SCD) [21]	2018	Korea	Cohort study[b]	2000–2013	AKIN	149	AKI: 38.5 ± 10.0 non-AKI: 39.4 ± 14.9	AKI: 3.5: 1 non-AKI: 1.6:1	40.3	DGF allograft survival eGFR
	Ko et al.* (ECD) [21]	2018	Korea	Cohort study[b]	2000–2013	AKIN	53	AKI: 56.7 ± 6.1 non-AKI: 58.4 ± 4.7	AKI: 3.5: 1 non-AKI: 1.2:1	40.3	DGF allograft survival eGFR
21	Gwon et al. [39]	2018	Korea	Cohort study[b]	2009–2015	AKIN	101	AKI: 46.2 ± 13.5 non-AKI: 51.0 ± 20.1	AKI: 7: 1 non-AKI: 1.3:1	-	DGF allograft survival eGFR
22	Torabi et al. [40]	2019	USA	Cohort study[b]	2014–2016	AKIN	285	AKI: 56.1 ± 13.7 non-AKI: 56.9 ± 12.1	AKI: 1.8: 1 non-AKI: 2:1	-	DGF acute rejection allograft survival eGFR
23	Domagala et al. [41]	2019	Poland	Cohort study[b]	2010–2011	tSCr	226	AKI: 42 ± 15 non-AKI: 47 ± 15	AKI: 4: 1 non-AKI: 1.2:1	60	DGF acute rejection allograft survival hospital stay
24	Hall et al. [42]	2019	USA	Cohort study[c]	2010–2013	AKIN	1,298	AKI: 41 ± 14 non-AKI: 42 ± 15	AKI: 1.7: 1 non-AKI: 1.5:1	48	allograft survival
25	Schütte-Nütgen et al. [43]	2019	Germany	Cohort study[b]	2004–2014	AKIN	214	AKI: 54.3 ± 17.2 non-AKI: 51.1 ± 16.5	AKI: 1.4: 1 non-AKI: 1.3:1	60	DGF allograft survival eGFR
26	van der Windt et al. [44]	2019	USA	Cohort study[b]	2013–2017	AKIN	333	AKI: 41.5 ± 12.9 non-AKI: 41.3 ± 13.7	AKI: 1.3: 1 non-AKI: 1.7:1	32	DGF acute rejection allograft survival eGFR
27	Liu et al. [45]	2020	USA	Cohort study[a]	2010–2013	KDIGO	25,323	AKI: 42 (28–52) non-AKI: 42 (27–52)	AKI: 1.7: 1 non-AKI: 1.7:1	60	DGF PNF allograft survival eGFR
28	Pei et al. [46]	2021	Australia & New Zealand	Cohort study[a]	1997–2017	KDIGO	5,744	AKI: 46 (30–58) non-AKI: 46 (30–58)	AKI: 2: 1 non-AKI: 1.2:1	62 (24–114)	DGF acute rejection allograft survival
29	Kim et al. [14]	2021	Korea	Cohort study[b]	2003–2016	KDIGO	376	AKI: 47.9 ± 14.1 non-AKI: 44.2 ± 16.0	AKI: 2.1: 1 non-AKI: 1.8:1	AKI: 78 (51–103) non-AKI: 96 (63–132)	DGF acute rejection allograft survival eGFR
30	Park et al.** (lKDPI) [22]	2021	Korea	Cohort study[c]	1996–2017	KDIGO	269	AKI: 36.4 ± 10.7 non-AKI: 34.8 ± 13.7	AKI: 3.6: 1 non-AKI: 2.3:1	48 (22.3–68)	DGF acute rejection allograft survival eGFR
	Park et al.** (hKDPI) [22]	2021	Korea	Cohort study[c]	1996–2017	KDIGO	338	AKI: 54.5 ± 8.3 non-AKI: 56.2 ± 10.0	AKI 2.5: 1 non-AKI: 1.2:1	48 (22.3–68)	DGF acute rejection allograft survival eGFR

[a]*National Transplant Registry analysis.*
[b]*Single-centre cohort study.*
[c]*Multi-centre cohort study.*
**Standard Criteria Donors (SCD); Extended Criteria Donors (ECD).*
***Low Kidney Donor Profile Index (lKDPI); High Kidney Donor Profile Index (hKDPI).*
AKI, Acute Kidney Injury; tSCr, (donor) terminal Serum Creatinine; pSCr—(donor) peak Serum Creatinine; DGF, Delayed Graft Function; PNF, Primary Non-Function; eGFR, estimated Glomerular Filtration Rate.

TABLE 2 | Quality assessment of non-randomised cohort studies (Newcastle-Ottawa Scale).

	Author (year)	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration outcome of interest not present at start	Comparability of cohorts	Assessment of outcome	Follow-up period	Follow-up adequacy	Total (out of 9)
1	Kayler et al. [18]	★	★	★	★	★	★	★	★	8
2	Rodrigo et al. [24]	★	★	★	★		★		★	6
3	Kolonko et al. [25]	★	★	★	★	★	★	★	★	8
4	Farney et al. [26]	★	★	★	★		★	★	★	7
5	Jung et al. [27]	★	★	★	★	★	★	★		7
6	Jacobi et al. [19]	★	★	★	★		★	★		6
7	Lee et al. [28]	★	★	★	★	★	★	★		7
8	Yu et al. [29]	★	★	★	★		★	★	★	7
9	Yuan et al. [30]	★	★	★	★	★	★	★		7
10	Molina et al. [31]	★	★	★	★	★	★	★		7
11	Ali et al. [32]	★	★	★	★	★	★	★	★	8
12	Benck et al. [33]	★	★	★	★	★	★			6
13	Hall et al. [34]	★	★	★	★	★	★	★	★	8
14	Heilman et al. [20]	★	★	★	★	★	★	★		7
15	Wiwattanathum et al. [35]	★	★	★	★	★	★	★		7
16	Boffa et al. [13]	★	★	★	★	★	★	★		7
17	Kim et al. [36]	★	★	★	★	★★	★	★		8
18	Bauer et al. [37]	★	★	★	★	★	★	★		7
19	Yeon et al. [38]	★	★	★	★	★	★	★		7
20	Ko et al. [21]	★	★	★	★	★	★	★	★	8
21	Gwon et al. [39]	★	★	★	★		★	★		6
22	Torabi et al. [40]	★	★	★	★	★★	★	★		8
23	Domagala et al. [41]	★	★	★	★		★	★	★	7
24	Hall et al. [42]	★	★	★	★		★	★	★	7
25	Schütte-Nütgen et al. [43]	★	★	★	★		★	★	★	7
26	van der Windt et al. [44]	★	★	★	★	★	★	★	★	8
27	Liu et al. [45]	★	★	★	★	★★	★	★	★	9
28	Pei et al. [46]	★	★	★	★	★★	★	★	★	9
29	Kim et al. [14]	★	★	★	★	★★	★	★		8
30	Park et al. [22]	★	★	★	★	★	★	★		7

Maximum of ★ awarded for each item except for "comparability" where a maximum of ★★ can be awarded.
A study scoring 7 and above was regarded as a good quality cohort study.
Acceptable follow up period was established at least 12 months for the endpoints.

donors (36% in the SCD with AKI, 41% in the ECD with AKI, compared to 21% and 32% in the same groups when AKI was not present). This was the first study to demonstrate that a raised level of tSCr in the donor had no impact on the long term-graft survival from SCD kidneys. Our meta-analysis also supports the same long-term outcomes.

In the ECD group however, worse outcomes were recorded suggesting that parenchymal chronic changes could have a significant effect. This is in keeping with the existing knowledge suggesting that recovery of renal function is inversely proportional to age. A population of donors >65 years with comorbidities may have less likelihood of recovery of function [48, 49].

Kayler et al. [18] also highlights an important observation that kidneys with good urine output and no chronic changes on biopsy had comparable outcomes to those in which the SCr stabilised in terms of PNF, DGF and 1-year graft survival. One of the important limitations in this study is the reliance on tSCr without taking into consideration initial or peak levels. As kidneys with high tSCr are generally considered "high-risk," this study was prone to selection bias. In addition, data on the donor urine output, RRT need, and perfusion technique, which are independent discriminating variables, have not been accounted for.

Rodrigo et al. [24] is the first study to apply the RIFLE (Risk, Injury, and Failure; and Loss, and End-stage kidney disease) criteria [50] to analyse the kidney damage. This classification considers the renal function dynamic as opposed to focusing on tSCr values. In this study, AKI donor kidney recipients had a higher risk of DGF, higher immediate creatinine levels and lower urine output. Importantly, these seem to normalise from 6 months onwards. The long-term graft function and 5-year graft survival were

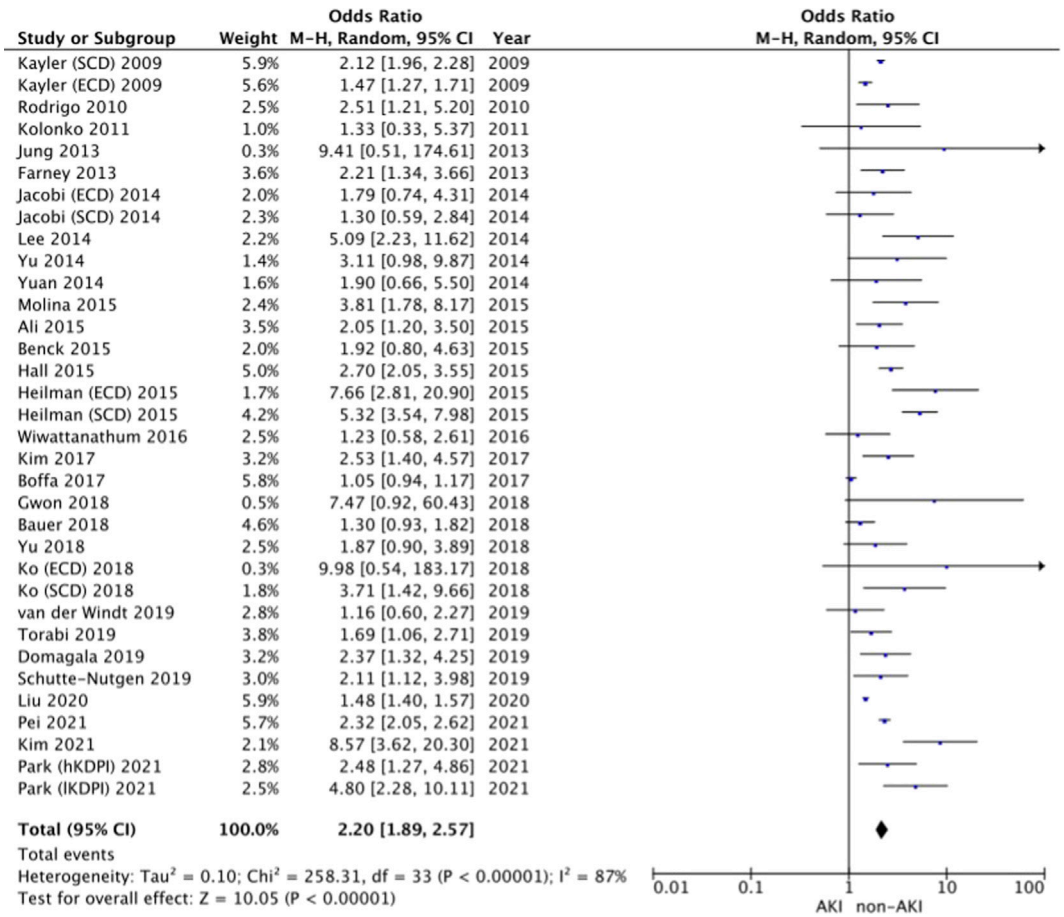


FIGURE 2 | Delayed graft function—Forrest plot.

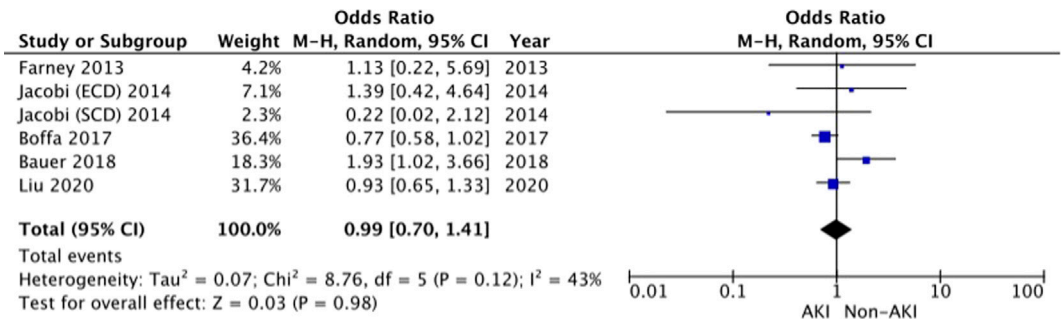


FIGURE 3 | Primary non-function—Forrest plot.

similar (58.4% AKI vs. 61.5% non-AKI kidneys). This study demonstrates that age, hypertension, a higher APACHE II score, hypotensive episodes, and length of ICU stay, are directly proportional with the chance of developing AKI. This is an important finding as the life expectancy and comorbidity status of the general population is on the rise. In this study, 85% of the donors had either traumatic head injury or a cerebrovascular accident (CVA), and this might not be representative of general ITU patient population but does probably represent standard donor population. In addition, it is important to note the limited population in the 'failure' category. Yu et al. [29] had a significantly higher cohort of patients in this category, and demonstrated no statistical difference in relation to PNF, DGF, acute rejection and renal function and graft survival. They observed that the risk of DGF rises exponentially with the increase in the AKI severity.

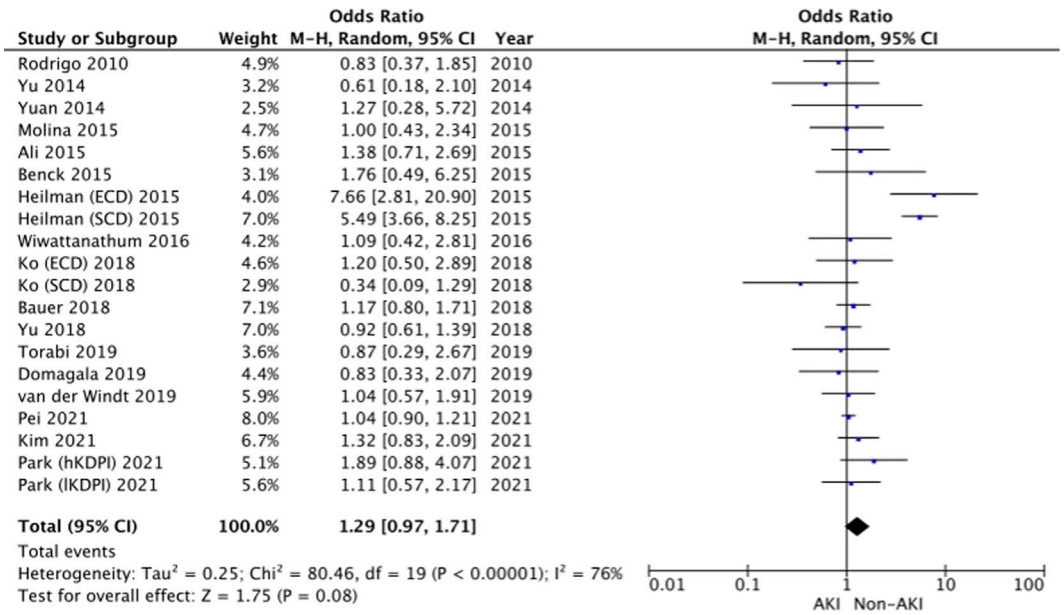


FIGURE 4 | Acute rejection—Forrest plot.

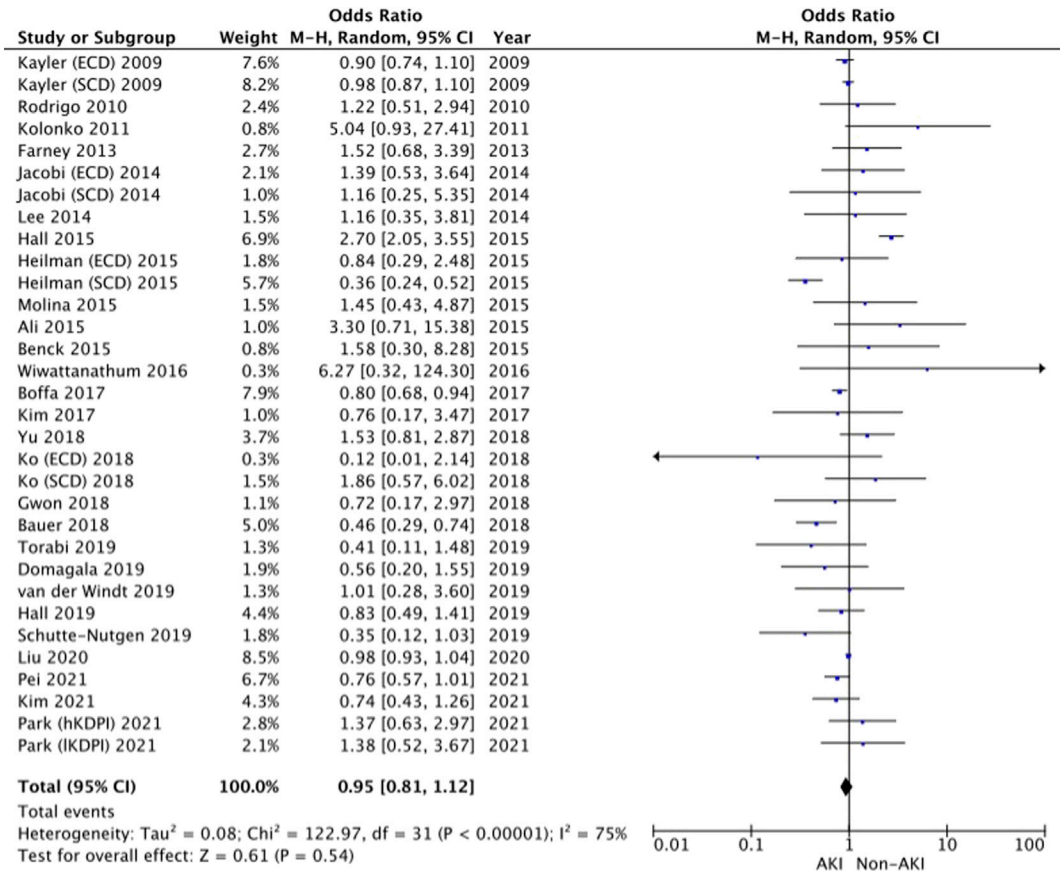


FIGURE 5 | Allograft survival–Forrest plot.

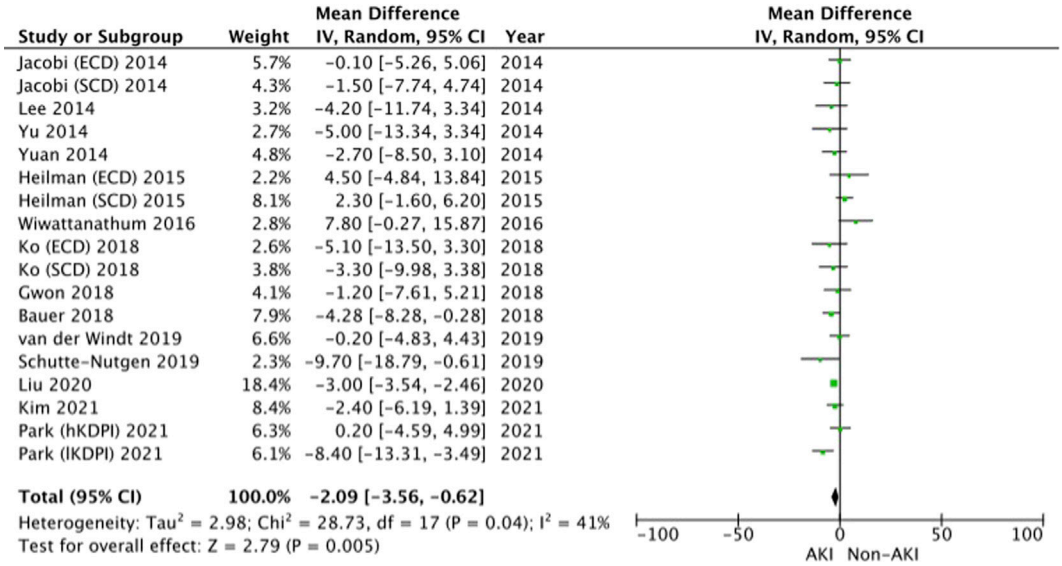


FIGURE 6 | eGFR—Forrest plot.

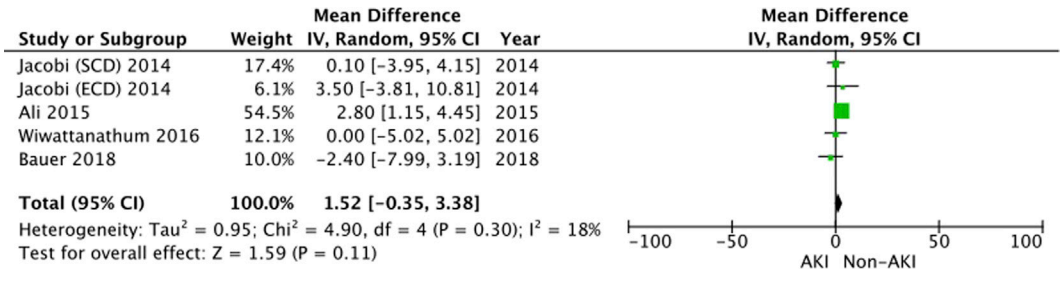


FIGURE 7 | Length of hospital stay—Forrest plot.

In contrast with Rodrigo et al. [24], Kolonko et al. [25] reported inferior immediate graft function in the AKI group (30% vs. 10%) and a higher risk of graft loss (28% vs. 7%). However, there was no substantial difference in the longer-term renal function (eGFR >12 months). These findings were also consistent with our meta-analysis.

The link between AKI and DGF was demonstrated by Farney et al. [26], 30% of the recipients of an AKI kidney being at risk of developing DGF compared to 13% in the non-AKI donor population. Although the study suggests a lower 3-year graft survival when DGF is present, there is no difference between the 2 populations (68% vs. 90% non-AKI with and without DGF vs. 89% and 91% AKI with and without DGF). The SCr levels are similar at 1- and 2-year post-transplantation across the entire cohort, reiterating the hypothesis that renal function recovery begins in the donor and continues post-transplantation. This study highlights the importance of knowing the baseline renal function as variation from it cannot be established in its absence.

In Jung et al. [27] the terminal serum creatinine (tSCr) was determined as an independent risk factor of DGF and slow graft function (19% in the AKI group vs. 5% in the non-AKI group). In accordance with the previous reported findings, the long-term allograft function and rejection-free survival do not significantly differ in this study. Jacobi et al. [19] demonstrated similar findings, although the study reported the lowest allograft survival rate in ECD population at 78%. Their subgroup analysis revealed that most graft losses were secondary to perioperative complications rather than due to the AKI status, which is an important confounding feature.

Lee et al. [51] was the first study to utilise the AKIN classification [52] as opposed to the previously used RIFLE

criteria or tSCr. By applying the AKIN criteria, the results remained consistent with the existing knowledge demonstrating a higher rate of DGF in the AKI group (42% vs. 12%) and a non-inferior medium-term graft survival. Similarly, Ali et al. [32] demonstrated an exponential increase in DGF, 60% of the AKIN stage 3 population developing DGF vs. 25% in the non-AKI donor group.

Benck et al. [33], Hall et al. [34] and Heilman et al. [20] also reported comparable findings in terms of DGF, allograft function and survival. An important advantage of the latter study is that it only excluded kidneys with cortical necrosis or moderate-severe chronic changes on biopsy. If these criteria would be extrapolated, this study estimates that a further 31% SCD and 22% ECD kidneys in the US could become transplantable.

With the emergence of the KDIGO classification, the question about whether previous AKI classification systems are inferior arose. Kim et al. [36] addressed this by comparing the KDIGO and AKIN criteria and demonstrated that although KDIGO criteria has a better predictive value for DGF, both provide similar predictive value with respect to allograft function and survival.

van Der Windt [44] investigated the link between AKI kidneys and histology, demonstrating a similar degree of fibrosis on biopsies obtained 1-year post-transplantation, reiterating that recovery continues in the recipient. The limitation of this study lies in their cohort of mainly AKIN stage 1 kidneys, rendering the study underpowered to draw a conclusion regarding higher degrees of AKI.

In the UK, Boffa et al. [13] published a large national transplant registry analysis comprising of 11,000 donors. This is the first study in the literature demonstrating contrasting results in the rates of graft failure at 1 year compared to the previous studies. They have reported a reduction in 1-year graft survival in the AKI group by 2% (89% vs. 91%), however the clinical significance of this remains limited particularly if balanced against the annual death rate by remaining on the waiting list. This is in contradiction with our meta-analysis which found no significant difference in 1-year graft survival. Their results also showed that approximately 28% of kidneys were not utilised, and AKI stage 3 kidneys being 20 times more likely to be discarded. In contrast to the previous studies linking age with the likelihood of developing CKD, the Cox-regression analysis did not identify age as an independent risk factor. Caution was advised regarding utilisation of AKI stage 3 donors given the higher rates of DGF (three times greater vs. non-AKI) and PNF (9% vs. 4%). They have suggested counselling patients in regards to the risks and benefits of AKI kidneys when considering the utilisation of AKI stage 3 kidneys. *Bauer et al.* [37] employed this strategy successfully, showing that in their cohort, none of the patients refused transplantation from such kidneys.

In contrast, Liu et al. [45], a large registry-based, propensity-matched cohort study of over 25,000 recipients, showed that AKI status had no correlation with death-censored graft failure (HR 1.01; 95% CI 0.95–1.08) or all-cause graft failure (HR 0.97; 95% CI 0.93–1.02), across all AKI stages.

These findings were replicated by Pei et al. [46], which demonstrated that donor AKI stage did not negatively correlate with post-transplant outcome (allograft failure, mortality, acute rejection), except for DGF (44% in the AKI donor group vs. 26% in the non-AKI group). However, interpretation of this remains limited in high stage AKI, given the lower numbers in the AKI stage 3 category. This study demonstrated acceptable overall outcomes when transplanting kidneys from donors with AKI in line with previously published data [53].

Our meta-analysis has several limitations. Firstly, there is a considerable heterogeneity in the included studies, particularly when reporting DGF, acute rejection and allograft survival. This is unavoidable due to the population and methodological diversity [54]. This was accounted for by using a random-effects model when performing this meta-analysis.

Secondly, all the studies included in this meta-analysis were retrospective cohort studies. A particular drawback of retrospective studies is selection bias. A larger proportion of 'lower risk' AKI kidneys could have potentially been selected as acceptable for transplant, particularly in the early studies as the AKI donor profile was emerging. However, a randomised control trial addressing this would be logistically and ethically challenging to perform.

The large number of pooled donors (over 110,000), provide a good population size and renders our meta-analysis findings both representative and generalisable. There appears to be no significant difference in the odds of allograft survival (OR 0.95, 95% CI = 0.81–1.12, $p = 0.54$) between the two groups. This data should be interpreted cautiously as the included studies reported a mixture of death-censored and non-death censored graft survival over variable lengths of time (ranging from 12 to 120 months). Hazard ratios (HR) could not be calculated due to under-reporting of specific values in the literature. In addition, subgroup analyses stratifying the risk according to the AKI stage or determining if there are different outcomes between current and recovering AKI was also not reported in most of the studies included in this meta-analysis.

The criteria utilised to define AKI was also inconsistent. This is unavoidable due to the temporal evolution of these classification systems (RIFLE, AKIN, KDIGO). However, multiple studies demonstrate no significant differences in the prognostic value of these systems [36, 55–58].

As the acceptable AKI kidney donor profile is developing, future research is required to determine the long-term outcomes, risk stratification and optimal selection methods of these kidneys. Development of accurate AKI biomarkers to predict post-transplant outcomes would aid the selection of AKI donor kidneys [59–61]. Novel perfusion strategies are also increasingly being utilised in the assessment and pre-conditioning of organs. Normothermic regional perfusion (NRP) is a promising emerging technique which could provide functional assessment and ischaemic pre-conditioning of donor organs. Early existing data supports this, demonstrating that NRP reduces the rates of DGF and PNF in the post-DCD transplantation population [62–64].

CONCLUSION

This study demonstrates that transplanting kidneys from donors with AKI can lead to satisfactory outcomes. The rates of DGF are higher in this population but does not seem to impact long-term allograft function and survival. With higher AKI stage kidneys, a

degree of caution is advised, however, these organs could be judiciously utilised discussing the potential benefits and risks on an individual basis. Donor kidneys with AKI remain an underutilised pool of resource which could help bridge the existing gap between supply and demand, ultimately improving outcomes and survival for transplant waitlisted patients.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors have contributed to the concept or design of this work. GN and JG contributed to the acquisition, analysis or interpretation of data for this work. All authors have contributed to writing this work and have approved its submission for publication. DD is the guarantor of this work.

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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GLOSSARY

ACR	Albumin:Creatinine Ratio
ADQI	Acute Dialysis Quality Initiative
AKI	Acute Kidney Injury
AKIN	Acute Kidney Injury Network
AR	Acute Rejection
ARF	Acute Renal Failure
AS	Allograft Survival
CEBM	(Oxford) Centre of Evidence-Based Medicine
CIT	Cold Ischaemia Time
CKD	Chronic Kidney Disease
DBD	Donation after Brainstem Death
DCD	Donation after Circulatory Death
DGF	Delayed Graft Function
ECD	Expanded Criteria deceased Donors
ESKD	End-Stage Kidney Disease
(e)GFR	(estimated) Glomerular Filtration Rate
KDIGO	Kidney Disease: Improving Global Outcomes
KDPI	Kidney Donor Profile Index
NHS	National Health Service
NHSBT	NHS Blood and Transplant
NICE	National Institute for Health and Care Excellence
NOS	Newcastle-Ottawa Scale
NRP	Normothermic Regional Perfusion
PNF	Primary Non-Function
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
pSCr	peak Serum Creatinine
QoL	Quality of Life
RIFLE	Risk-Injury-Failure-Loss-End stage kidney disease
RRT	Renal Replacement Therapy
SaBTO	Advisory Committee on the Safety of Blood, Tissues and Organs
SCD	Standard Criteria deceased Donors
SCr	Serum Creatinine
tSCr	terminal Serum Creatinine
UO	Urine Output

ESOT | Transplant International

ORIGINAL RESEARCH
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How Large is the Potential of Brain Dead Donors and what Prevents Utilization? A Multicenter Retrospective Analysis at Seven University Hospitals in North Rhine-Westphalia

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Organ donation after brain death is constantly lower in Germany compared to other countries. Instead, representative surveys show a positive attitude towards donation. Why this does not translate into more donations remains questionable. We retrospectively analyzed all potential brain dead donors treated in the university hospitals of Aachen, Bielefeld, Bonn, Essen, Düsseldorf, Cologne and Münster between June 2020 and July 2021. 300 potential brain dead donors were identified. Donation was utilized in 69 cases (23%). Refused consent (n = 190), and failed utilization despite consent (n = 41) were reasons for a donation not realized. Consent was significantly higher in potential donors with a known attitude towards donation (n = 94) compared to a decision by family members (n = 195) (49% vs. 33%, p = 0.012). The potential donor´s age, status of interviewer, and the timing of the interview with decision-makers had no influence on consent rates, and it was comparable between hospitals. Refused consent was the predominant reason for a donation not utilized. Consent rate was lower than in surveys, only a known attitude towards donation had a significant positive influence. This indicates that survey results do not translate well into everyday clinical practice and promoting a previously documented decision on organ donation is important.

Keywords: organ donation, brain death, consent, opt-in, decision-maker

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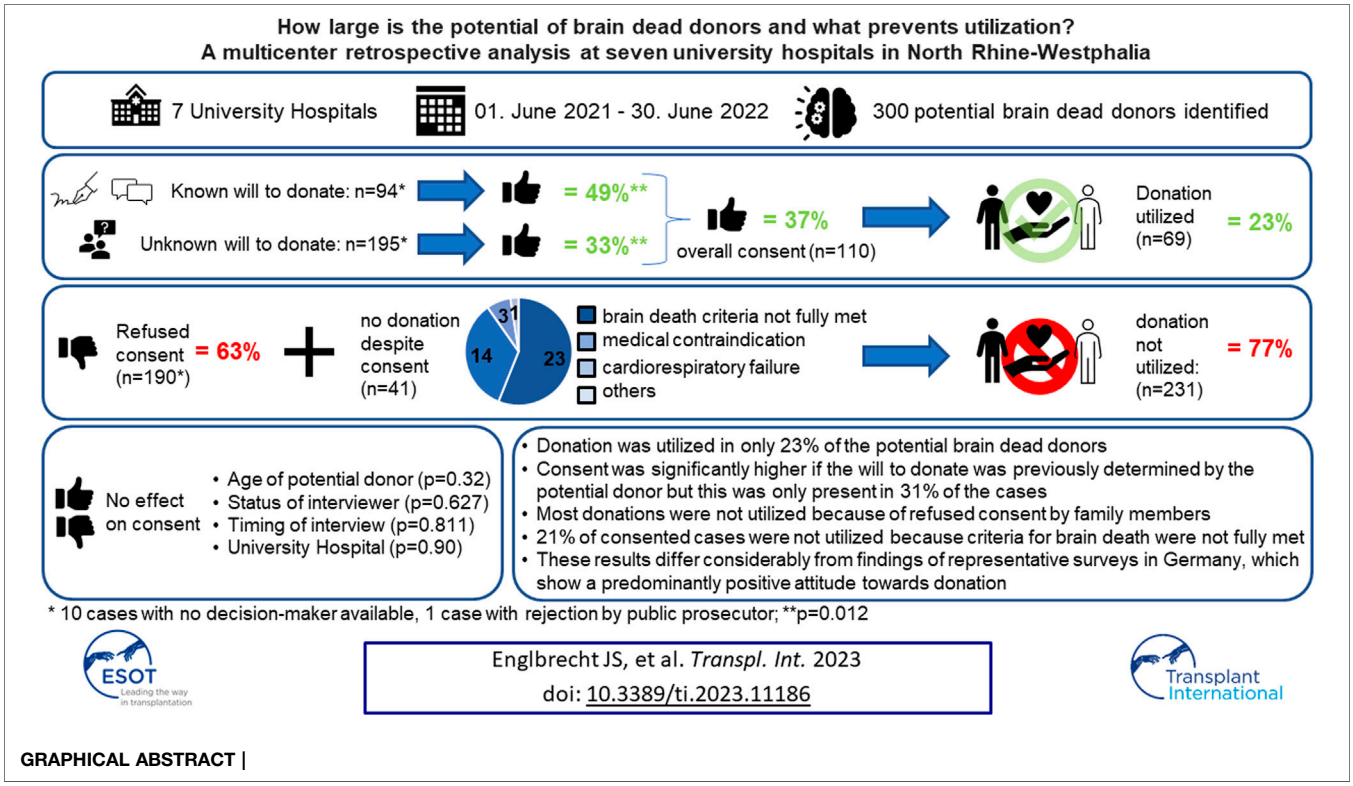

GRAPHICAL ABSTRACT |

INTRODUCTION

The number of utilized donations after brain death (DBD) has remained at a low level in Germany in recent years and is comparatively low in contrast to other countries [1, 2]. Spain realized 40.2 donations per one million inhabitants in 2021, the United States of America 41.9 and Germany 11.2, respectively [1]. There was a remarkable drop in utilized DBD in 2012 because of an organ allocation scandal in Germany [3] and donations since then have not returned to previous levels [2].

On the other hand, results from representative surveys by the Federal Centre for Health Education ("Bundeszentrale für gesundheitliche Aufklärung" - BZgA) show a constantly positive attitude towards organ donation, which was not significantly influenced by the scandal [3]. In 2020, 82% of the German population had a positive attitude towards donation. A share of 62% of the respondents said they had already made a decision and of these, 71% would agree to donate [4]. In its annual report 2021, the German Organ Procurement Organization ("Deutsche Stiftung Organtransplantation" - DSO) states that the rate of refusals among a total of 1,280 "qualified organ donors" (defined by the DSO as deceased persons in whom brain death has been determined and medical contraindications are absent) is only just under 19% [2].

The reasons for the discrepancy between the positive attitude and the low level of refused consent on the one hand, and the low rate of utilized donations on the other, remain unclear. This has been described in other countries with few organ donors, such as Switzerland [1, 5]. The country-specific legislation is one factor, that is repeatedly discussed [6–8]. Consent to donation in Germany is based on an opt-in system. In May 2012, an amendment to the Transplantation Act was introduced. The decision solution ("Entscheidungslösung") as a modification of the opt-in consent was established in August 2012. The population is regularly informed about organ donation by their health insurers and they receive an organ donor card [8]. There are no formal requirements if or how the will to donate is registered. This can be done by filling out the donor card, documenting the decision in an advance directive or communicate the will with family members or witnesses. If the will is unknown, family members are asked to decide in accordance with the presumed will of the donor or their own values [9]. Another aspect discussed to explain the low numbers is the fact, that only DBD is allowed in Germany, whilst donation after circulatory death (DCD) is refused by the German Medical Association ("Bundesärztekammer" – BÄK) [10, 11].

An inadequate identification of potential DBD, not considering to diagnose brain death, or disregard of a possible wish to donate organs in the context of end-of-life decisions could also contribute to the low numbers [7, 11–13].

Published preliminary data from our workgroup revealed, that consent is significantly dependent on whether and how the potential DBD has documented his will to donate. Highest consent rate is found when a will to donate is previously confirmed in writing by the potential DBD whereas it is lowest for a decision by family members if the will of the donor is unknown [14]. The present analysis intents to

provide further answers on the question of how many potential DBD exist in the participating hospitals, how many donations can be utilized, and what factors influence consent and utilization of organ donation after brain death.

PATIENTS AND METHODS

Identification of Potential Organ Donors and Inclusion Criteria

The Madrid resolution, introduced in 2011, defines a critical pathway for assessing the potential of deceased donation and for the identification of possible deceased donors. It describes among other things a definition of a potential DBD donor ("a person whose clinical condition is suspected to fulfill brain death criteria") [15]. The BÄK-guideline on donor identification, which is mandatory for all physicians in Germany, describes a comparable definition of a potential DBD donor ("a patient with primary and/or secondary brain damage, who is mechanically ventilated and treated in an intensive care unit (ICU), who is eligible as organ donor according to medical assessment, in whom brain death is imminent, suspected to have already occurred or in whom brain death has already been diagnosed") [16].

All potential DBD who met this definition and who were treated in the ICU of the University Hospitals of Aachen, Bielefeld, Bonn, Düsseldorf, Essen, Cologne and Münster between 1st June 2020, and 30th June 2021, were retrospectively included into the analysis. Identification and medical assessment of whether a patient was a potential DBD was made by the attending physician and supported by the inhouse transplant coordinator ("Transplantationsbeauftragter" - TxB), who was a mandatory participant in every case due to obligations by the BÄK-guideline [16].

Data were collected retrospectively from the medical files and from the records of the TxB. The completeness of the study cohort was confirmed with a computer program ("Transplant Check") provided by the DSO, which retrospectively identifies all in hospital deaths of patients with primary and/or secondary brain damage from the patient data according to § 21 Hospital Remuneration Act ("Krankenhausentgeltgesetz") (a law that legally regulates the charges for full and partial inpatient hospital services) [11].

Parameters and Variables

It was evaluated if and how the potential DBD had previously defined his will to donate. If the will was unknown, it was evaluated if family members existed, who were authorized to decide about a potential donation according to the presumed will or their own values. Consequently, the number of consented and utilized donations, reasons for a donation not utilized despite consent and predefined variables potentially influencing consent to donation were recorded (**Table 1**).

Ethics Committee and Registration

The Ethics Committee of the University of Muenster approved the study protocol (file number 2021-801-f-S). In addition, the study was registered in the German Register of Clinical Trials (DRKS) (DRKS-ID of the study: DRKS00027854).

Statistical Methods

Statistical analysis was performed using SigmaPlot 14.0 (Systat Software, Inc., San Jose, California, USA). Parameters were expressed as mean ± standard deviation in case of normal distribution, otherwise as median [25%; 75% percentile]. By means of a chi-square (χ^2)-test, the consent rate for organ donation was analyzed as a function of the individual variables (**Table 1**) and a statistical significance was assumed at $p < 0.05$.

RESULTS

Potential Brain Dead Donors

During the observation period, a total of 300 potential DBD (male: $n = 152$, female: $n = 148$) were identified in the seven university hospitals (**Figure 1**; **Table 2**).

Consent to Organ Donation

Overall consent to organ donation was found in 110 of the 300 cases (37%). The proportion of men was significantly higher in this collective (men/women: $n = 64/46$, $p = 0.035$), and the rate of consent tended to be higher in men than in women (42% vs. 31%, $p = 0.063$, **Table 2**). No consent could be obtained in the remaining 190 cases.

In 94 cases (31%), the potential DBD had previously defined his will to donate, leading to 46 consents (49%). In 195 cases (65%) the family members were to be involved because the will to donate was not determined by the potential DBD, resulting in 64 consents (33%). In ten cases, no decision-maker was available. In one case, consent was rejected by the public prosecutor. Consent rate was significantly higher, if the decision to donate was made by the potential DBD, compared to a decision by family members (49% vs. 33%, $p = 0.012$, **Table 2**).

Utilized Organ Donation

Organ donation was utilized in 69 out of the 300 potential DBD (23%). In 41 cases, donation could not be utilized despite consent (14% of all cases or 37% of all consented cases). Reasons for this were preserved brain stem reflexes ($n = 21$) or inconclusive diagnosis of brain death ($n = 2$), medical contraindications ($n = 14$), or cardiovascular instability ($n = 3$). In one case, the reason was not documented.

Variables Influencing Consent

The age of the potential DBD, the status of the interviewer, and the timepoint of the interview with family members about a decision when the will of the potential donor was unknown (before or after determination of brain death) had no influence on the consent rate, nor did it differ between the participating hospitals (**Table 3**).

DISCUSSION

The results of this retrospective analysis of utilized organ donations in potential DBD provide new explanations of the low number of donations in Germany, the apparent discrepancy to the positive attitude in representative surveys and the low

TABLE 1 | Parameters and variables recorded in the study cohort.

Parameter	Variable
Decision on organ donation	- Consent - Refusal
Organ donation utilized after consent	- Yes - No
Age of potential DBD	- Years
Gender	- Male - Female
Decision-maker	- Potential DBD - Family members - No decision-maker available - Public prosecutor
Timing of interview with family members to evaluate consent	- Before diagnosis of brain death - After diagnosis of brain death
Status of Interviewer	- Consultant - Fellow - Resident - TxB
University Hospital	- Aachen - Bielefeld - Bonn - Düsseldorf - Essen - Cologne - Münster

DBD, donation after brain death; TxB, inhouse transplant coordinator.

refused consent rate published by the DSO. A donation could only be utilized in 23% of all identified potential DBD. In 37% of the consented cases, donation was nevertheless not possible. In 63% of all cases, consent was refused. Consent rate was significantly higher when the attitude towards donation was known, but only 31% of all potential DBD had previously determined their attitude towards organ donation. The age of the potential DBD, the status of the interviewer and the timing of the interview with family members to evaluate the will to donate had no significant influence on the consent rate, which was also comparable between the participating hospitals.

Potential and Utilized DBD Donors

A total of 69 donations could be utilized in this cohort of 300 potential DBD (23%). Information about the total number of potential DBD in Germany is scare [11], partly due to the lack of epidemiologic studies and missing data about ICU-mortality [17]. Data from other countries show that the proportion of potential donors among deceased in the ICU ranges from 1.4% in Canada (with 36% utilized donors) [18], to 2% in Australia (33% utilized) [19] and 1.5%–2.4% in the Netherlands (26%–35% utilized) [20], respectively. Harvesting hospitals in Germany report annually to the DSO about their donation activities. The data analysis in these reports is based on numbers generated by the DSO tool "Transplant Check" (see Method section) [2, 11]. In 2020, a total of 14.164 death with documented brain damage were detected in all harvesting hospitals of NRW and 168 donations were utilized (1.2%) [21]. The corresponding numbers from the participating hospitals and the proportion of potential DBD donors identified in this study are shown in **Table 4**. Based on this data reference from 2020, the total proportion of potential DBD in this study was around 12% (ranging from 7% to 19% for each hospital) of all deceased with brain damage. This indicates that the low number of utilized donors is not a problem of failure to identify a potential DBD, at least in this cohort. However, it must be mentioned that the proportion could be different, if the number of potential donors were put in relation to all deceased in the ICU and not to all in hospital deceased with brain damage.

In an older work, Wesslau et al found 600 utilized and 1,285 potential DBD in 2019 deceased with brain damage in the north east donor region of Germany between 2002 and 2005, indicating a higher proportion of utilized (47%) and potential DBD (64%) than in our cohort [17]. Notably, Wesslau et al defined potential DBD as "those for whom the diagnosis of brain death had been initiated and/or completed and no contraindications existed". We used the definition of potential DBD according to the BÄK guideline on donor identification (see Method section). This could partially explain the lower rate of utilized DBD in our cohort because with our definition more potential DBD are identified. In our opinion this reflects a more precise definition of potential DBD and in turn a more realistic calculation of a representative consent rate, quite apart from the fact that the BÄK guideline also makes this definition mandatory [16]. The higher proportion of potential donors in Wesslau´s study might be due to fact, that they performed a prospective study, where only deceased in the ICU with a relevant brain damage were included by the attending physician rather than all deceased of a hospital with brain damage identified retrospectively by "Transplant Check."

The most frequent reason for a donation not being realized in our cohort was refused consent in 190 cases (63%), including 48 (51%) refusals by the potential DBD and 131 (67%) refusals by family members, respectively. Wesslau et al found refused consent in 38% of potential DBD, but again this calculation was based on a different definition of potential DBD. Somewhat surprisingly, only refusals by family members were reported but no decisive information about refusals by the potential DBD are found in their analysis [17].

Additionally, an organ donation could not be utilized in 41 (37%) of the 110 consented cases in our cohort, in 17 of these cases due to the medical condition of the potential DBD. In 23 cases, the criteria for determining brain death were not fully met. Following the German legislation, an organ donation was thus not possible. In this constellation with severe brain damage, a therapy limitation due to an unfavorable prognosis usually leads to death from cardiovascular arrest within a short time. In many countries, organ donation would be permissible in such situations after planned therapy withdrawal (potential DCD donor). Twenty-one percent of all consented cases can be considered a relevant amount. At maximum utilization, the number of donors in this cohort would have increased from 69 (23%) to 92 (31%). Consequently, the fact that DCD is not possible may contribute to the overall low number of organ donations in Germany [6]. Although concerns have been raised that the success of

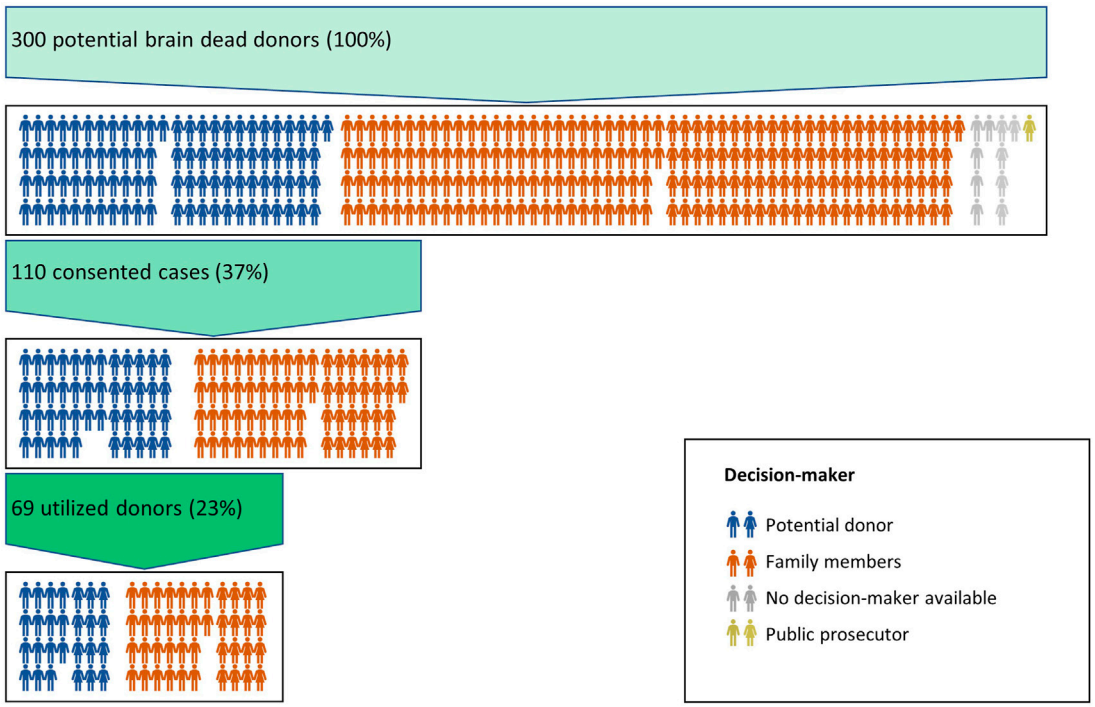

FIGURE 1 | Overview of the study cohort. Each icon ( ) represents one male/female case. The colour of the icon indicates the basis of the decision about a donation in each case.

TABLE 2 | Demographics in the study cohort and basis of decision.

Cohort	N	Age[b]	Male	Female	*p*-value	Basis of decision[a]				*p*-value
						Organ donor card	Advance directive/ other document	Communicated orally	Family members	
Potential DBD	300	61 [50; 77]	152	148		27	16	51	195	
Consented DBD	110 (37%)	60 [48; 71]	64 (42%)	46 (31%)	0.035 0.063	23	7 ((23+7+16) / (27+16+51)=49%)	16	64 33%	0.012
Utilized DBD	69 (23%)	53.2 ± 19.6	41	28						

[a]*10 cases with no decision-maker available, one case with refused consent by public prosecutor.*
[b]*Age in years is displayed as average ± standard deviation in case of normal distribution, otherwise as median [25%; 75% percentile].*
DBD, donation after brain death.

implementing DCD would be at the expense of DBD donors [22], recent studies do not support these concerns [22–25]. A study from the United States identified almost 10.000 potential uncontrolled DCD donors per year, resulting in doubling the number of deceased donors, if maximally utilized [26]. In the United Kingdom, intended DCD donors (defined as patients who progressed to at least the organization of a theater team) increased from 1,187 between 2004 and 2009 to over 4,500 between 2009 and 2014 without a reduction in intended DBD donors [25].

Consent to Organ Donation

Consent rate was 37% in this cohort, and a previously determined decision about donation was only found in 31% of all cases. These results differ considerably from surveys of the BZgA, in which 62% of the respondents stated that they had already made a decision and 71% of this group would agree to donate [4]. According to the 2021 DSO annual report, a consent to donate was achieved in 81% of the qualified DBD donors and in 42% the decision was made based upon a known will to donate [2].

The low number of realized donations despite the positive attitude to organ donation in surveys and the low rate of refused consent in reports from the DSO is repeatedly emphasized in the literature [7,11–13,27]. Subtly and sometimes explicitly, the reproach is voiced against hospitals that they are not sufficiently committed to increase the number of organ donations or that they do not identify eligible organ donors

TABLE 3 | Consent rate as a function of parameters and variables.

Parameter	Variable	Potential DBD [n]	Consent [n]	%	*p*
Age [years]	0–9	7	4	57	0.32
	10–19	3	0	0	
	20–29	19	8	42	
	30–39	18	8	44	
	40–49	27	10	37	
	50–59	56	21	38	
	60–69	65	28	43	
	70–79	53	16	30	
	80–89	48	15	31	
	90–99	4	0	0	
Status of Interviewer	Consultant	151	61	40	0.627
	Fellow	31	9	29	
	Resident	44	15	34	
	TxB	64	25	39	
	No interview[a]	10	0	0	
Timing of Interview[b]	Before brain death	169	56	33	0.811
	After brain death	26	8	31	
University Hospital	Aachen	35	10	29	0.90
	Bielefeld	49	17	35	
	Bonn	47	16	34	
	Düsseldorf	58	22	38	
	Essen	43	17	40	
	Cologne	27	12	44	
	Münster	41	16	39	

[a]*No decision-maker available.*
[b]*Interview with family members about the will to donate in 195 of the 300 cases, because the attitude towards donation was not previously determined by the potential donor.*
DBD, donation after brain death; TxB, inhouse transplant coordinator.

TABLE 4 | Deceased with documented primary and/or secondary brain damage in 2020 in the participating hospitals, compared to the potential DBD donors identified in this study.

	Aachen	Bielefeld	Bonn	Düsseldorf	Essen	Cologne	Münster	Total
Deceased*	358	263	429	311	376	415	307	2,459
Contraindication to donation*	49	10	46	25	41	58	36	265
No mechanical ventilation*	94	79	136	81	97	131	89	707
No relevant brain damage*	40	37	28	18	31	81	9	244
Remaining cases with relevant brain damage*	175	137	219	187	207	145	173	1,243
Utilized donors*	5	10	3	6	10	15	5	54
Potential DBD in this study	35	49	47	58	43	27	41	300
Proportion of potential DBD/deceased	10%	19%	11%	19%	11%	7%	13%	12%

*DBD, donation after brain death; *numbers from 2020 provided by the German Organ Procurement Organization [21].*

[11–13]. Based on the results of this study, this accusation seems – at least in part – not justified.

First, in this cohort, it was mainly family members who had to make the decision, and their consent rate was significantly lower. Lower rates of consent by family members when the donor's will is unclear were also found previously by others [5, 28, 29]. It is obviously different to decide for oneself in a survey rather than for someone else, especially in the stressful situation where family members are asked to make a decision in an end-of-live setting, but only the presumed will or one's own values can serve as the basis for this decision [30, 31]. Data from England and the USA have shown that knowledge about a person's attitude to organ donation is one of the most important factors in consent by family members [28, 32]. In Switzerland, in 56% of the cases rejected by family members, it was stated they might have consented if there had been a documented will to donate [5]. Moreover, surveys on such a sensitive topic as the willingness to donate organs could lead to a bias in response behavior in favor of a perceived social desirability [33]. Whether the 2012 organ allocation scandal in Germany is still negatively influencing family members' decisions because of a prevalence of mistrust in the transplant process cannot be answered from our data, but surveys suggest that public support for and confidence in organ donation and transplantation recovered quickly after the scandal [3].

Second, it is questionable to conclude from the consent rates reported by the DSO that similarly high consent rates must be found in everyday clinical practice. This is done in the literature to make a prediction about how many donations would be feasible [11]. In 3,132 organ donation-related contacts with the DSO in the year 2021, 1816 cases did not result in organ donation, in 29% of these cases because of a known refusal [2]. As contact with the DSO prior to conducting the diagnosis of brain death is optional, this refusal rate is not representative due to an unclear number of unreported cases [11]. For cases in which brain death has been diagnosed, there is a legal obligation to report to the DSO [34]. In these cases, the overall refusal rate according to the DSO was 19%, or 33% in the cases, where family members had to decide because of an unknown will of the potential DBD [2]. In our cohort, family members had to decide in 195 (65%) cases because of an unknown will and their rejection rate was 67% overall. However, in this cohort, the evaluation with family members about the will to donate was mostly carried out before brain death was confirmed (in 169 (87%) of the 195 cases). This approach is in accordance with the BÄK guideline on donor identification [16], recommending that a patient's will for organ donation be explored at an early stage, at the latest when treatment limitations are being discussed. If a refused consent is communicated in this treatment phase, diagnose of brain death is often no longer performed, a palliative treatment concept is initiated, and the case is probably not reported to the DSO as organ donation is not possible. However, the DSO can only determine valid consent rates for cases with a completed diagnosis of brain death, as only then there is a legal obligation to report [34]. This could create a significant selection bias, as a negative attitude among potential donors who are not submitted to the diagnose of brain death may not be reported to the DSO [27]. Consequently, this can result in lower refused consent rates in their reports.

Third, in countries with opt-out consent, higher donor numbers can be achieved [35–37], although this positive effect is not demonstrable everywhere [38,39]. However, the opt-in consent used in Germany could have a negative impact on donor numbers, especially with regard to the significantly higher refusal rate by family members if the will of the potential donor is unknown [17]. Some politicians tried to address this issue with a legislative proposal that would introduce an opt-out system in Germany, but the majority of members of the German parliament voted against it in 2020 [40].

Finally, the DSO states the number of qualified donors in the 300 harvesting hospitals of North Rhine-Westphalia in 2020 to be 264 in total [41]. In this survey of seven university hospitals from North Rhine-Westphalia, 300 potential organ donors were identified, but not all of them were reported to the DSO, as a refused consent was already known before a pending determination of brain death. A lack of commitment in identifying potential organ donors cannot therefore be generally accused, although this is sometimes explicitly done [12, 13, 40].

Age and Gender of the Donor

Mean age of the potential DBD was 61 and 60 years for the entire cohort and consented cases respectively. The DSO only provides numbers of age groups of utilized organ donations, with most donors aged between 16 and 55, but they give no information about the age of potential donors prior to the determination of brain death [2]. Others report an average age of potential donors of 55.1 years in Germany, and 50.5 years in consented organ donors, with a higher percentage of refusals in older age groups [17]. In this cohort, the age of the potential DBD had no significant influence on the consent rate. As expected, the cohort of utilized donations was younger than the group of potential DBD, presumably because medical contraindications are more common in older potential donors [17].

The proportion of male decedents with consent was significantly higher and the consent rate tended to be higher than for females. Others report significantly higher rates of consent among younger, male potential DBD [17], which is often also associated with a higher rate of consent after traumatic brain injury [32]. However, as the type of brain injury was not recorded in our retrospective survey, this hypothesis cannot be supported with the available data.

Status of the Interviewer

The status of the interviewer when evaluating the will to donate with family members in the absence of a determined will had no significant influence on the consent rate in this cohort. Others could show that a combined approach by hospital staff and coordinators from an organ procurement organization resulted in the highest consent rate [42]. The United Kingdom provides specialist nurse training programs to train the communication with family members of potential organ donors [43]. Higher consent rates can be achieved when these specialists are involved in the decision-making process with family members [44].

Decision-making is usually a longer process with several communications with family members. In this retrospective study, only information on the conversation in which the decision was finally documented was collected. Since there is no information about any conversations that may have taken place before this process, the results of this cohort should not be over-interpreted. However, it seems generally undisputed that staff who are trained in dealing and communicating with family members of potential donors achieve higher consent rates. The status of the interviewer seems to be secondary in this respect [17, 42].

Timing of Interview

In this cohort, the consent rate was comparable if the interview with the family members to evaluate possible consent in the absence of a known will of the potential DBD was conducted before or after the diagnosis of brain death. Other studies have also shown that the timing of the interview had no relevant influence on the consent rate [45]. There are only indications suggesting that there is a negative influence on consent if the question about organ donation is asked directly in the context of death notice or notification of the completed brain death diagnosis [42].

Treating University Hospital

Consent rate did not differ significantly between the participating university hospitals. Calculations of conversion rates (realized organ donations/contact rates with organ procurement organization) or realization rates (realized organ donations/

qualified organ donors) are often used to assess a "donation commitment" of an individual hospital [12]. These calculations show considerable differences between hospitals [12, 40].

However, the basis of this calculation gives rise to discussion. Contact with the DSO is not bindingly defined (according to the DSO's procedural instructions, contact is required if the potential DBD is "eligible for organ donation according to medical assessment" [34]). This makes an objective comparison between hospitals based on conversion rates impossible. It is also questionable to use the number of "qualified organ donors" as a basis for comparison. By definition of the DSO, a "qualified organ donor" is one who has been diagnosed brain dead and who has no medical contraindications to donation [2]. This means that a hospital´s commitment to realize a donation is not captured for a case where a potential DBD is identified in advance of a possible brain death, but due to a known refused consent, brain death is not diagnosed. In our cohort, 113 refused consents were transmitted by family members prior to a pending diagnosis of brain death. Thus, using realization rates as an indicator for the "donation commitment" of a hospital should be treated with caution.

Limitations

In this retrospective study, relevant factors possibly influencing results may not have been completely recorded, especially in such a difficult field as organ donation (e.g., no information on religious affiliation, type of brain damage or interviews prior to the final decision). It also cannot be completely ruled out that a possible consent to donate was not recorded due to lack of information about it. Only data from university hospitals in North Rhine-Westphalia were collected. It is possible that the results are not representative for the whole of Germany, as donor numbers may vary depending on the regions and the level of care provided by the hospital [11, 13]. The high proportion of identified potential DBD donors found in this study may not be generalizable to all harvesting hospitals in Germany, in part because there is evidence that the TxB are often not involved in the donation process, particularly in smaller hospitals [11] and they are not always given sufficient time off from their other duties to support donor identification [46].

CONCLUSION

Following the recommended definition of a potential DBD, a donation could only be utilized in 23% of all potential DBD. The refusal rate was remarkably higher than results from representative surveys would suggest. Consent was significantly higher when the attitude towards donation was known but this was only available in 31% of all cases. Most refusals were communicated by family members before a pending diagnosis of brain death. A notable number of consented cases could not be transferred into utilized donations. These results suggest that attitudes to organ donation found in surveys and refusal rates provided by the DSO can only be transferred to everyday clinical practice to a limited extent. A clear definition of whether to involve the DSO and a requirement to involve the DSO early in the donation process when indicated, in combination with using internationally standardized definitions could provide more valid data on donor potential and consent rates in Germany. Better support for the work of the TxB might increase identification of potential DBD, and enabling DCD could be a promising option to increase donations. Considering the high rate of refused consent by family members in the absence of a known will, the implementation of an opt-out system should be discussed, as recently suggested by the German Federal Minister of Health [47]. As long as opt-in consent is used, promoting the documentation of a will to donate is essential to increase donations in Germany.

DATA AVAILABILITY STATEMENT

Datasets are available on request from the corresponding author. Requests to access these datasets should be directed to jan.englbrecht@ukmuenster.de.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics committe of the Westfälische Wilhelms-Universität Münster. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JE, DaS, and MaS were involved in planning and supervised the work, JE, HK, and MaS processed the data, and performed the analysis. JE and MaS drafted the manuscript and designed the figures. MeS, DiS, and FB aided in interpreting the results and worked on the manuscript. All authors discussed the results and contributed to the final manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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

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ORIGINAL RESEARCH
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Counselling on Conceiving: Attitudes and Factors Influencing Advice of Professionals in Transplantation

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Pregnancy after kidney transplantation (KT) conveys risks of adverse pregnancy outcomes (APO). Little is known about performance of pre-pregnancy counselling after KT. This study investigated perceptions of risk, attitudes towards pregnancy and factors influencing advice given at pre-pregnancy counselling after KT. A web-based vignette survey was conducted among nephrologists and gynaecologists between March 2020 and March 2021, consisting of five vignettes containing known risk factors for APO and general questions on pre-pregnancy counselling after KT. Per vignette, attitudes towards pregnancy and estimation of outcomes were examined. In total 52 nephrologists and 25 gynaecologists participated, 56% from university hospitals. One third had no experience with pregnancy after KT. All gave positive pregnancy advice in the vignette with ideal circumstances (V1), versus 83% in V2 (proteinuria), 81% in V3 (hypertension), 71% in V4 (eGFR 40 ml/min/1.73 m^2). Only 2% was positive in V5 (worst-case scenario). Chance of preeclampsia was underestimated by 89% in V1. 63% and 98% overestimated risk for graft loss in V4 and V5. Professionals often incorrectly estimated risk of APO after KT. As experience with pregnancy after KT was limited among professionals, patients should be referred to specialised centres for multidisciplinary pre-pregnancy counselling to build experience and increase consistency in given advice.

Keywords: kidney transplantation, nephrological care, pregnancy, counselling, gynaecologist

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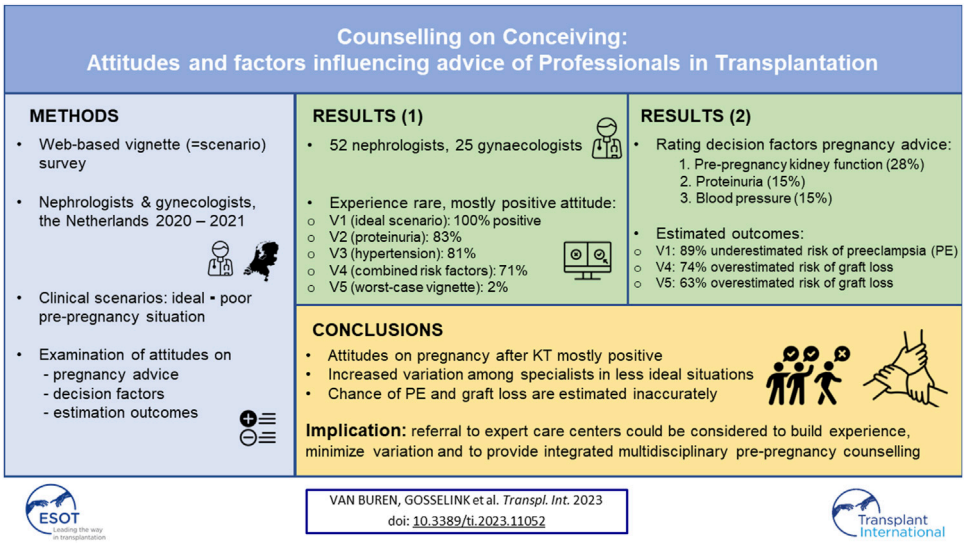
INTRODUCTION

Pregnancy after kidney transplantation (KT) is challenging from both an obstetric and renal point of view. Higher incidences of adverse pregnancy outcomes (APO) have been described, such as preeclampsia, foetal growth restriction and preterm birth (1–3). Pregnancy does not seem to negatively affect graft function or graft loss when pre-pregnancy kidney functioning is good (4).

Abbreviations: APO, adverse pregnancy outcomes; BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; IQR, interquartile range; KT, kidney transplantation; SCr, serum creatinine; SD, standard deviation.



GRAPHICAL ABSTRACT |

Since the first successful pregnancy after KT in 1958 (5), annual numbers of pregnancy after KT have been rising. In the US, on average 220 women conceive and give birth after KT per year, in the Netherlands on average 12 women per year (6).

Although challenging, women after KT have the same desire for children and often have considered their plans for pregnancy to a greater extent than women in the healthy population (7, 8). Therefore, pre-pregnancy counselling is an important aspect of clinical care for kidney transplant recipients. Our previous study showed that kidney transplant recipients rely on their nephrologists' pregnancy advice and that attitudes towards pregnancy vary between nephrologists (9).

According to the best practice guidelines from 2002 (European) and 2005 (United States) (10, 11) optimal timing of pregnancy after KT is at least 1–2 years after transplantation, in women with good kidney function, little/no proteinuria, normal blood pressure, no recent acute rejection, good compliance to medication and no use of teratogenic drugs. When the situation does not meet these criteria, practice guidelines advise evaluation on case-by-case basis.

While guidelines describe the ideal candidate for pregnancy after KT, little is known about pregnancy in less ideal situations. Furthermore, physicians do not always follow clinical practice guidelines (12, 13). This cross-sectional survey vignette study was designed to examine the variation in attitude of medical specialists regarding pregnancy after KT in varying situations. Also, factors influencing their attitude and pregnancy advice were examined.

MATERIALS AND METHODS

Study Design

A cross-sectional survey vignette study (14–17) was conducted between March 2020 and March 2021. To determine variation in pre-pregnancy counselling between medical specialists in the Netherlands, five clinical vignettes were constructed. Participants were invited by e-mail to complete a web-based questionnaire concerning these vignettes. LimeSurvey software was used to create the survey and collect data (18). The Checklist for Reporting Results of Internet E-Surveys (CHERRIES) was used for reporting the results of our study (**Supplementary File S3**) (19).

Participants

Nephrologists and gynaecologists practicing in public hospitals were invited to participate. Of note, post-KT care in the Netherlands is mainly carried out by university medical centres during the first year after KT. After 1 year, patients are referred to general hospitals for further care. Therefore, patients with a wish to conceive may be undergoing treatment either in university or in general hospitals. To enable inclusion of participants in both settings, the survey was sent to the regional network of the research group. Participants were invited by an initial e-mail to fill in the questionnaire, followed by two reminders. Responses were also included if the questionnaire was not fully completed. The survey was only accessible for the invited participants and was protected by a password.

Vignettes

Vignette studies use short scenarios (vignettes) for respondents in surveys to express their views and attitudes on these scenarios. By systematically varying the levels of theoretically important vignette characteristics, a sample of different vignettes is available for respondents to judge (17).

For our study, vignettes were carefully constructed according to several steps. First, vignettes were designed based on previous literature and clinical expertise (4). (1, 20, 21) Then, vignettes

were evaluated by two experienced specialists in counselling for pregnancy after KT: one obstetrician and one transplant nephrologist. The vignettes were then reviewed by a health psychologist involved in survey research, to check for clear wording and corresponding questions and answer categories. Finally, a study pilot was conducted by sending the survey to three transplant professionals to test understanding and acceptability. According to these responses, the vignettes and questions were revised.

The vignettes described the same case of a woman of reproductive age after KT, coming to the outpatient clinic with a wish to conceive. In each vignette, one factor was adjusted to assess factors influencing attitudes towards pregnancy and advice. Although the decision making process is complex and multifactorial, only the most important risk factors for adverse pregnancy outcome (1, 2, 4, 22) could be included in this study because of the expected number of respondents. Vignettes varied on the following characteristics: presence of hypertension (blood pressure >140/>90 mmHg), proteinuria (>500 mg/L), poor kidney functioning (eGFR <60 mL/min/1.73 m^2) and rejection in the past year (21). The first vignette described the ideal situation for pregnancy after KT, with no risk factors for poor outcomes. The second to fifth vignette introduced, respectively proteinuria, hypertension, poor kidney function (eGFR 40 mL/min/1.73 m^2) and a combination of risk factors (hypertension, eGFR 25 mL/min/1.73 m^2, proteinuria, rejection in the past year). In the supplementary data file, the vignettes and questionnaire are shown (**Supplementary Files S1, S2**).

Survey

The survey consisted of three parts: first, questions regarding participants' characteristics and their experience with pregnancy after KT. Furthermore questions were asked about counselling style and responsibility. Additionally, participants were asked to rank the factors that influence their advice regarding pregnancy from a scale of 1–5 (Likert scale). These factors were identified from current literature (1, 4, 10, 11) (**Supplementary File S1**). Second, vignettes were displayed and per vignette, participants were asked whether their attitude towards a pregnancy for this patient would be negative or positive. Also, the weight of decision factors for their attitude was examined (on a scale of 1–5). Furthermore, participants had to predict the pregnancy outcome of the given vignette with respect to gestational age, birth weight, chance of developing preeclampsia and chance of graft loss within 2 years after pregnancy. Lastly, participants were asked to name and rate (on a scale of 1–5) the most important factor influencing pregnancy advice after KT per vignette (**Supplementary File S2**).

Ethics

There were no patients involved in this study. Personal information of participants was pseudo-anonymized. Data was collected and stored in a secured database. The study was approved by the Ethical Committee of the Erasmus Medical Centre: MEC-2020-0194.

Analytical Approach

Continuous values are reported as means (SD) when they were normally distributed. Variables with a non-normal distribution are reported as median with interquartile range (IQR). For each vignette, positive and negative attitudes towards pregnancy were analysed. Per vignette, the study group was divided into a positive attitude group and negative attitude group and groups were cross-tabulated against participants' demographic characteristics. Also, per vignette, participants' estimated outcomes were compared with observed pregnancy outcomes after KT in the PARTOUT-dataset, in which all pregnancies after KT and their outcomes of the past 40 years in the Netherlands are included (22). Unfortunately, only for vignette 1, 4 and 5 a comparison with current literature could be made since for vignette 2 (proteinuria) and vignette 3 (hypertension) no comparative data were available. Furthermore, a ranking was made per specialty for factors influencing pregnancy counselling and advice. Significance between groups was determined by a T-test or Chi-square test. Significance was corrected for multiple testing with the Bonferroni correction (23). Analyses were performed using IBM SPSS Statistics, version 25.0.0. Graphs and figures were established with GraphPad Prism, version 8. Free text-responses were categorized.

RESULTS

Participant Characteristics

In total, 265 medical specialists were invited to participate in this questionnaire. After removal of non-existing and duplicate email addresses and participants that opted out, 77/240 participated (32% response rate, **Figure 1**). Participant characteristics are reported in **Table 1**. The study group consisted of 52 (68%) nephrologists and 25 (32%) gynaecologists.

Experience and Opinions on Counselling

Experience With Pregnancy Outcomes

Overall, 76/77 of participants answered the question regarding their experience of treating women who became pregnant after KT. The majority (57%) reported good experiences with pregnancy after KT. 35% of respondents indicated having too little experience with pregnancy after KT to answer this question. Furthermore, participants were asked to clarify their definition of good pregnancy outcomes. Regarding the child, answers varied from being "born at term" to "birth after 36 weeks without complications for the child or growth retardation." Regarding the mother, quotes varied from "birth without complications" to "stable graft function, uncomplicated pregnancy and being able to enjoy the pregnancy and birth." Regarding the graft, definitions of poor outcome ranged from "decline in eGFR" to "renal replacement therapy." One nephrologist stated: "transplant survival is not the only important outcome in life." Another stated: "Pregnancy after transplantation is not a pink cloud, but a medical obstacle course where parents should make a conscious decision. But if you make this choice with the right guidance, the outcome can be successful."

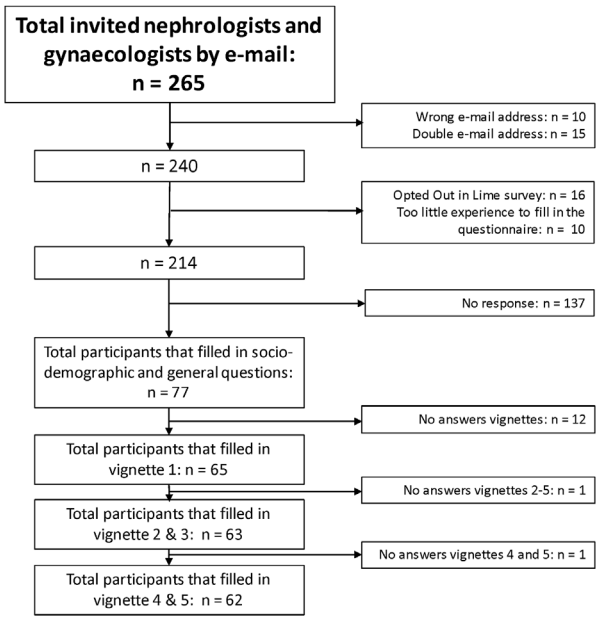


FIGURE 1 | Flowchart of this study. In total, 77/240 (32%) participants replied to the questionnaire.

Counselling Style

Participants were questioned about their counselling style. Most participants responded to be more informing and coaching than directive: "Informing, but more guiding when there are great risks." Also, participants indicated that styles differed per type of patient.

Responsibility of Decision Making

The majority of participants see themselves as "responsible" to "very responsible" in the decision-making process when a patient is wishing to conceive after KT (64%). However, 8% felt no responsibility "as long as the patient is not in need of assisted fertility, she alone is responsible" and 29% felt little responsibility "it is the decision of the patient, it is her life." Regarding responsibility for a pregnancy, most participants indicated that the professional/clinician is responsible for informing the patient about several scenarios of outcomes. The responsibility for the final decision to conceive lays with the patient: "The doctor advises, the patient decides, always." Also, a difference was made in spontaneous conception versus assisted pregnancies, "if there is enough proof for a negative medical pregnancy advice then you should have the guts to offer no fertility treatments, that is really a responsibility of the doctor." Only a few participants (two nephrologists) thought that the clinician was responsible for the final decision, because of his/her medical expertise.

Factors Influencing Counselling Advice

The results are shown in **Figure 2**. Gynaecologists ranked "graft rejection in the past" significantly more important than nephrologists ($p = 0.002$).

Furthermore, participants were asked to rank their three most important factors for pre-pregnancy counselling andadvice. Of all these factors, pre-pregnancy eGFR was considered most important (28%), followed by pre-pregnancy proteinuria (15%) and pre-pregnancy blood pressure (14.5%). Co-morbidity, obstetric history, mental health, smoking, BMI, attitude towards potential adverse pregnancy outcomes were also factors that were taken into consideration.

Vignettes

For each vignette, the number of positive attitudes towards pregnancy after KT is shown in **Figure 3**.

In the first vignette (ideal situation), all participants had a positive attitude towards pregnancy after KT. In the second vignette (proteinuria) 83% was positive. As shown in **Figure 3**, while more nephrologists (10/41, 24%) had a negative attitude than gynaecologists (1/22) ($p = 0.045$), this difference was not significant. Reasons for negative advice included: "risk of graft failure," "examine reason for proteinuria before getting pregnant" or "inform patient regarding high risk of graft failure and preeclampsia." In the third and fourth vignette (hypertension and poor kidney function), respectively 81% and 71% were positive. In the last vignette (worst case), 98% of participants had a negative attitude towards pregnancy. A nephrologist stated "do not become pregnant, unless the woman is of higher age and is not able to wait any longer, and only if she knows this could lead to the loss of her kidney graft." The one gynaecologist who would give positive advice for this vignette explained: "in the end it is a patient's choice, but counselling should be very attentive with all concerns thoroughly explained: it will be a high-risk pregnancy with high chance of complications." No significant associations were found between demographic characteristics and attitude towards pregnancy.

Estimated Outcomes of the Vignettes

In **Figure 4**, for vignette 1, 4 and 5, participants' predictions of outcomes are shown, compared to the observed outcomes in the PARTOUT-dataset and current literature. The dark grey bars are the "true" results from the PARTOUT dataset (22).

In vignette 1, the majority (62%) predicted a higher gestational age than observed in the PARTOUT-dataset (estimated >37 weeks versus mean gestational age PARTOUT-data 36 weeks). In vignettes 4 and 5, estimated birthweight corresponded with the PARTOUT-data. The chance of developing pre-eclampsia was underestimated in vignette 1 (ideal situation): 89% of participants estimated the chance of preeclampsia <30% while the PARTOUT-dataset showed an incidence of 39%. Estimated outcomes of vignette 2: proteinuria, and vignette 3: uncontrolled hypertension could not be compared with current literature, since no comparative data on these parameters were available. Although the difference was smaller, in vignette 4 (eGFR 40 mL/min/1.73 m^2) the incidence of preeclampsia was underestimated as well (estimated 10%–30% versus PARTOUT-data 33%–39%). The chance of graft loss was overestimated in vignette 4 (eGFR 40 mL/min/1.73 m^2) and 5 (worst case) by respectively 63% and 98% of participants.

TABLE 1 | Participants' baseline characteristics.

Demographic variable	N = 77 (n/%)
Medical centre	
- University Hospital	43 (56%)
- General Hospital	34 (44%)
Function	
- Gynaecologist	25 (33%)
- Nephrologist	52 (68%)
Year of graduation medical training (median, IQR)	2006 (10)
Age (IQR) (median, IQR)	47 (13)
Gender	
- Male	39 (51%)
- Female	38 (49%)
Children of their own	71 (92%)
Dutch nationality	76 (99%)
Religion, of which:	17 (22%)
- Christianity	16 (21%)
- Islam	1 (1%)
Working experience in KT, years (median, IQR)	12 (14)
Number of women with pregnancy after KT treated by the participant (median, IQR)	3 (15)

KT, kidney transplantation; IQR, inter quartile index.

Lastly, predicted outcomes were compared between nephrologists and gynaecologists. After adjusting for multiple testing, $p < 0.0025$ was considered significant. In vignette 5 (worst case), nephrologists predicted a higher birth weight than gynaecologists ($p = 0.046$, $p = 0.020$). Furthermore, nephrologists estimated a lower chance of developing preeclampsia than gynaecologists in vignette 4 [eGFR 40 mL/min/1.73 m^2 ($p = 0.004$)]. These differences were not significant after adjusting for multiple testing. No association was found between years of experience and prediction of outcomes.

DISCUSSION

Main Conclusion

This study, focusing on attitudes among professionals towards pregnancy after KT, has four major findings. First, professionals had little experience with pregnancy after KT. Among those with experience, attitudes towards pregnancy after KT were positive. Second, pre-pregnancy kidney function, proteinuria and blood pressure are considered most important factors influencing pregnancy advice after KT. Third, despite participants' overall positive attitude towards pregnancy after KT, in less ideal situations, there was less agreement on pregnancy advice. Fourth, participants seem to underestimate the chance of developing preeclampsia and overestimate the chance of graft loss within 2 years after pregnancy. As pregnancy after KT is rare, referral to expert care centers could be considered to build experience and to provide combined pre-pregnancy care and counselling by a nephrologist and gynaecologist together.

Comparison With Current Literature

In the Netherlands, the incidence of pregnancy in women who are transplanted under the age of 45 is approximately 10% (9).

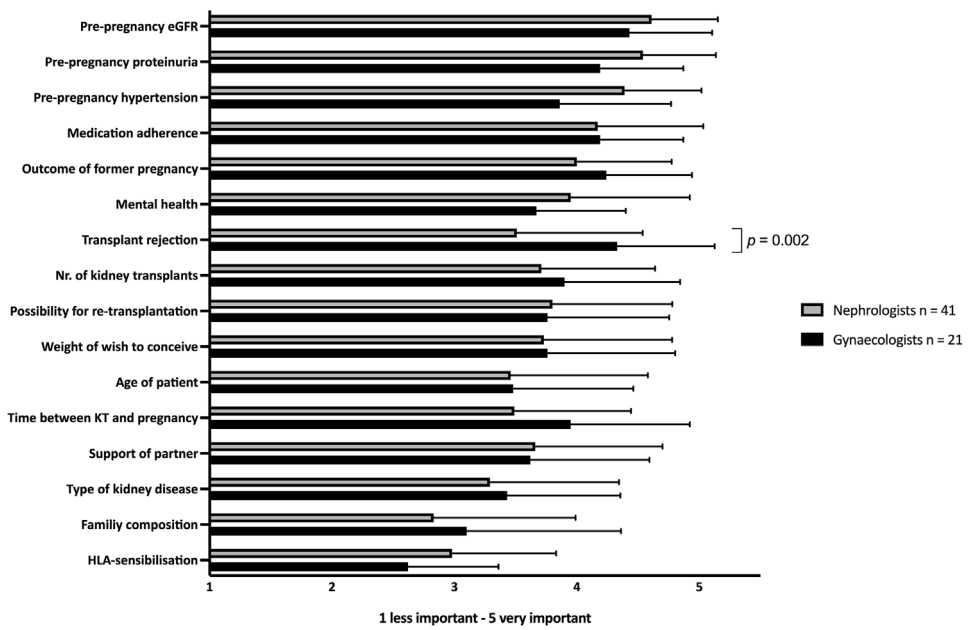


FIGURE 2 | Importance of factors in pre-pregnancy counselling after kidney transplantation in general, according to clinicians. Scale 1 (less important) to 5 (very important). Gynaecologists scored "history of transplant rejection" significantly more important than nephrologists ($p = 0.002$). Significance was determined by T-test. The use of Bonferroni's post-test correction adjusted significance level to (0.05/16) $p < 0.0031$.

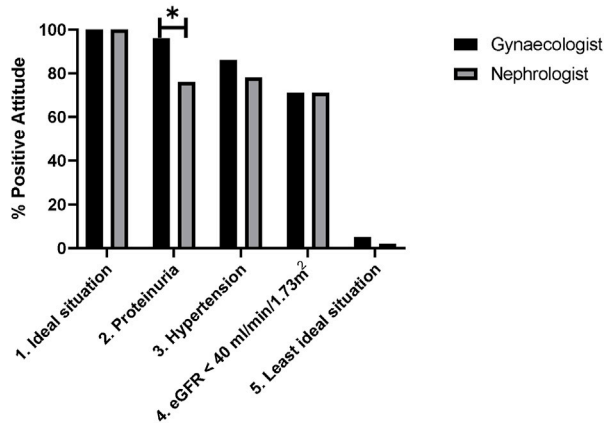


FIGURE 3 | Attitudes of clinicians towards pregnancy in kidney transplant recipients per vignette. *In Vignette 2 (proteinuria), gynaecologists were more positive than nephrologists, $p = 0.045$ considered not significant. Significance was determined by Chi-square. The use of Bonferroni's post-test correction adjusted significance level to (0.05/5) adjusted the significance level to $p < 0.01$.

Therefore, pregnancy after KT is a rare phenomenon in daily practice, especially for nephrologists and gynaecologists in general hospitals. This was also shown in our study, with 30% of clinicians having no experience with pregnancy after KT. Nevertheless, these clinicians can also be confronted with questions regarding pregnancy from KT recipients in daily practice when patients express their wish to conceive to their treating physician. When experience is lacking, clinicians need to turn to guidelines and consensus statements. Unfortunately, these guidelines describe only ideal situations (10, 11). This makes it difficult to counsel more complex cases such as patients with some proteinuria and/or lower kidney function. Furthermore, a previous study among CKD-patients regarding fertility care showed a relationship between knowledge of clinicians on fertility care and the amount of fertility care that was given (24). From this study, it can be hypothesized that with little experience, a clinician might be less attentive to the subject of pregnancy after KT. This may also help explain why, in our previous study, women after KT reported a lack of initiative among clinicians to broach the subject and experienced a high threshold to discuss their wish to conceive with their nephrologist (9).

The ranking of kidney function, proteinuria and blood pressure as the three main important factors for counselling and for risk identification matches current literature and guidelines (1, 4, 10, 11). Clinicians' estimations were compared to the Dutch PARTOUT-cohort for two reasons. First, to ensure a representative comparison of estimations and reported outcomes on a national scale. Second, to ensure an optimal comparison given the availability of many vignette-parameters in the PARTOUT-data that were lacking in other published cohorts. In this comparison, the chance of developing pre-eclampsia and preterm birth was underestimated by clinicians (22). However, when comparing clinicians' estimations regarding preeclampsia to outcomes reported in the study by Stoumpos et al, clinicians' estimations seem more adequate. Nevertheless, Stoumpos' incidence of preterm birth (61%) was similar to the PARTOUT-dataset (25). Also, the cohort of KT-pregnancies reported by Piccoli et al could be matched to vignette 1 (ideal situation), showing a higher incidence of preterm birth than the PARTOUT-data (26). Thereby, when comparing clinicians' estimations regarding preterm birth internationally to different cohorts, their predictions remain an underestimation. Unfortunately, to our knowledge, other estimated parameters such as risk of graft loss within 2 years after delivery were not available in other published cohorts for vignette-comparison.

We reported an overestimation of the risk of graft loss within 2 years after delivery compared to the PARTOUT-cohort. There is a relationship between kidney function and the risk for graft loss (27), but in our recent study there was a small but non-significant difference in eGFR-slope before and after pregnancy (28). Additionally, a recent meta-analysis showed no difference in graft loss between women with and without pregnancy after KT (4, 25). Unfortunately, literature on proteinuria and pregnancy outcomes after KT is lacking.

The majority of clinicians had a positive attitude towards pregnancy after KT. This contrasts earlier studies on pre-pregnancy counselling among KT recipients, where respectively one-third and a quarter of female KT recipients reported to have been counselled against pregnancy (29, 30). While the intentions of these clinicians remain unknown, their opinion counts and negative information can be overwhelming for women. It is important that clinicians are aware of their influence and that they have adequate counselling skills. Even a negative tone might lead to cancelling pregnancy plans. Wiles et al also investigated pre-pregnancy counselling in CKD-patients. They found that the clinicians' positive or negative attitude towards pregnancy had an influence on the decision to become pregnant (31). Taking this influence into account, it is desirable that clinicians are well informed on most recent findings and have up-to-date knowledge on this subject.

Of note, though this study focuses on counselling KT recipients who want to become pregnant, some KT recipients get pregnant without planning. While in Netherlands termination of pregnancy at an early stage of pregnancy is legal, this is not the case in all countries. A recent editorial on the impact of the reversal of *Roe v. Wade* in the United States, further emphasized the importance of reproductive care and pre-pregnancy counselling for women with CKD in countries or states where abortion is not legal (32).

Based on our findings we recommend that in more complex clinical cases pregnancy counselling and care should be carried out in multidisciplinary teams with an individualised approach for the patient wishing to conceive. This is in line with the previous advice by Cabiddu et al. regarding pregnancy after KT in less ideal situations (33).

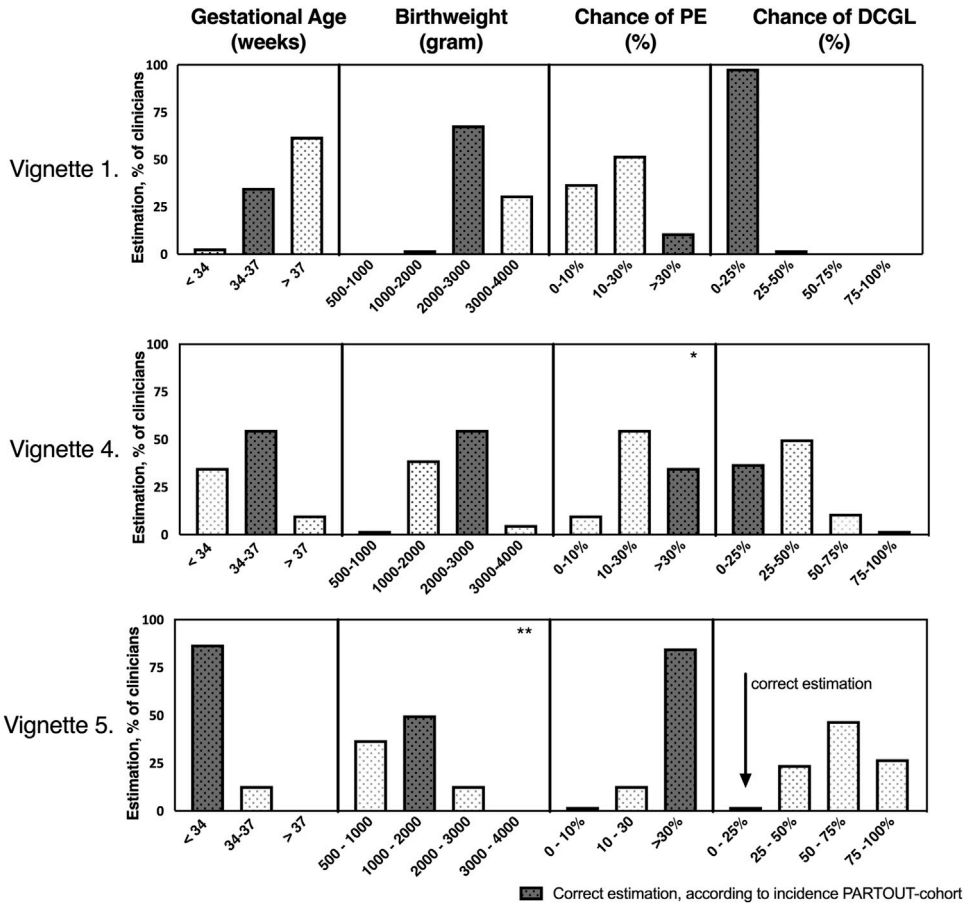


FIGURE 4 | Clinicians' estimations regarding pregnancy outcomes per vignette compared to the true incidences of pregnancy outcomes in the PARTOUT-cohort. Vignette 1 = ideal situation eGFR 65 ml/min/1.73 m^2 (n = 65). Vignette 4 = eGFR <40 ml/min/1.73 m^2 (n = 62). Vignette 5 = least ideal situation (combination of factors), eGFR 25 ml/min/1.73 m^2 (n = 62) For each vignette, the dark bar represents the correct estimation according to the PARTOUT-dataset. PE: preeclampsia, DCGL: Death Censored Graft Loss within 2 years postpartum. * Chi-square: p = 0.004 nephrologists estimated lower chance of developing preeclampsia than gynaecologists. ** Chi-square: p = 0.020 nephrologists estimated higher birthweight than gynaecologists. Both considered not significant. The use of Bonferroni's post-test correction adjusted significance level to (0.05/20) $p < 0.0025$.

Strengths and Limitations

To date, this is the first study investigating attitudes and factors influencing pre-pregnancy counselling after KT among nephrologists and gynaecologists. Another strength is the elaborate and methodical vignette construction. With expertise from experienced transplant professionals (a nephrologist and a gynaecologist), a health psychologist and a pilot study, vignettes were improved and refined. Therefore, vignettes were constructed that fit the research questions. However, despite these efforts, feasibility of the survey required simplification of scenarios and options for advice. Therefore, the fictitious vignettes did not cover the full range of complex dilemmas, possible factors influencing counselling and advice in daily practice. To address this limitation, factors -based on current literature and expert opinion-were ranked by participants next to the vignettes. Another limitation is the low participation rate (32%). This is in all likelihood because pregnancy after KT is highly specialised care. The questionnaire was sent to all nephrologists and gynaecologists in the regional network of the PARTOUT-network. Part of the invitees might not have felt compelled to participate in this study because they were lacking experience with pregnancy after KT. This could have led to selection bias. Although the relationship between prediction of pregnancy outcomes and clinicians' experience in the transplant field or with pregnancy after KT seems intuitive, this could not be demonstrated. A possible explanation might be the relatively small sample size causing low statistical power. Despite considerable limitations, this study is unique and can contribute to a broader focus on how pre–pregnancy counselling should be performed.

Implications and Further Research

In order to promote informed shared-decision making, more information needs to be available for patients and clinicians.

With outdated guidelines, providing accurate information to the patient is a challenge. On top of this, although the number of women getting pregnant after KT is rising, yearly numbers are still relatively low. Though the recent publication of the PARTOUT-data will assist in counselling for pregnancy after KT, larger international datasets on pregnancy outcomes are needed. Furthermore, to capture the different attitudes in the dilemmas of daily practice more thoroughly, this study could be expanded internationally, to evaluate additional factors that may influence counselling to the vignettes. Although this study does not directly demonstrate experienced professionals predicting pregnancy outcomes more accurately, we suggest pre-pregnancy counselling to be centralized in specialised centres for multidisciplinary pre-pregnancy counselling. This in order to build experience as pregnancy after KT is scarce and often complicated.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Committee of the Erasmus Medical Centre: MEC-2020-0194. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MvB: participated in research design and performance, data analysis and interpretation, and writing of the article. MG: participated in research design and performance, data analysis and interpretation, and writing of the article. EM: participated in research design, data analysis and review of the article. JW: participated in research design and performance, data interpretation, and writing of the article. AL: participated in research design and performance, data interpretation, and writing of the article.

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.2023.11052/full#supplementary-material

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ORIGINAL RESEARCH
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Pregnancy Complications and Impact on Kidney Allograft After Kidney Transplantation in IgA Nephropathy

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Pregnancy in kidney transplantation (KT) recipients has been challenging because of the high risk of maternal, fetal, and renal complications. Although patients with immunoglobulin A nephropathy (IgAN)-chronic kidney disease (CKD) are at a high risk for hypertension in pregnancy (HIP), the maternal risk in KT recipients with IgAN as the etiology remains unclear. We retrospectively reviewed the medical records of pregnant KT recipients who delivered at our hospital. The incidence of maternal and fetal complications and the impact on kidney allografts between the group with IgAN as the primary kidney disease and the group with other primary diseases were compared. The analysis included 73 pregnancies in 64 KT recipients. The IgAN group had a higher incidence of HIP than the non-IgAN group (69% vs. 40%, $p = 0.02$). IgAN as primary kidney disease and interval from transplantation to conception were associated with HIP (OR 3.33 [1.11–9.92], $p = 0.03$, OR 0.83 [0.72–0.96], $p < 0.01$, respectively). The 20-year graft survival or prevention of CKD stage 5 in group with IgAN was lower than that in the group with other primary disease ($p < 0.01$). KT recipients should be informed of the risk of HIP and possibility of long-term worsening of postpartum renal function.

Keywords: kidney transplantation, graft survival, pregnancy, IgA nephropathy, pregnancy complications

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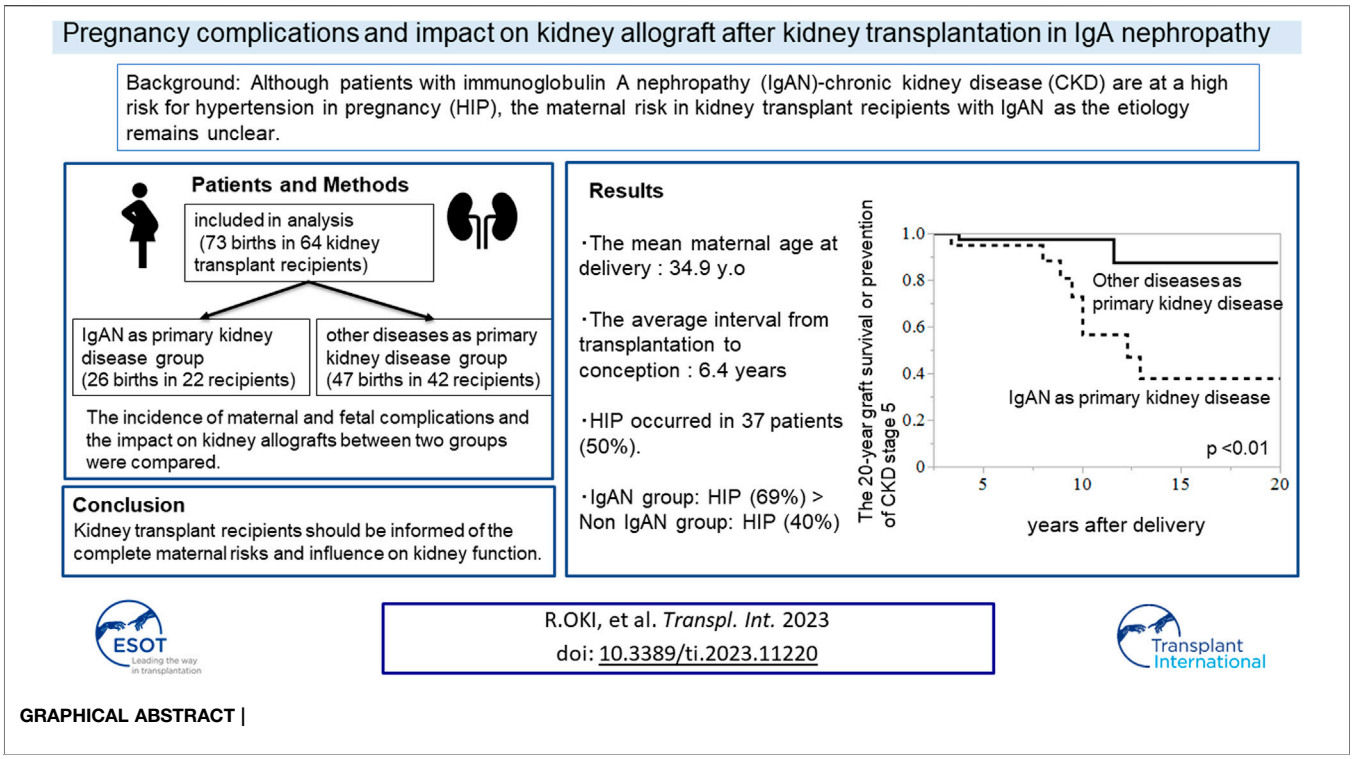
INTRODUCTION

Female patients with end-stage kidney disease (ESKD) are known to have lower fertility rates due to disruption of hypothalamic-gonadal axis (1). Earlier studies have revealed that the probability of delivering a live-born baby may be rounded to 1:100 for women on dialysis compared to the overall Italian population; (2). Meanwhile, women with functioning kidney grafts have a 10-fold higher probability of delivering a live-born baby than patients on dialysis; (2). Thus, kidney transplantation (KT) deserves special attention because it provides a hope for women with ESKD who desire for childbearing. Along with increase in KT, increasing number of post-KT recipients in Japan who experienced pregnancy and childbirth have been observed, with over 500 cases (3, 4).

However, pregnancy in KT recipients remains challenging because it might severely affect graft kidney function, fetal development, and maternal health; in particular, the risk of deterioration of allograft function and/or occurrence of antibody-mediated rejection exists throughout pregnancy (5). Mohammadi et al. have reported that one-third had deterioration in graft dysfunction during pregnancy, more than 60% of which did not return to baseline (6). Pregnant KT recipients are also



GRAPHICAL ABSTRACT |

reportedly at a higher risk of gestational diabetes, hypertension during pregnancy (HIP), preeclampsia (PE), cesarean section, and preterm delivery (7).

Immunoglobulin A nephropathy (IgAN) is the most common glomerulonephritis, which is one of the leading causes of ESKD in the younger generation. Therefore, IgAN would also be the common primary disease of ESKD for KT recipients (8), in particular among young women of childbearing age.

In general, for pregnant women (non-KT patients), IgAN is considered as a risk factor for adverse outcomes. A systematic review by Piccoli et al. has revealed that the incidence of adverse pregnancy-related outcomes, including HIP or PE, was ten-fold higher in pregnancy with IgAN (non KT patients), than in control groups (9). In this study, baseline kidney function (estimated glomerular filtration rate [eGFR]) was relatively well preserved (9). Another study has reported that pregnant women with IgAN (non-KT patients) were at a higher risk of having preterm birth, PE and small for gestational age babies (10). However, whether IgAN might lead to an increased rate of adverse pregnancy-related outcomes in post-KT pregnancy remains unclear. There are a lot of KT female recipients with IgAN desire pregnancy. Therefore, clarifying the adverse pregnancy-related outcomes in KT recipients with IgAN may be necessary for preconception counseling in postpartum care.

This study was conducted to evaluate pregnancies in KT recipients and their impact on the mother, fetus, and graft function after delivery in our hospital. We focused on the difference in the incidence of maternal/fetal complications and the impact on kidney allograft, depending on the presence or absence of IgAN as primary kidney disease. We also examined the factors related to HIP in KT recipients.

MATERIALS AND METHODS

Patients and Data Collection

This retrospective cohort study examined post-KT pregnant women who were on regular prenatal care and delivered at our hospital from 1 January 2001 to 31 December 2019. This study, conducted at a single center (Tokyo Women's Medical University Hospital), was approved by the Institutional Review Board of the Tokyo Women's Medical University Hospital (#2022-0084). This study retrospectively collected data from the medical records; therefore, informed consent was waived by the Institutional Review Board of the Research Ethics Committee of the Faculty of Medicine of Tokyo Women's Medical University Hospital. All methods of research procedures were performed in accordance with the Declaration of Helsinki. The exclusion criteria were as follows: 1) follow-up period from delivery of <3 years; 2) pregnancies resulting in birth at <22 weeks of gestation; 3) miscarriage; 4) abortion; and 5) stillbirth. Stillbirth was defined as the intrauterine death of a fetus at ≥22 weeks of gestation.

The basic information of patients, pregnancy, and neonates (age at transplantation or pregnancy, information on transplantation, cause of ESKD, donor type, type of immune-suppressive therapy during the perinatal period, data on pregnancy/delivery/neonates, and kidney function pre-pregnancy/at postpartum/at 1 year and up to 3 years postpartum) were collected from the patients' medical records. Latent IgA deposition from donor was not considered IgAN as primary disease.

Calcineurin inhibitors (tacrolimus or cyclosporine), mycophenolate mofetil (MMF) and prednisolone were used as maintenance immunosuppressants at our hospital. MMF was discontinued at least 6 weeks prior to the planned pregnancy and replaced with azathioprine (AZA), considering a risk of the teratogenicity. The date of preparation for pregnancy was defined as the date when MMF was discontinued or replaced with AZA on medical charts.

Pregnancy data included the incidence of HIP, gestational diabetes (diagnosed according to the recommendations of the International Society for the Study of Hypertension in Pregnancy [ISSHP] (11), cesarean section, gestational age at delivery, and preterm birth (babies born alive before 37 weeks of pregnancy). Blood pressure was measured with a brachial sphygmomanometer at home.

Data on neonates included birth weight, incidence of low birth weight (defined by the World Health Organization [WHO] as weight at birth of <2500 g), APGAR-score (Appearance, Pulse, Grimace, Activity, and Respiration), and umbilical cord blood pH at delivery. The maternal indications for cesarean section include severe HIP, deterioration of kidney graft function, prolonged labor (defined by the WHO as active labor that lasts >12 h (12), and previous cesarean delivery. The fetal indications for cesarean section included fetal growth restriction, fetal malposition, and non-reassuring fetal status. The interval between KT and pregnancy was calculated separately for each pregnancy.

Kidney function, including serum creatinine, eGFR, and data on proteinuria measured qualitatively, was evaluated pre-pregnancy, postpartum, and at 1 year and up to 3 years postpartum. The pre-pregnancy serum creatinine level was defined as the latest result within 3 months before conception. Postpartum serum creatinine levels were measured the day after delivery. Graft loss after pregnancy was defined as returning to dialysis or undergoing second transplantation. The indications for graft biopsy after delivery included time-dependent protocol biopsy or episode biopsy.

The Luminex single antigen beads assay (One Lambda Inc., Canoga, Park, CA, United States) was used to detect *de novo* donor-specific antibody (DSA). The assay was also conducted according to the manufacturer's instructions as previously described. Positive DSA was defined as a mean fluorescence intensity >1000.

Pregnancy Outcomes

This study compared the incidence of HIP between patients with IgAN as the primary kidney disease and those with other primary diseases. HIP is defined as chronic (predating pregnancy or diagnosed before 20 weeks of pregnancy) or *de novo* (either PE or GH), according to the ISSHP guidelines.

Renal Outcomes

The primary outcome was the composite of all-cause graft loss and chronic kidney disease (CKD) stage 5 (eGFR<15 mL/min/1.73 m^2) within 20 years postpartum. The occurrence and date of the first observed outcome postpartum were also investigated. For recipients who underwent twice deliveries, the duration from the first delivery was included in the analysis. Patient survival was examined at graft loss, the date when CKD stage 5 was detected, or the date of the last follow-up.

Statistical Analysis

All statistical analyses were conducted using software (JMP®, Version<16.0>; SAS Institute Inc., Cary, NC, 1989–2021). Continuous data were expressed as mean ± standard deviation or median (interquartile range). Student *t*-tests or Mann–Whitney U-tests were used to compare continuous variables. The chi-square test or Fisher's exact test was used to compare the categorical variables. Univariate and multivariate logistic regression analyses were performed to examine significant factors associated with HIP. The Kaplan-Meier method and log-rank test were used to compare differences in graft survival or CKD stage 5 within 20 years postpartum between groups. A paired-samples t-test was used to compare kidney function at each point (pre-pregnancy, delivery, 1–3 years postpartum). Values for which p was less than 0.05 were inferred as significant.

RESULTS

Characteristics of Study Participants

Of the 110 pregnancies during the study period, a total of 73 births in 64 patients were included in the analysis (**Figure 1**). Nine recipients experienced two deliveries, and three recipients delivered twins, resulting in 64 patients delivering 76 neonates by 73 pregnancies. In total, 26 births were recorded in 22 patients in a group with IgAN as the primary kidney disease, and 47 births were recorded in 42 patients in a group with other diseases as the primary kidney disease. The baseline characteristics of all participants are presented in **Table 1**. The mean patient age at transplantation was 28.1 ± 6.1 years old. The most common primary kidney disease was IgAN (n = 22, 34%), followed by glomerulonephritis (n = 13, 20%) [chronic glomerulonephritis, focal segmental glomerular sclerosis (FSGS), membranous proliferative glomerulonephritis, and rapid progressive glomerulonephritis], and congenital anomalies (n = 5, 8%). All the patients were recipients of living related KT, and most donors were the recipients' parents.

Pregnancy Outcomes

Table 2 presents the comparison of pregnancy outcomes of IgAN between the primary kidney disease group and group with other primary diseases. Four patients exhibited IgA deposition in a zero-hour biopsy without mesangial proliferative changes. No significant difference in incidence of latent IgA deposition was observed between the two groups.

The average interval from transplantation to conception was 6.4 ± 4.0 years, and 60% of pregnancies were observed ≥5 years after transplantation. The average period from the date of preparation for pregnancy (discontinuation of MMF or replacement with AZA) to pregnancy was 1.1 ± 1.1 years. However, there were six cases, in which MMF was suspended or changed after pregnancy was confirmed. The mean maternal

age at delivery was 34.9 ± 4.22 years old. No significant difference in mean maternal age was observed between the two groups. HIP occurred in 37 patients (50%), of which 14 patients had hypertension before pregnancy, and 23 patients developed *de novo* hypertension. As presented in **Table 2**, the most commonly used antihypertensive drug during pregnancy was methyldopa, which was subsequently replaced with a calcium blocker or angiotensin-receptor blocker.

The IgAN group had a higher incidence of HIP (69% vs. 40%, $p = 0.02$) and significantly lower gestational age and birth weight (mean gestational age; 35.0 weeks vs. 37.7 weeks, $p = 0.04$; average birth weight, 2008 g vs. 2416 g, $p = 0.03$) than the other primary diseases group. Additionally, more neonates in the IgAN group had a low APGAR score <7 at 5 min after birth.

Logistic regression models were used to evaluate factors related to HIP (**Tables 3**, **4**). The analyses were adjusted for factors reported to be associated with an increased risk of HIP as follows (13, 14): maternal age at delivery, diabetes mellitus, CKD (kidney dysfunction before pregnancy), and interval from transplantation to conception. IgAN as the primary kidney disease and the interval from transplantation to conception were found to be related significantly to HIP in all the models (**Table 4**).

Renal Outcomes

The median serum creatinine [mg/dL] level at pregnancy was 1.05, and the median eGFR [mL/min/1.73 m^2] was 49.6. Only four cases (6%) had proteinuria (quantitative test 1+) before pregnancy (**Table 5**). Although eGFR [mL/min/1.73 m^2] at delivery was significantly lower than that at pre-pregnancy (42.7 vs. 49.6, $p < 0.01$), it recovered to baseline level within 1 year postpartum (**Table 5**). Once renal function was recovered, it gradually and significantly deteriorated after 2 years, compared with that before pregnancy. No significant difference in renal function at each point was observed between the groups with IgAN as the primary disease (**Table 5**).

Screening for panel reactive antibody was performed in 36 of 64 patients, and 8% ($n = 3$) of them had *de novo* DSA postpartum. No significant difference in the rate of *de novo* DSA was detected between the two groups (**Supplementary Table S1**) Kidney biopsy was performed in 36 of 64 patients postpartum. Biopsy-proven rejection developed in nine cases (chronic active antibody mediated rejection; N = 6, antibody mediated rejection; N = 3). The average interval from delivery to rejection was 5.7 years. More patients in the IgAN group had biopsy proven rejection than those with other primary diseases (50% vs. 12.5%, $p < 0.01$) (**Supplementary Table S2**).

Among the patients in this study population, 10 recipients eventually experienced graft failure or progression to CKD stage 5 within 20 years postpartum. The causes of graft loss or CKD stage 5 included rejection ($n = 2$, 20%), recurrent IgAN ($n = 1$, 10%), calcineurin inhibitor toxicity ($n = 1$, 10%), and secondary (FSGS) ($n = 1$, 10%) and unknown causes ($n = 5$, 50%). Compared to the non-IgAN group, the IgAN group had a higher rate of graft loss or incidence of progression to CKD stage 5 [36% (8/26 patients) vs. 5% (2/37 patients), $p < 0.01$] (**Table 5**). Graft loss or progression to CKD stage 5 within 20 years postpartum was compared between the groups with IgAN as the primary kidney disease group and the group (N = 22) with other primary diseases (N = 42) using Kaplan–Meier analysis and log-rank testing (**Figure 2**). The results revealed that the 20-year graft survival or the rate of CKD stage 5 prevention in the IgAN group was significantly lower than that in the group with other primary diseases ($p < 0.01$).

DISCUSSION

In this retrospective observational study, we analyzed 73 pregnancies occurring in 64 KT recipients to examine factors related to HIP. The results of this study demonstrated that IgAN as the primary kidney disease and the interval from transplantation to conception were associated with HIP in KT recipients. Additionally, IgAN was significantly associated with a higher rate of graft loss or CKD stage 5 within 20 years postpartum, although no significant difference in the short term renal prognosis was noted. All patients were properly managed for kidney function and lifestyle-related diseases, including hypertension by attending physician during the perinatal and postpartum periods. To the best of our knowledge, this is the first study to demonstrate the possible contribution of IgAN to adverse pregnancy and renal outcomes in KT recipients.

The number of pregnancies among KT recipients reported worldwide has steadily increased, which has received considerable attention (15). Pregnant recipients are widely known to be at an increased risk for adverse maternal complications. In a review by Shah et al. of 6712 pregnancies in 4174 KT recipients, the mean maternal age was 29.6 ± 2.4 years (7), whereas it was 34.9 ± 4.22 years in our study. The rate of pregnancy outcomes was reported in a review by Shah et al. as follows; PE (21.5%), GH (24.1%), cesarean section (62.6%) and preterm delivery (43.1%) (7), which were compatible with our data, despite the high rate of late child bearing in our facility.

The prevalence of PIH in generally reproductive-aged women is approximately 7%–10% (16). HIP remains one of the major complications of pregnancy, which might cause maternal and perinatal morbidity and mortality (17). HIP-associated short-term complications include intrauterine growth restriction, small for gestational age, low birth weight and preterm birth (17). Although no significant difference in the rate of preterm birth or low birth weight was observed in our study, the median gestational age and mean birth weight were significantly lower in the IgAN group, than in the group with other primary diseases. (35 weeks vs. 37.7 weeks, $p = 0.04$, 2008g vs. 2416g, $p = 0.03$, respectively) Thus, it is presumed that these findings might reflect the high incidence of HIP in the group with IgAN.

Reportedly, the risk of GH or PE was 10 or 11 times higher in women with IgAN despite relatively well preserved kidney function (9). According to another report, IgAN was associated with an increased risk of preterm birth and cesarean section (10). Our pregnancy outcomes are in line with previous studies that have demonstrated the possible link of IgAN in pregnancy to adverse pregnancy outcomes.

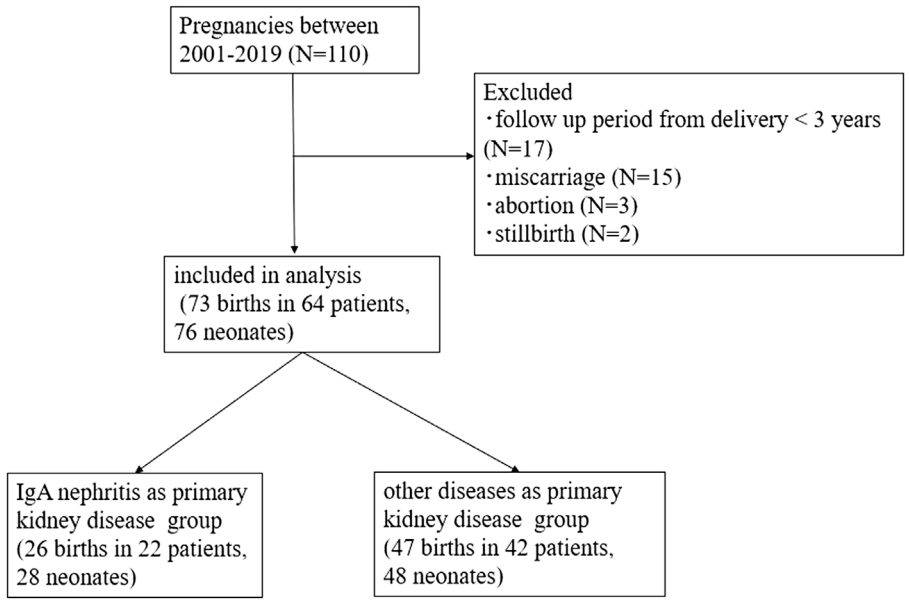


FIGURE 1 | Chart showing flow of the study.

TABLE 1 | Basic information of the pregnant kidney transplant recipients (N = 64).

Variable	
Age at transplantation [y.o.]	28.1 ± 6.1
Primary kidney disease	
IgA nephropathy	22 (34%)
Glomerulonephritis[a]	13 (20%)
Congenital anomaly	5 (8%)
Vesicoureteral reflux	4 (6%)
Interstitial nephritis	3 (5%)
Diabetes mellitus	3 (5%)
Alport syndrome	2 (3%)
Others	3 (5%)
Unknown	9 (14%)
Living kidney donor	64 (100%)
Donor	
Parent	58 (91%)
Sibling	3 (5%)
Spouse	1 (1%)
Other	2 (3%)

[a]*Glomerulonephritis; except for IgA nephropathy. chronic glomerulonephritis (N = 5), focal segmental glomerular sclerosis (N = 4), membranoproliferative glomerulonephritis (N = 2), and rapid progressive glomerulonephritis (N = 1).*

Although the underlying pathophysiology of HIP has not been elucidated to date, placental ischemia and imbalance of angiogenic factors seem to be responsible for it (16). First, uteroplacental perfusion is reduced because of abnormal cytotrophoblast invasion of spiral arterioles. Next, placental ischemia leads to widespread activation/dysfunction of the maternal vascular endothelium, which contributes to enhance endothelial dysfunction (16). In particular, the release of antiangiogenic factors, including soluble fms-like tyrosine-kinase 1 (sFlt-1) and endoglin induces maternal vascular endothelial dysfunction (18). sFlt and endoglin reportedly increase before the onset of PE and correlate with disease severity (18). Zhai et al have demonstrated that excess sFlt-1 levels in patients with IgAN correlated with proteinuria and hypertension and elevated sFlt-1 levels contributed to endothelial injury in IgAN (19, 20). Circulating sFlt-1 levels are increased in various degrees of kidney dysfunction including post-KT (21). Moreover, a clinical association has been identified between circulating sFlt-1 and endothelial dysfunction in patients even after KT (21). An excess sFlt-1 level might be observed in post-KT recipients with IgAN, which could induce HIP. However, we did not obtain data of sFlt1 in our study, and we cannot substantiate it.

As for the activity or severity of IgAN before pregnancy, no significant differences in creatinine level and rate of proteinuria before pregnancy were observed between the groups with and without IgAN. Moreover, whether recurrent IgAN occurred in the kidney allograft in all pregnancy cases with IgAN remains unclear. Since 2019, we have routinely performed pre-pregnancy kidney biopsies to detect whether pregnancy was possible. However, the biopsy results before 2018 were insufficient because they were not determined in our hospital. Only 3 of 8 cases with pre-pregnancy biopsy results in the IgAN group had recurrent IgAN. Altogether, no evident mechanism between IgAN in KT recipients and HIP can be inferred from the results of this study.

In addition to the presence of IgAN, the interval from transplantation to conception was associated with HIP in KT recipients in our study. In the logistic analysis, the odds ratio associated with each 1-year increase in interval from transplantation to conception was 0.84 (95% confidence interval 0.73–0.96, $p < 0.01$), independent of maternal age. The optimal timing of pregnancy after KT remains

TABLE 2 | Pregnancy outcomes stratified by groups according to whether IgA nephropathy was the primary disease.

Variables	All (*N* = 73)	IgA nephropathy (*N* = 26)	Non-IgA nephropathy (*N* = 47)	*p*
Latent IgA deposition in zero-hour biopsy	4 (5%)	3 (12%)	1 (2%)	0.09
Interval from transplantation to conception [years]	6.4 ± 4.0	6.2 ± 4.1	6.5 ± 4.0	0.77
<2 years	12 (17%)	4 (15%)	8 (17%)	0.86
2–5 years	17 (23%)	7 (27%)	10 (21%)	0.59
≥5 years	44 (60%)	15 (62%)	29 (62%)	0.74
Interval from preparation for pregnancy to conception [years]	1.1 ± 1.1	1.2 ± 1.1	1.0 ± 1.2	0.48
Average maternal age at delivery [y.o.]	34.9 ± 4.22	35.3 ± 3.39	34.7 ± 4.64	0.57
Immunosuppressive regimens during pregnancy				
TAC, AZA and PSL	52 (71%)	19 (73%)	33 (70%)	0.80
CyA, AZA, and PSL	2 (3%)	1 (4%)	1 (2%)	—
TAC and PSL	11 (15%)	1 (4%)	10 (21%)	0.046
TAC and AZA	3 (4%)	1 (4%)	2 (4%)	—
CyA and AZA	4 (5%)	3 (12%)	1 (2%)	—
CyA, MZ, and PSL	1 (1%)	1 (4%)	0 (0%)	—
hypertension in pregnancy	37 (50%)	18 (69%)	19 (40%)	0.02
Antihypertensive drugs during pregnancy				
Methyldopa	19 (26%)	10 (38%)	9 (19%)	0.62
Calcium blocker	1 (1%)	1 (4%)	0 (0%)	—
βblocker	1 (1%)	0 (0%)	1 (2%)	—
Antihypertensive drugs in follow-up period				
Calcium blocker	22 (30%)	11 (42%)	11 (23%)	0.84
Angiotensin receptor blocker	6 (8%)	5 (19%)	1 (2%)	0.06
Methyldopa	5 (11%)	2 (8%)	3 (6%)	0.68
Gestational diabetes	1 (1%)	0 (0%)	1 (2%)	—
Delivery				0.62
Vaginal birth	28 (38%)	9 (35%)	19 (40%)	
Caesarean section	45 (62%)	17 (65%)	28 (60%)	
Indication of caesarean section				0.27
Maternal	36 (80%)	15 (88%)	21 (75%)	
Fetal	9 (20%)	2 (12%)	7 (25%)	
Gestational age [weeks]	37.4 (32.7, 38.7)	35.0 (29.1, 38.3)	37.7 (34.1, 38.9)	0.04
Preterm birth	31 (42%)	14 (54%)	17 (36%)	0.14
Birth weight [g][a]	2266 ± 783	2008 ± 887	2416 ± 681	0.03
Low birth weight[a]	42 (55%)	18 (64%)	24 (50%)	0.23
umbilical cord blood pH at delivery (livebirths)[a]	7.29 (7.25, 7.33)	7.28 (7.25, 7.31)	7.30 (7.26, 7.34)	0.14
APGAR score after 5 min[b]				0.03
7–10	67 (93%)	21 (84%)	46 (98%)	
4–6	3 (4%)	3 (12%)	0 (0%)	
0–3	2 (3%)	1 (4%)	1 (2%)	

AZA, azathioprine; CyA, cyclosporine; IgA, immunoglobulin A; MZ, mizoribine; TAC, tacrolimus; PSL, prednisolone.
Continuous data are presented as the mean ± SD or median (IQR).
Nine patients had two pregnancies. Each pregnancy was calculated separately.
[a]*Three patients had twins. Each neonate was evaluated separately.*
[b]*Four values were missing for APGAR score in 5 min.*

controversial. The American and European guidelines for advising transplant recipients suggest that conception could be considered as early as 1–2 years post-transplantation, under a stable general condition (22, 23). Deshpande et al. have reported that obstetric complications including PE and gestational diabetes, were the highest in the <2-year interval following KT, and delivery outcomes including cesarean section rate and preterm birth rate were also less favorable in this interval (14). However, waiting the timing of pregnancy after KT might increase the risk of late childbearing and miss a chance of pregnancy because the fertility window could be narrow. Therefore, preconception counseling and care including family planning are warranted for safe and successful pregnancies in KT recipients.

Regarding the impact on postpartum kidney function, our results indicate that serum creatinine levels significantly decreased at delivery and then recovered at 1 year postpartum. A slight significant increase in serum creatinine level in 2–3 years was observed after delivery (Δserum creatinine 0.04 mg/dL, pre-pregnancy to post 3 years postpartum). This trend is similar as that previously reported (24). Buren et al have reported a rise in serum creatinine within 2 years postpartum of 0.18 mg/dL, which did not decrease for up to 10 years postpartum (24).

The rate of graft loss or CKD stage 5 within 20 years postpartum was significantly higher in the IgAN group than in the other primary disease. What is the rationale behind this result? First, the rejection rate in the IgAN group was higher than that in the group with other primary diseases (50% vs. 12.5%, p =

TABLE 3 | Results of the univariate logistic regression analyses for hypertension in pregnancy.

Variables	OR (95% CI)	*p*
Age at transplantation	0.95 (0.85–1.06)	0.38
Primary kidney disease		
IgA nephropathy	3.32 (1.20–9.16)	0.02
Diabetes mellitus	0.47 (0.04–5.45)	0.55
Interval from transplantation to conception	0.85 (0.75–0.97)	<0.01
<2 years	3.54 (0.87–14.3)	0.08
2–5 years	2.12 (0.69–6.51)	0.19
≥5 years	0.28 (0.10–0.77)	0.01
Interval from preparation for pregnancy to conception	1.43 (0.89–2.29)	0.13
Average maternal age at delivery	0.95 (0.85–1.06)	0.38
Pre-pregnancy		
Cre	0.97 (0.13–7.30)	0.98
BUN	1.08 (0.98–1.20)	0.13
eGFR	0.99 (0.96–1.03)	0.77
Proteinuria	0.97 (0.13–7.31)	0.98

OR, odds ratio; CI, confidence interval; IgA, immunoglobulin A; Cre, creatinine; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate.

TABLE 4 | Results of the multivariate logistic regression analyses for hypertension in pregnancy.

Variables	Model 1		Mode1 2		Model 3	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
IgA nephropathy	3.33 (1.11–9.92)	0.03	3.90 (1.29–11.8)	0.02	3.91 (1.28–11.9)	0.02
Interval from transplantation to conception	0.83 (0.72–0.96)	<0.01	0.84 (0.73–0.96)	<0.01	0.83 (0.73–0.96)	<0.01
Diabetes mellitus	0.29 (0.02–3.79)	0.35				
Average maternal age at delivery			0.92 (0.81–1.04)	0.16		
Pre-pregnancy Cre					0.48 (0.05–4.81)	0.53

OR, odds ratio; CI, confidence interval; IgA, immunoglobulin A; Cre, creatinine.
Model 1: IgA nephropathy, interval from transplantation to conception, diabetes mellitus.
Model 2: IgA nephropathy, interval from transplantation to conception, average maternal age at delivery.
Model 3: IgA nephropathy, interval from transplantation to conception, pre-pregnancy creatinine.

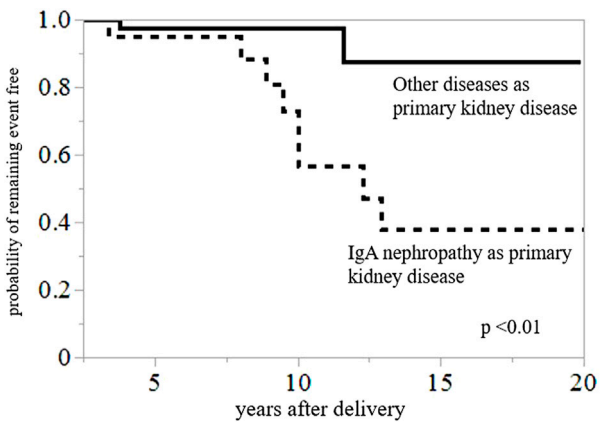


FIGURE 2 | Kaplan–Meier analysis for composite outcomes of graft loss or CKD stage 5 within 20 years postpartum. Graft survival or probability of avoidance of CKD stage 5 during 20 years postpartum was significantly lower in the group with IgAN than in the group with other primary diseases ($p < 0.01$).

0.01). However, we cannot insist that recipients with IgAN are prone to rejection postpartum, because only 36 of 64 patients underwent kidney biopsy postpartum. Second, on whether IgAN as the primary disease affects graft survival, several studies have revealed that comparatively short-term graft survival (within 10–12 years) for IgAN patients was similar to that of patients with other primary disease (25, 26), but long-term graft survival (after 12–15 years) became worse (25, 27). Graft loss could be attributed to recurrent IgAN on long-term follow-up in their studies (25, 27). In our study, only one patient with graft loss or CKD stage5 within 20 years of delivery had recurrent IgAN. Third, the impact of bipara or twins may affect graft function. However, no significant difference in the percentage of recipients who had two deliveries or twins was observed between the two groups. Furthermore, the Kaplan–Meier analyses showed no significant difference in the 20-year graft survival or prevention of CKD stage 5 between unipara and bipara ($p = 0.66$). Collectively, whether IgAN as a primary kidney disease has a higher likelihood of deteriorating kidney function or graft loss after delivery is difficult to be explained. Pregnant recipients with IgAN should be paid special attention for kidney function postpartum.

The present study has several limitations. First, we might not have investigated or collected sufficient data on the unknown factors affecting the relationship between IgAN and HIP. Second, as mentioned above, the severity or activity of IgAN before delivery was not sufficiently

TABLE 5 | Renal outcomes compared by groups according to whether IgA nephropathy was the primary disease.

Variables	All (*N* = 73)	IgA nephropathy (*N* = 26)	Non-IgA nephropathy (*N* = 47)	*p*
Pre-pregnancy				
Cre [mg/dL]	1.05 (0.95, 1.22)	1.16 (0.98, 1.33)	1.03 (0.94, 1.22)	0.20
BUN [mg/dL]	16.1 (13.6, 21.0)	16.7 (13.8, 21.3)	15.6 (13.5, 20.7)	0.41
eGFR [mL/min/1.73 m^2]	49.6 (41.6, 57.6)	46.0 (38.0, 58.0)	50.3 (42.4, 57.7)	0.29
Proteinuria+	4 (6%)	2 (8%)	2 (5%)	0.56
Delivery				
Cre [mg/dL]	1.20 (0.98, 1.52)	1.27 (1.03, 1.48)	1.16 (0.96, 1.60)	0.77
BUN [mg/dL]	17.4 (12.5, 22.1)	17.7 (12.0, 21.9)	17.0 (13.1, 23.6)	0.90
eGFR [mL/min/1.73 m^2]	42.7 (32.8, 53.6)	40.5 (33.3, 53.3)	45.9 (30.9, 54.4)	0.75
Proteinuria	28 (38%)	11 (42%)	17 (36%)	0.53
Proteinuria +	16 (22%)	8 (31%)	8 (17%)	
Proteinuria 2+	9 (12%)	2 (7%)	7 (15%)	
Proteinuria 3+	3 (4%)	1 (4%)	2 (4%)	
1 year postpartum				
Cre [mg/dL]	1.07 (0.91, 1.41)	1.13 (0.90, 1.39)	1.03 (0.90, 1.42)	0.82
BUN [mg/dL]	17.8 (13.3, 22.7)	17.4 (12.8, 22.7)	18.1 (13.6, 23.0)	0.62
eGFR [mL/min/1.73 m^2]	48 (36.2, 61.8)	44.3 (36.3, 65.7)	49.3 (35.7, 61.5)	0.74
proteinuria	11 (15%)	4 (16%)	7 (15%)	0.44
Proteinuria +	8 (11%)	2 (8%)	6 (13%)	
Proteinuria 2+	3 (4%)	2 (8%)	1 (2%)	
2 years postpartum				
Cre [mg/dL]	1.08 (0.91, 1.34)	1.15 (0.92, 1.34)	1.05 (0.90, 1.35)	0.61
BUN [mg/dL]	18.0 (14.3, 22.8)	19.0 (13.8, 22.8)	17.3 (14.4, 23.1)	0.86
eGFR [mL/min/1.73 m^2]	46.9 (36.5, 58.4)	44.3 (36.5, 56.5)	48.6 (36.3, 59.1)	0.51
Proteinuria	10 (14%)	5 (19%)	5 (11%)	0.30
Proteinuria +	9 (13%)	4 (15%)	5 (11%)	
Proteinuria 2+	1 (1%)	1 (4%)	0 (0%)	
3 years postpartum				
Cre [mg/dL]	1.09 (0.90, 1.42)	1.18 (0.98, 1.47)	1.03 (0.89, 1.41)	0.19
BUN [mg/dL]	18.0 (14.8, 23.1)	19.5 (16.3, 24.2)	17.0 (14.4, 22.7)	0.34
eGFR [mL/min/1.73 m^2]	46.0 (34.1, 57.0)	41.2 (32.9, 57.5)	50.5 (35.0, 57.0)	0.19
Proteinuria	10 (14%)	4 (15%)	6 (13%)	0.35
Proteinuria +	9 (12%)	3 (11%)	6 (13%)	
Proteinuria 3+	1 (1%)	1 (4%)	0 (0%)	
Graft loss or CKD stage5 within 20 years postpartum[a]	10 (15%)	8 (36%)	2 (5%)	<0.01

IgA, immunoglobulin A; Cre, creatinine; BUN, blood urea nitrogen; eGFR, glomerular filtration rate; CKD, chronic kidney disease.
[a]*Evaluation based on pregnant kidney transplant recipients (N = 64), only the first pregnancies were analyzed.*

considered because of the lack of data on kidney biopsy. We only had data on urinary protein as a qualitative test because quantitative tests are not routinely performed for general follow-up in outpatient clinics. Moreover, the race-dependent difference in IgAN was not considered as this study was performed in a single Japanese medical facility, which enrolls only Asian patients. Third, the generalizability of the results remains unconfirmed because this retrospective study was conducted in a single institution. Hence, larger studies including other facilities or different races are needed in the future to test our findings.

Within these limits, our study presents several clinical implications. Our findings suggest that female recipients of childbearing age wishing to consider pregnancy should be informed of the complete maternal risks and influence on kidney function by an expert multidisciplinary team. The best outcomes could be likely achieved under careful pre-pregnancy evaluation, planning, and perinatal management. We hope that our findings may guide periconceptional counseling on clinical decision-making and quality of life in KT patients.

DATA AVAILABILITY STATEMENT

Raw data were generated at Tokyo women's medical university. Derived data supporting the findings of this study are available from the corresponding author (KU) on request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of The Tokyo Women's Medical University (#2022-0084). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

RO, KU, and HS conceived the idea of the study. RO and KU developed the statistical analysis plan and conducted statistical analyses. JK, HB, TB, TY, TK, TH, KO, and KK contributed to the

interpretation of the results. RO drafted the original manuscript. JH, TT, and HI supervised the conduct of this study. All authors reviewed the manuscript draft and revised it critically on intellectual content. All authors approved the final version of the manuscript to be published.

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.2023.11220/full#supplementary-material

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ORIGINAL RESEARCH
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Can We Predict Graft Intolerance Syndrome After Kidney Transplant Failure? External Validation of a Previously Developed Model

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Previously we established a prediction model for graft intolerance syndrome requiring graft nephrectomy in patients with late kidney graft failure. The aim of this study is to determine generalizability of this model in an independent cohort. The validation cohort included patients with late kidney graft failure between 2008 and 2018. Primary outcome is the prognostic performance of our model, expressed as the area under the receiver operating characteristic curve (ROC-AUC), in the validation cohort. In 63 of 580 patients (10.9%) a graft nephrectomy was performed because of graft intolerance. The original model, which included donor age, graft survival and number of acute rejections, performed poorly in the validation cohort (ROC-AUC 0.61). After retraining of the model using recipient age at graft failure instead of donor age, the model had an average ROC-AUC of 0.70 in the original cohort and of 0.69 in the validation cohort. Our original model did not accurately predict the graft intolerance syndrome in a validation cohort. However, a retrained model including recipient age at graft failure instead of donor age performed moderately well in both the development and validation cohort enabling identification of patients with the highest and lowest risk of graft intolerance syndrome.

Keywords: prediction model, graft intolerance syndrome, kidney graft failure, external validation, graft nephrectomy

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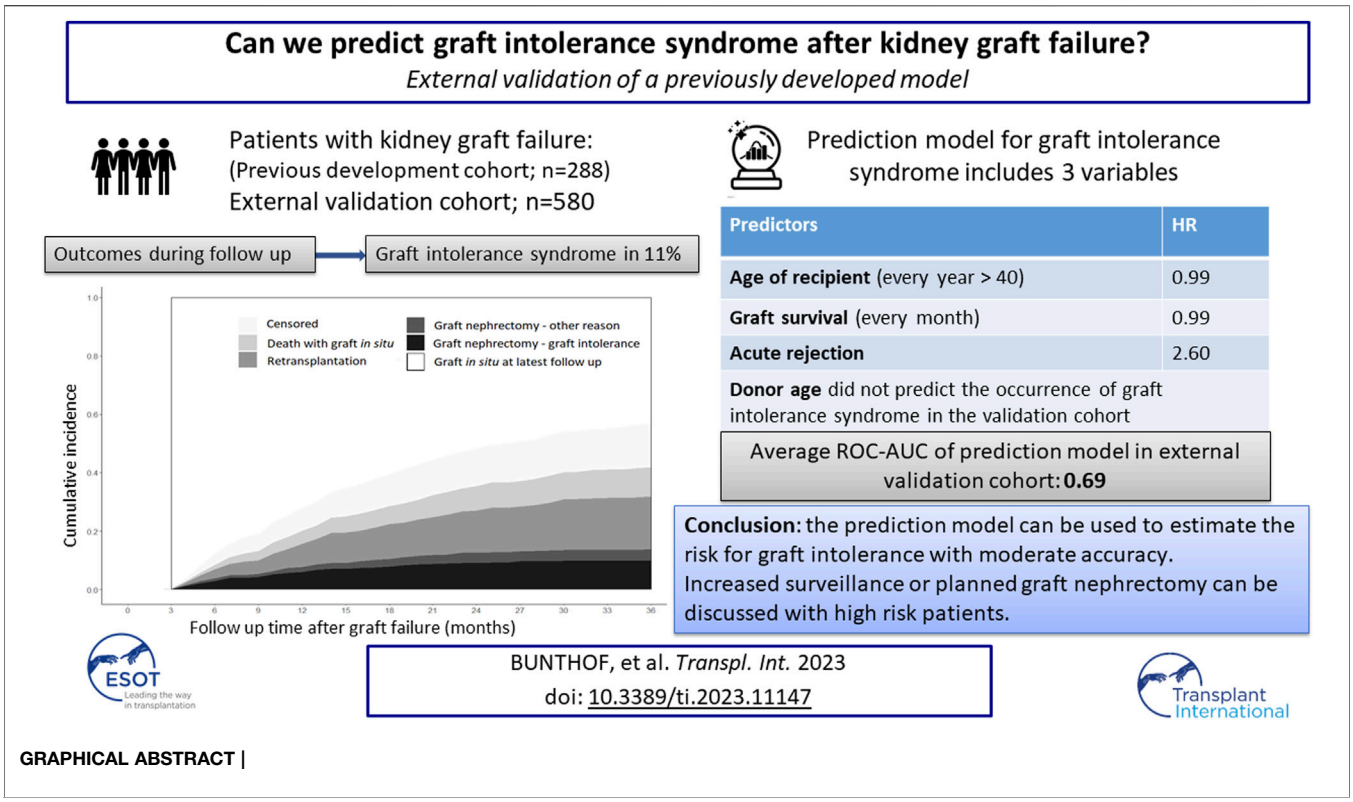
INTRODUCTION

Although kidney graft survival has improved over the last decades, recent data indicate that the incidence of kidney graft failure within 5 years after transplantation is still 12% and 20% for living and deceased donor kidneys, respectively (1, 2). After reinstitution of dialysis, the failed graft can be removed or left *in situ*. When to perform graft nephrectomy is controversial and often depends on local clinical practice. In general, graft nephrectomy is recommended after early graft failure (within 3–6 months) in order to avoid systemic and local effects of acute rejection. After late graft failure, the risk of acute rejection is presumably much smaller, and the graft is usually left *in situ*. However, in some cases late graft nephrectomy becomes necessary. Accepted indications for graft nephrectomy are to create space for re-transplantation, to enable immediate complete withdrawal of



GRAPHICAL ABSTRACT |

immunosuppression, graft malignancy, recurrent transplant pyelonephritis, and graft intolerance syndrome (3–6). Graft intolerance syndrome is characterized by the presence of pain or swelling of the graft, hematuria, fever, malaise, or refractory anemia, all in the absence of an infectious process. The syndrome is reported in 30%–50% of patients with graft failure and occurs mostly within the first year after initiation of dialysis. It reflects a chronic inflammatory state induced by the retained graft and is mostly associated with discontinuation of immunosuppression. However, also patients with (low dose) immunosuppression can present with a graft intolerance syndrome. Graft intolerance syndrome is associated with high morbidity and in most cases an urgent graft nephrectomy is required. Perioperative mortality and morbidity are substantially higher for urgent graft nephrectomy than for elective graft nephrectomy (7, 8). If the need for graft nephrectomy could be predicted, this could help clinicians in deciding to perform a pre-emptive graft nephrectomy, as a planned intervention may minimize the risk of peri-operative morbidity and mortality compared to an urgent procedure.

In a previous study we used data from a single center to develop a model to predict the need for graft nephrectomy because of graft intolerance syndrome (9). The training study cohort included 288 patients with kidney graft failure, of whom 48 (16.7%) suffered from graft intolerance syndrome requiring graft nephrectomy. We used Fine and Gray regression analysis to evaluate the association between this outcome and baseline characteristics. Our final model included donor age, number of acute rejections, and graft survival (time interval between transplantation and graft failure) as predictors. External validation of a prediction model is essential to support general applicability and implementation in clinical practice. Therefore, the aim of the present study was to determine generalizability of this prediction model for graft intolerance syndrome requiring graft nephrectomy.

PATIENTS AND METHODS

Study Population

The validation cohort included adult patients who experienced kidney graft failure at least 6 months post kidney transplantation between 2008 and 2018, and were treated in one of the following Dutch Transplant Centers: Erasmus University Medical Center (Rotterdam), Amsterdam University Medical Centers (Amsterdam), and University Medical Center Utrecht (Utrecht). Additionally, we included patients from the Radboud university medical center (Nijmegen) who were not included in the training cohort. In general, after graft failure and start of dialysis treatment, immunosuppression was gradually tapered to zero or to low dose steroids. In all patients a watchful waiting policy was followed regarding graft nephrectomy. In- and exclusion criteria were identical to those used for the training cohort. We excluded patients with one of the following events within 3 months after graft failure: re-transplantation, graft nephrectomy, death, or loss of follow up. Patients gave informed consent for sharing data in the National Organ Transplantation Registry (NOTR). This registry includes data

about all national transplantation programs and is used for quality assurance and scientific research. This study was approved by each local medical ethics committee. The trial was conducted in accordance with the Declaration of Helsinki and approved by the research ethics committee of the Radboud University Medical Center, Nijmegen (2018-4732).

Data Collection

We collected the following data from the NOTR and local patient files: age, gender, donor age, duration of graft survival, number of acute rejection episodes, and the occurrence of graft nephrectomy after graft failure. A rejection episode was defined as the need for anti-rejection therapy with or without biopsy-proven rejection. Treatment of rejection (either biopsy-proven or clinical diagnosis) after an interval of at least 3 months without acute rejection was considered to represent a new rejection episode. Indications for graft nephrectomy were retrieved from patients' files. Graft intolerance syndrome was defined as the presence of one or more of the following clinical criteria in the absence of another plausible explanation after routine clinical examination: pain or swelling of the graft, hematuria, fever, malaise, or refractory anemia. Follow-up ended in case of a competing event (death or re-transplantation) or when patients were lost to follow-up.

Sample Size Calculation

There are no generally accepted approaches to estimate the sample size requirement of validation studies of risk prediction models. The number of outcome events dictates the effective sample size. Our sample size was determined by the available data from participating transplant centers, and we did not choose a sample size on statistical grounds. Limited evidence suggests that a minimum of 100 events is needed to adequately quantify the performance of an existing model in other data, but more events are preferred (10).

Statistical Analysis

The prediction rule below was applied to the patients in the validation cohort.

Log baseline cumulative hazard

$$(ln\,ln\,H_0(t)) = -2.0252 - 32.3433t^{-2} + 0.0126t^{-0.5}$$

Prognostic index (PI) = $0.027 \times donor\ age\,[in\ years] - 0.011 \times graft\ survival\ [in\ months] + 0.336 \times total\ number\ of\ rejections$

Risk of graft nephrectomy at time t:

$$R(t) = 1 - exp\ [-exp\,exp(ln\,H_0(t))]^{exp\ (PI)}$$

We also recalibrated our model in the validation cohort by adjusting the baseline cumulative hazard without changing predicting factors. The performance of the model, expressed as the area under the receiver operating characteristic curve (ROC-AUC), and the calibration were assessed. A visual impression of the calibration of model predictions in the validation set was obtained by plotting the observed versus predicted probabilities. Finally, we retrained the original model with recipient age at the time of graft failure instead of donor age. We used the same training data and method (Fine and Gray regression) as with the previous model which was published by Bunthof et al. (10). We externally validated the retrained model with the data collected for the present study. The full analysis scripts can be accessed on https://github.com/JanvandenBrand/tect_validate.

RESULTS

Study Population

Our study cohort included 2,166 kidney graft failures between 2008 and 2018 (**Figure 1**). Patients with death as the cause of graft failure (n = 1,094) and patients with graft failure within 6 months after kidney transplantation (n = 219) were excluded from analyses. Graft nephrectomy was performed <3 months after graft failure in 62 patients and follow up ended <3 months after graft failure in 211 patients because of death, re-transplantation, or loss of follow up. Finally, we included 580 patients for validation of our model. In 98 patients of our validation cohort (16.9%) a graft nephrectomy was performed. Indications for graft nephrectomy were graft intolerance syndrome (n = 63), to create space for re-transplantation (n = 14), infection (n = 13), and other reasons (n = 8). The incidence of graft intolerance syndrome requiring a graft nephrectomy was 10.9%. Stacked cumulative incidence curves for various events during follow-up are shown in **Figure 2**. Patient and transplantation characteristics are shown in **Table 1**. Median duration of follow-up (time from graft failure to graft nephrectomy) was 10.4 and 20.1 months in patients with graft nephrectomy for graft intolerance and for other indications, respectively. In patients with a retained failed graft, median duration of follow-up (time from graft failure to death, retransplantation, or loss to follow-up) was 33 months. Compared with patients with a retained failed graft, patients with graft intolerance syndrome had a lower age at graft failure (median 43 vs. 50 years), had more acute rejection episodes, and a shorter graft survival (median 45 vs. 77 months).

Prediction Model

We applied our original prediction rule on the validation cohort. The obtained ROC-AUC was only 0.61 in the validation population with a poor calibration at every time point after follow up. In both cohorts, patients with a graft intolerance syndrome were younger at graft failure as compared to patients with a retained graft. Above the age of 40 years, the risk of graft intolerance syndrome requiring a graft nephrectomy decreased linearly. In our original analysis, age at time of graft failure was a significant factor in univariate analysis with a hazard ratio for graft intolerance of 0.97 for every additional year of age. It was not included in the original prediction rule because the model with donor age performed slightly better. We retrained our prediction model by replacing donor age by the age of the recipient at the time of graft failure. In this model the risk for graft intolerance changes only for patients aged above 40 years with a decrease for every additional year of age. In addition to age at graft failure this retrained prediction model included graft survival (in months) and the occurrence of any acute rejection. Hazard ratios for these factors are shown in **Table 2**. The model prognostic index (PI) for our retrained model is calculated by: PI = −0.0098 (age of recipient −40) (only included if age of

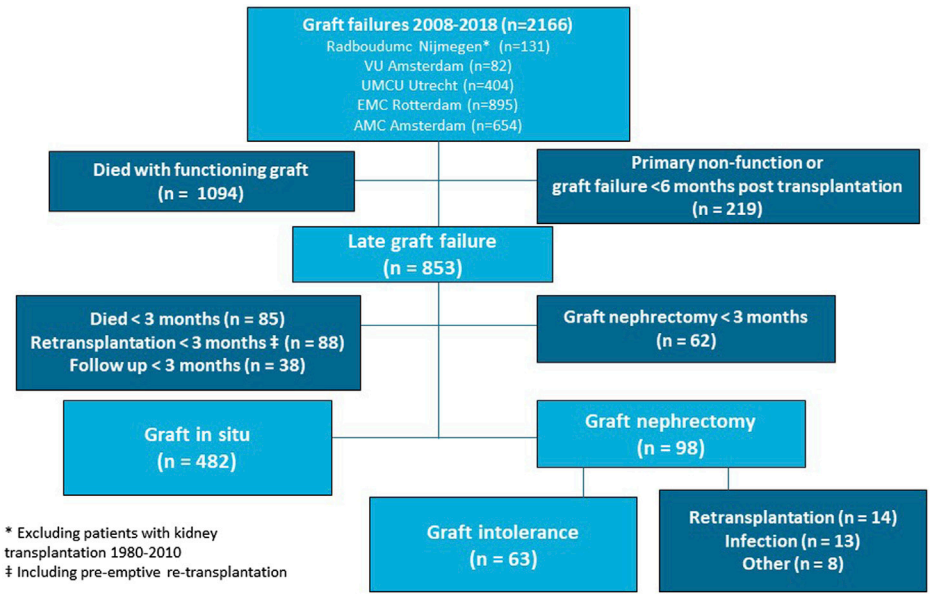


FIGURE 1 | Patient inclusion.

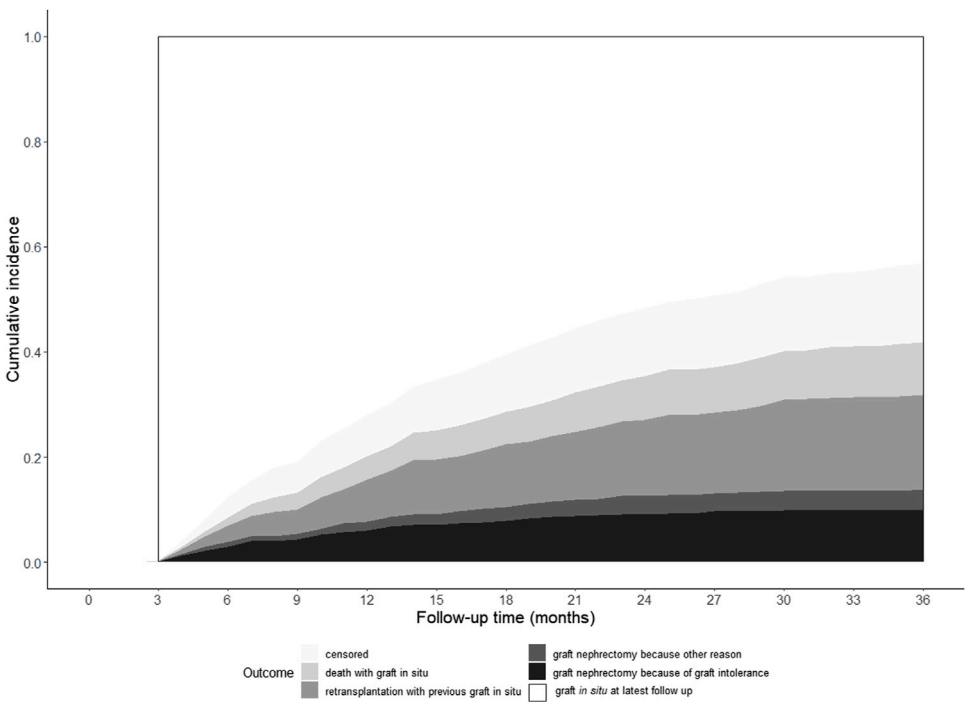


FIGURE 2 | Cumulative incidence curves of study outcomes.

recipient is ≥ 40 years at time of graft failure) −0.0094 (graft survival in months) + 0.9569 (if any acute rejection occurred) The ROC-AUC of this adjusted prediction rule is on average 0.70 in the original training cohort (compared to 0.69 of the original prediction model in the training cohort) and 0.69 in the validation cohort (**Figures 3A, B**).

The model object can be downloaded from https://github.com/JanvandenBrand/tect_validate/blob/main/output/fgr_model_final.RData for integration into a local machine learning operations platform. The prediction model is also available as a mobile friendly, web based RShiny application at https://jvandenbrand.shinyapps.io/predicttect/, allowing to estimate the risk of graft

TABLE 1 | Patient and transplantation characteristics of the validation cohort.

	Allograft *in situ* n = 482	Graft nephrectomy		
		Graft intolerance n = 63	Other indication *n* = 35	*p**
Patient characteristics				
Male (%)	286 (59.3)	31 (49.2)	19 (54.3)	0.13
Age at graft failure (median ± IQR)	50 (40–62)	43 (33–54)	48 (38–57)	0.001
Transplantation characteristics				
Donor age (median ± IQR)	54 (44–61)	50 (40–58)	45 (24–57)	0.09
Number of acute rejections (%)				0.05
0	237 (49.2)	23 (36.5)	12 (34.3)	
1	176 (36.5)	29 (46.0)	21 (60.0)	
2	61 (12.7)	9 (14.3)	2 (5.7)	
>2	8 (1.7)	2 (3.2)	0	
Graft survival in months (median ± IQR)	77 (43–136)	45 (26–70)	108 (50–144)	<0.001
Follow up time in months (median ± IQR)	33 (12–59)	10.4 (5.7–19.0)	20.1 (10.5–39.8)	<0.001
Center (%)				0.005
AMC, Amsterdam	149 (30.9)	24 (38.1)	10 (28.6)	
Erasmusmc, Rotterdam	233 (48.3)	22 (34.9)	15 (42.9)	
Radboudumc, Nijmegen	16 (3.3)	0	1 (2.9)	
UMCU, Utrecht	70 (14.5)	10 (15.9)	6 (17.1)	
VU, Amsterdam	14 (2.9)	14 (3.0)	3 (8.6)	

TABLE 2 | Hazard ratios for factors included in our retrained model in our validation cohort.

	Hazard ratio	95%-confidence interval
Age of recipient (every year)	0.99	0.98–1.00 (p = 0.11)
Graft survival (every month)	0.99	0.98–1.00 (p = 0.006)
Acute rejection	2.60	1.17–5.80 (p = 0.02)

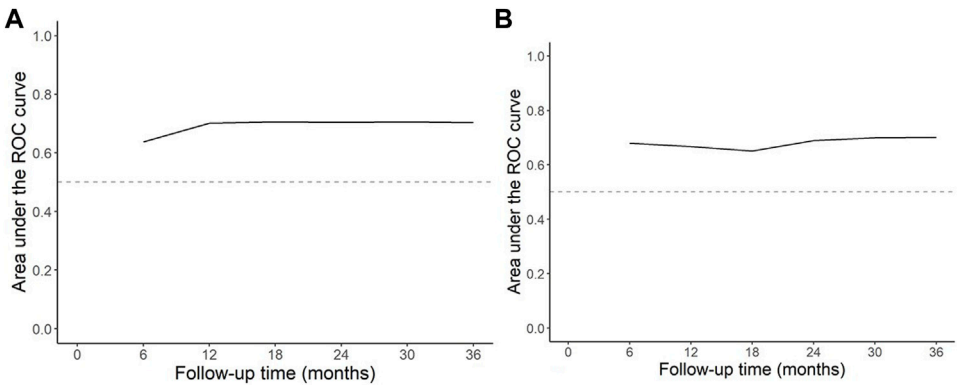


FIGURE 3 | AUC of ROC curve by follow up time **(A)** retrained model in the development cohort **(B)** retrained model in the validation cohort. The above figures show the ROC-AUC estimates at various time points during follow-up. The discriminative performance is reasonably constant throughout follow-up with an average of 0.70 in the training data, and 0.69 in the validation data.

nephrectomy due to graft intolerance after entering age at graft failure, graft survival, and history of acute rejection.

DISCUSSION

The aim of this retrospective cohort study was to validate our earlier published prediction model for the need for graft nephrectomy because of graft intolerance syndrome after graft failure. The originally developed prediction model including graft survival (in months), donor age (in years), and number of acute rejections, did not predict the occurrence of a graft nephrectomy in an external validation cohort. However, an adjusted model in which donor age was replaced by recipient age at the time of graft failure performed moderately well in both the training and validation cohorts.

Patient and transplant characteristics in patients with a graft intolerance syndrome requiring a graft nephrectomy were different from patients with a retained failed kidney graft. Patients who required a graft nephrectomy because of graft intolerance syndrome, had a shorter graft survival (median 45 months vs. 77 months) and almost 65% of them had experienced one or more acute rejection episodes. These differences were also found in our original dataset and reflect a more complicated course of the kidney transplant in patients ultimately requiring graft nephrectomy. However, unlike in our original dataset, donor age did not differ significantly and was in fact numerically lower instead of higher in the group with a graft intolerance syndrome. This may explain the poor performance of the original model in the validation cohort. The validation cohort included patients with graft failure between 2008 and 2018 with a median donor age of 53 years (IQR 42–61), while the training cohort included patients with a kidney transplantation over a time span of 3 decades (1980–2010) with a median donor age of 44 years (IQR 27–54). With increasing age of the donors over time, the discriminating potential of donor age appeared to decline. With this knowledge we reanalysed our original data and noticed that both in the training and in the validation cohort the age of the recipient at time of graft failure was lower in the group with a graft intolerance syndrome. There was a linear decrease in the incidence of graft nephrectomy above the age of 40 years. A possible explanation for this finding is that older patients have a less robust immune system, also referred to as immunosenescence (11, 12) We retrained the original model using recipient age at graft failure (for recipients >40 years) instead of donor age as predictive factor and tested this in the validation model. This resulted in an average ROC-AUC of 0.69, which is similar to the performance of this model in the original cohort.

The incidence of graft intolerance in the validation cohort was relatively low. Previous studies report variable incidence rates of 30%–50% in patients with kidney graft failure (8, 13). However, we studied a selected population by excluding patients with a short graft survival (<6 months), and patients with a graft nephrectomy within 3 months after kidney graft failure, because we would like to predict graft intolerance for patients without an obvious indication for graft removal. We also observed that the overall incidence of graft intolerance syndrome in the validation cohort was lower compared to the training cohort, while in- and exclusion criteria were similar. We hypothesize that in the more recent past immunosuppression was more often continued after graft failure in order to prevent immunisation, especially in patients who qualified for a re-transplantation, resulting in a lower incidence of the graft intolerance syndrome. Unfortunately, follow-up data on immunosuppression withdrawal after graft failure were too limited to test this hypothesis and we advocate a more systematic data collection in these patients.

Prognostic models with the aim to improve the prediction of clinical events are increasingly developed and published. External validation to confirm the reproducibility and generalizability of a prediction model for different patients was found to lack in 95% of studies on prediction models(14). We performed external validation in a large cohort of patients with kidney graft failure in a recent decade treated in different centers. An important similarity between the training and validation cohort was the 'watchful waiting strategy' with respect to graft nephrectomy and our prediction model of a graft intolerance syndrome is therefore clinically relevant.

A limitation of this study is the low event rate. Whereas 63 events were included, we hoped to include at least 100 events for a reliable validation of the prediction model. However, there are no absolute guidelines on the event number needed to perform an external validation and it remains uncertain whether a higher number of event rates would have resulted in a better prediction model. Another limitation of this study is its retrospective nature. The documentation of patients after kidney graft failure is usually poor. Data about the withdrawal of immunosuppressive medication and the occurrence of clinically relevant problems like the graft intolerance syndrome are generally not well recorded. Nevertheless, we are fairly sure that the large majority of patients who underwent a graft nephrectomy was identified.

Morbidity and mortality in patients with a failed kidney allograft are high (15–19). The population with kidney graft failure is very heterogeneous and evidence to guide clinicians is limited. The sole guideline on this topic is published by the British Transplant Society (BTS) and contains only weak recommendations (7). An unanswered question remains whether or not to remove the failed graft. Our model reasonably differentiates between patients with a low or high risk of a graft intolerance syndrome in our training and validation cohort s. The general policy on immunosuppressive treatment in both cohorts was to taper immunosuppression to zero or to low dose steroids. Hypothetically, continuation of more intensive immunosuppression could prevent the occurrence of graft intolerance syndrome with the need of a graft nephrectomy. However, evidence to support this hypothesis is lacking. Recent studies showed that patients still experienced rejection episodes and sensitization despite the continued use of immunosuppressants beyond the first year after transplant failure (20, 21) Prospective interventional trials are needed to compare the occurrence of graft intolerance between patients with different immunosuppressive treatment strategies. In the meantime, risks and benefits of a pre-emptive graft nephrectomy could be discussed individually with patients who have no prospect of a retransplantation in the near future and a high predicted risk of graft intolerance syndrome according to our model. Additionally an elevated risk creates awareness and can prompt more active surveillance for the possible occurrence of graft intolerance syndrome. In case of early recognition graft nephrectomy could be performed before deterioration of patients with ongoing inflammation. In conclusion, the incidence of graft intolerance syndrome in patients with late graft failure (i.e., graft survival >6 months) and an initial "watchful waiting policy" regarding graft nephrectomy was 11%. Our

retrained model including recipient age at time of graft failure, the occurrence of any acute rejection during latest transplant, and graft survival in months, can be used to estimate the risk of a graft intolerance syndrome with moderate accuracy. The estimated risk can be used to discuss the indication for pre-emptive removal of a failed kidney graft in individual patients.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by research ethics committee of the Radboud University Nijmegen Medical Centre. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

KB: Study design, data collection, data analyis and writing the paper, KS: Data collection, contributed to writing the paper, JW: data collection, contributed to writing the paper, AN: Data collection, contributed to writing the paper, FB: data collection, contributed to writing the paper, AZ: Data collection, contributed to writing the paper, JB: Data analysis, graphics, MB: Study design, supervision of the writing of the paper LH: Study design, data collection, supervision of the writing of the paper.

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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The Tacrolimus Concentration/Dose Ratio Does Not Predict Early Complications After Kidney Transplantation

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Early-on post kidney transplantation, there is a high risk of graft rejection and opportunistic viral infections. A low tacrolimus concentration/dose (C/D) ratio as a surrogate marker of fast tacrolimus metabolism has been established for risk stratification 3 months post-transplantation (M3). However, many adverse events occurring earlier might be missed, and stratification at 1 month post-transplantation (M1) has not been investigated. We retrospectively analyzed case data from 589 patients who had undergone kidney transplantation between 2011 and 2021 at three German transplant centers. Tacrolimus metabolism was estimated by use of the C/D ratio at M1, M3, M6, and M12. C/D ratios increased substantially during the year, particularly between M1 and M3. Many viral infections and most graft rejections occurred before M3. Neither at M1 nor at M3 was a low C/D ratio associated with susceptibility to BKV viremia or BKV nephritis. A low C/D ratio at M1 could not predict acute graft rejections or impaired kidney function, whereas at M3 it was significantly associated with subsequent rejections and impairment of kidney function. In summary, most rejections occur before M3, but a low C/D ratio at M1 does not identify patients at risk, limiting the predictive utility of this stratification approach.

Keywords: kidney transplantation, tacrolimus, acute graft rejection, C/D ratio, BKV nephritis

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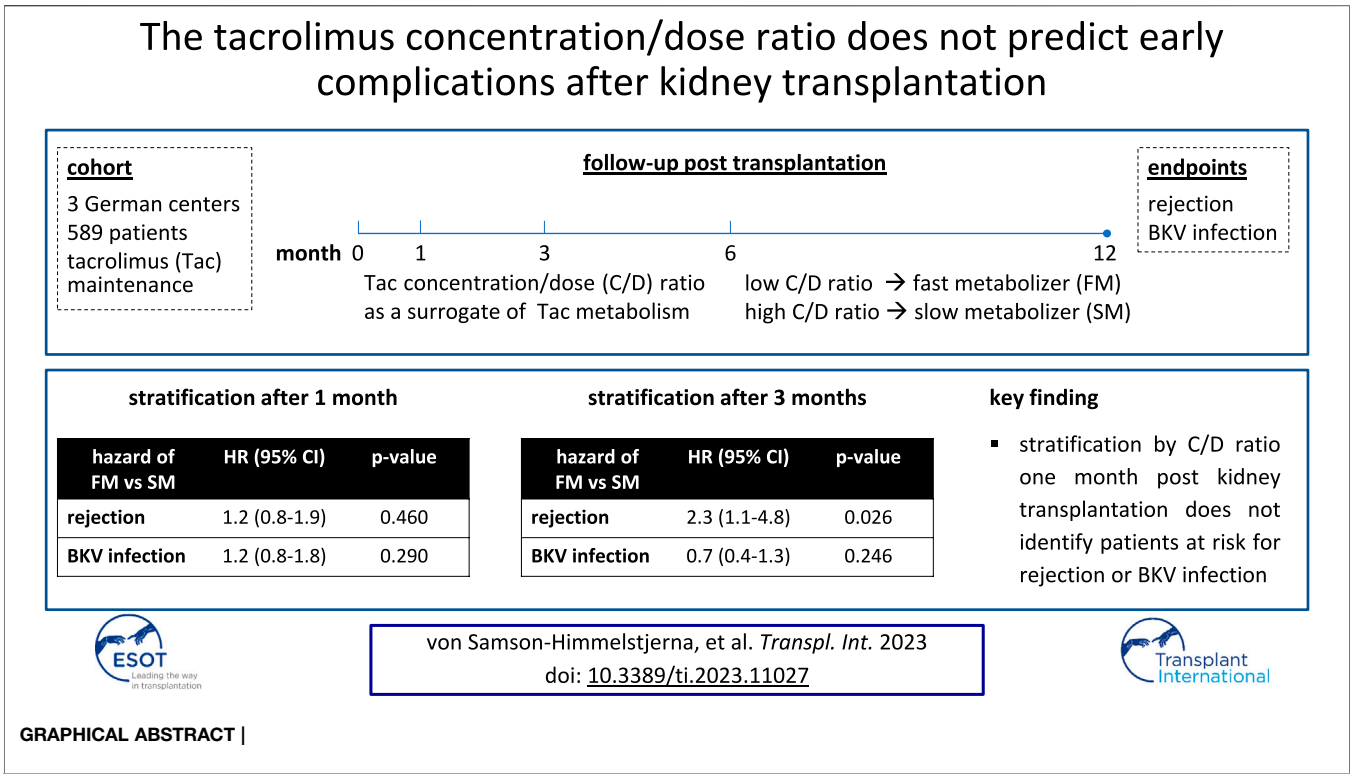
Abbreviations: ANOVA, analysis of variance; ATG, anti-thymocyte globulin; BKV, BK virus; BKVN, BKV nephritis; BMI, body-mass-index; C/D, concentration/dose; CI, confidence interval; CKD-EPI, chronic kidney disease epidemiology collaboration; CMV, cytomegalovirus; eGFR, estimated glomerular filtration rate; Eve, everolimus; FM, fast metabolizer; HR, hazard ratio; IM, intermediate metabolizer; IR, immediate-release; FM, fast metabolizer; M1/3/6/12, 1/3/6/12 months post-transplantation; MMF, mycophenolate mofetil; ns, not significant; p, value of probability; PCR, polymerase chain reaction; PR, prolonged-release; Predni, prednisone; SD, standard deviation; SM, slow metabolizer; Tac, tacrolimus; TX, transplantation.



GRAPHICAL ABSTRACT |

INTRODUCTION

Kidney transplantation is the best long-term renal replacement therapy for quality of life and survival (1). Although advances in immunosuppression therapy have increased graft tolerance, acute and chronic rejections remain the greatest threat to graft survival (2). The calcineurin inhibitor tacrolimus is a mainstay of the most common combination of immunosuppressants to maintain graft tolerance (3). Tacrolimus is mainly metabolized by the hepatic enzymes CYP3A4 and CYP3A5, the activity of which can be influenced by several factors such as comedication, diet, and genetic polymorphisms (4). If not monitored closely, drug levels can therefore be inadequate. Low tacrolimus levels lead to insufficient immunosuppression with the risk of graft rejection, whereas high levels can have nephrotoxic effects and increase the risk of opportunistic infections (5). Infections with cytomegalovirus (CMV) and with the polyomavirus BK virus (BKV) are particularly common and are most frequent during the first month post-transplantation (6, 7). In immunocompromised patients, CMV can cause life-threatening organ diseases such as pneumonitis and colitis, whereas BKV causes BKV nephritis (BKVN) with potentially severe graft dysfunction (7, 8). Consequently, tailoring the right dose of tacrolimus to each patient is a balancing act.

The tacrolimus metabolism rate varies extensively between kidney graft recipients. A simple way of estimating the metabolism rate was proposed by Thölking et al., who suggested using the concentration/dose (C/D) ratio as a surrogate marker (9). The authors found an increased risk of acute graft rejection, impairment of kidney function, and incidence of BKVN in patients with a low C/D ratio, who were defined as fast metabolizers (9–12). In a French cohort, fast metabolism was associated with death-censored graft failure (13). The C/D ratio could therefore be used as a readily available risk stratification tool. Previous studies have used 3 months (M3) to 6 months (M6) post-transplantation as the time to divide patients into metabolizer groups (11–13). However, because many opportunistic infections and rejections occur prior to M3 and M6, the practical value of the C/D ratio may be higher if an earlier time for stratification such as 1 month post-transplantation (M1) was established. This would require a stable tacrolimus metabolism at M1, but unfortunately, the stability of the C/D ratio during the early phase post-transplantation has not been well studied thus far, and the available literature yields contradictory results: Jouve et al. report a highly stable metabolism between M3, M6, and 12 months post-transplantation (M12), while a smaller case-control study showed a tendency of increasing C/D ratios from M1 to M6 (10, 13). Therefore, we intended to answer the following questions in this retrospective, multicenter study.

i) How stable is the C/D ratio throughout the first year after kidney transplantation?
ii) Can early determination of the C/D ratio at M1 predict viral infections?
iii) Can early determination of the C/D ratio at M1 predict acute graft rejections?

PATIENTS AND METHODS

Study Population

We screened 824 patients who had received kidney transplants from deceased or living donors at the three participating centers

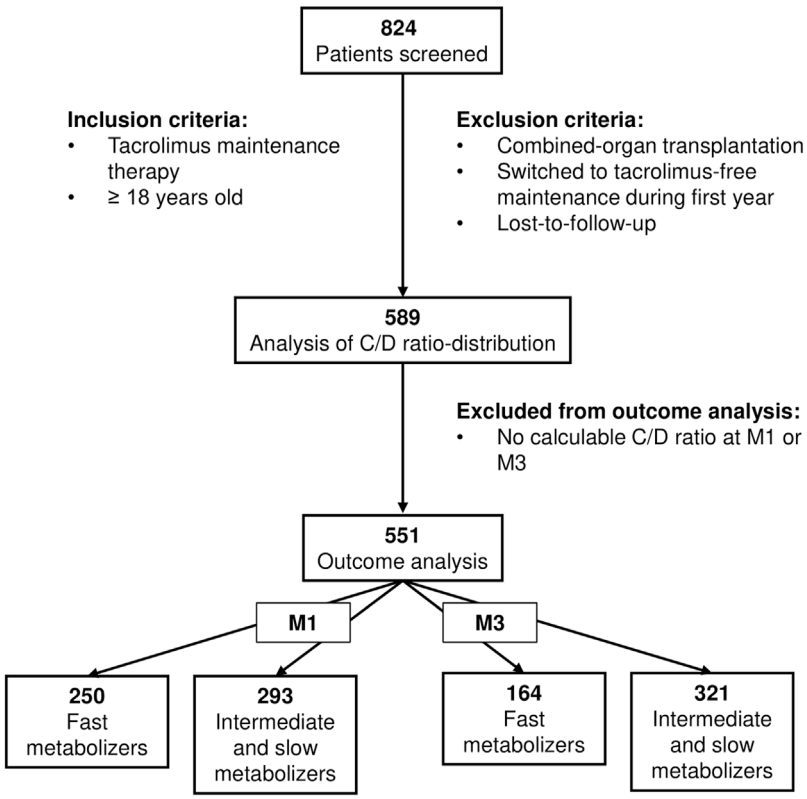


FIGURE 1 | Flowchart of patient enrollment. Kidney transplant recipients (deceased or living donors) between 2011 and 2021 were screened. Patients with tacrolimus maintenance therapy throughout the first year post-transplantation were included for analysis of the C/D ratio (n = 589), of which 38 patients were excluded from subsequent outcome analyses because of absence of calculable C/D ratios at M1 or M3. C/D, concentration/dose; M1/3/6/12, 1/3/6/12 months post-transplantation.

in Kiel, Essen, and Cologne-Merheim between February 2011 and July 2021. The standard immunosuppressive protocol at these centers included a twice-daily immediate-release formulation of tacrolimus (8–12 ng/mL during the first month, then subsequently 5–8 ng/mL), mycophenolate mofetil or mycophenolic acid, and prednisone (tapered to and continued at 5 mg/d by M6). Patients lost-to-follow-up, patients with combined-organ transplantations such as pancreas-kidney transplantation, and patients who had been switched to tacrolimus-free immunosuppression during the first year post-transplantation were not included in the study. We included 589 adult patients for analysis of tacrolimus metabolism who had at least one calculable C/D ratio available at M1, M3, M6, or M12. The subsequent analyses of 1-year outcomes contained only 551 patients because 38 patients did not have a calculable C/D ratio at M1 or M3 (**Figure 1**).

C/D Ratio

Similar to Thölking et al's study, C/D ratios were determined by dividing the tacrolimus trough level by the total daily dose. A C/D ratio <1.05 ng/(mg*ml) was considered as suggestive of fast tacrolimus metabolism, a C/D ratio between 1.05 and 1.55 ng/(mg*ml) as suggestive of intermediate metabolism, and a ratio ≥1.55 ng/(mg*ml) as suggestive of slow metabolism (9).

Outcomes

We defined BKV or CMV viremia as any infection with a detectable copy load in the plasma by using polymerase chain reaction (PCR). Severe BKV infection was defined as a copy load exceeding 100,000 U/mL. BKVN was defined as biopsy-proven BKVN. CMV organ infection was defined as a clinical diagnosis of CMV esophagitis, enteritis, encephalitis, hepatitis, pneumonitis, or retinitis. Acute graft rejections were defined as biopsy-proven acute graft rejections of any entity according to the most recent Banff classification at the respective time that required immunosuppressive treatment and/or plasmapheresis, including borderline rejections (14). The estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (15). Kidney failure was defined as the permanent necessity of renal replacement therapy or re-transplantation. Death was defined as all-cause mortality.

Ethical Approval

This study was approved by the ethics committee of the Christian-Albrecht University of Kiel (D 429/18).

Statistical Analysis

All statistical analyses were conducted using GraphPad Prism (version 5.0). Two-sided t-test was used for normally distributed

TABLE 1 | Baseline characteristics of patients included in the study.

Characteristic	Full cohort *n* = 551	M1			M3		
		FM *n* = 250	IM & SM *n* = 293	*p*	FM *n* = 164	IM & SM *n* = 321	*p*
Age mean (SD)	52.1 (±14.3)	49.9 (±14.6)	54.0 (±13.8)	***	49.4 (±15.3)	51.4 (±14.6)	ns
BMI (SD)	25.8 (±5.0)	25.5 (±5.1)	26.1 (±4.8)	ns	25.7 (±4.8)	25.9 (±5.1)	ns
Male	342 (62.1%)	146 (58.4%)	190 (64.8%)	ns	94 (57.3%)	205 (63.9%)	ns
Female	209 (37.9%)	104 (41.6%)	103 (35.2%)	ns	70 (42.7%)	116 (36.1%)	ns
Deceased donor graft	400 (72.6%)	177 (70.8%)	216 (73.7%)	ns	110 (67.1%)	242 (75.4%)	ns
Living donor graft	151 (27.4%)	73 (29.2%)	77 (26.3%)	ns	54 (32.9%)	79 (24.6%)	ns
First TX	514 (93.3%)	235 (94.0%)	272 (92.8%)	ns	151 (93.1%)	304 (94.7%)	ns
≥ Second TX	37 (6.7%)	15 (6%)	21 (7.2%)	ns	13 (7.1%)	17 (5.3%)	ns
Donor CMV+	278 (50.5%)	124 (49.7%)	151 (51.5%)	ns	79 (48.2%)	168 (52.3%)	ns
Recipient CMV+	329 (49.5%)	147 (58.8%)	178 (60.8%)	ns	97 (59.1%)	194 (60.4%)	ns
Induction therapy							
ATG	109 (19.8%)	52 (20.8%)	54 (18.4%)	ns	34 (20.7%)	61 (19.0%)	ns
Basiliximab	442 (90.2%)	198 (79.2%)	241 (82.3%)	ns	130 (79.3%)	260 (81.0%)	ns
Initial maintenance							
Tac, MMF, Predni	527 (95.6%)	242 (96.8%)	282 (96.2%)	ns	156 (95.1%)	309 (96.3%)	ns
Tac, Eve, Predni	21 (4.4%)	8 (3.2%)	11 (3.8%)	ns	5 (3%)	12 (3.7%)	ns
Maintenance at M12							
Tac, MMF, Predni	515 (93.5%)	235 (94.0%)	274 (93.5%)	ns	155 (94.5%)	299 (93.1%)	ns
Tac, Eve, Predni	31 (5.6%)	12 (4.8%)	19 (6.5%)	ns	9 (5.5%)	22 (6.9%)	ns
Prednisone (mg/d)							
M1	15.8 (±9.56)	17.1 (±10.8)	14.7 (±8.4)	**	16.27 (±10.9)	15.3 (±8.8)	ns
M3	8.4 (±4.3)	8.6 (±4.8)	8.2 (±3.9)	ns	8.9 (±5.4)	8.2 (±3.6)	ns
M6	6.6 (±6.9)	6.4 (±6.9)	6.7 (±7.0)	ns	6.1 (±2.2)	6.6 (±6.6)	ns
M12	5.7 (±4.4)	5.6 (±4)	5.8 (±4.8)	ns	5.9 (±5.6)	5.5 (±2.6)	ns
Tac dose (mg/d)							
M1	9.7 (±5.0)	13.1 (±4.6)	6.8 (±2.7)	****	13.3 (±5.1)	8.0 (±3.8)	****
M3	6.7 (±3.7)	9.0 (±3.7)	4.9 (±2.3)	****	10.5 (±3.3)	4.8 (±2.0)	****
M6	5.5 (±3.3)	7.2 (±3.7)	4.0 (±2.0)	****	8.4 (±3.5)	3.9 (±1.7)	****
M12	4.7 (±2.8)	6.0 (±3.1)	3.6 (±1.9)	****	7.1 (±3.1)	3.6 (±1.8)	****
Tac level (ng/mL)							
M1	9.6 (±3.1)	8.6 (±2.5)	10.4 (±3.3)	****	9.3 (±3.1)	9.7 (±3.0)	ns
M3	8.2 (±2.8)	8.3 (±2.3)	8.2 (±3.2)	ns	7.2 (±1.9)	8.8 (±3.0)	****
M6	7.0 (±2.1)	7.0 (±2.1)	7.0 (±2.1)	ns	7.0 (±2.2)	7.1 (±2.0)	ns
M12	6.7 (±2.0)	6.9 (±2.2)	6.6 (±1.8)	*	6.9 (±1.7)	6.7 (±2.1)	ns

*Patients with calculable C/D ratios were stratified at M1 and M3. Significance as indicated: ns p-value ≥0.05, * p-value <0.05, ** p-value <0.01, *** p-value <0.001, ****p-value <0.0001. ATG, anti-thymocyte globulin; BMI, body mass index; CMV+, IgG positive for cytomegalovirus; Eve, everolimus; FM, fast metabolizer; IM, intermediate metabolizer; M1/3/6/12, 1/3/6/12 months post-transplantation, MMF, mycophenolate mofetil; Predni, prednisone; SD, standard deviation; SM, slow metabolizer; Tac, tacrolimus; TX, transplantation.*

linear variables, Mann-Whitney test was used for non-normally distributed linear variables, and chi-square test (95% confidence level) was used for categorical variables, respectively, when two groups were compared. One-way ANOVA with Bonferroni *post hoc* test was used for the comparison of multiple groups. Log-rank (Mantel-Cox) tests were used for analysis of Kaplan-Meier survival curves. All hazard ratios (HR) were calculated at a 95% confidence level. Values of probability (p) <0.05 were considered statistically significant.

RESULTS

The C/D Ratio Increases Throughout the First Year

Baseline characteristics of the study group are shown in **Table 1**. Fast metabolizers stratified at M1 were significantly younger, had received more prednisone, and had lower tacrolimus trough levels than slow metabolizers. Stratifying at M3, there were no age differences between fast metabolizers and slow metabolizers, but fast metabolizers had lower trough levels. Regarding other baseline parameters, no significant differences were detected. Most patients had received a deceased donor transplantation with basiliximab induction therapy. Maintenance immunosuppression almost exclusively consisted of prednisone, mycophenolate, and tacrolimus. At M1, fast metabolizers represented the largest group (47%, **Figure 2A**). While 35% of the fast metabolizers at M1 underwent conversion to slow metabolism by M12, only 4% of slow metabolizers at M1 had developed fast metabolism by M12. The relative number of patients classified as fast metabolizers reduced quickly initially, from 47% at M1 to 35% at M3, but stabilized thereafter, declining to 29% and 23% at M6 and M12, respectively. Throughout the first year post-transplantation, the mean C/D ratio in the full cohort increased from 1.28 ng/(mg*ml) at M1 to 1.92 ng/(mg*ml) at M12 (**Figures 2A,B**).

Fast Metabolizer Status is Not Associated With Viral Infection

Throughout the year, BKV viremia occurred in 20.1% of all patients and BKVN occurred in 4.9% (**Table 2**). CMV viremia

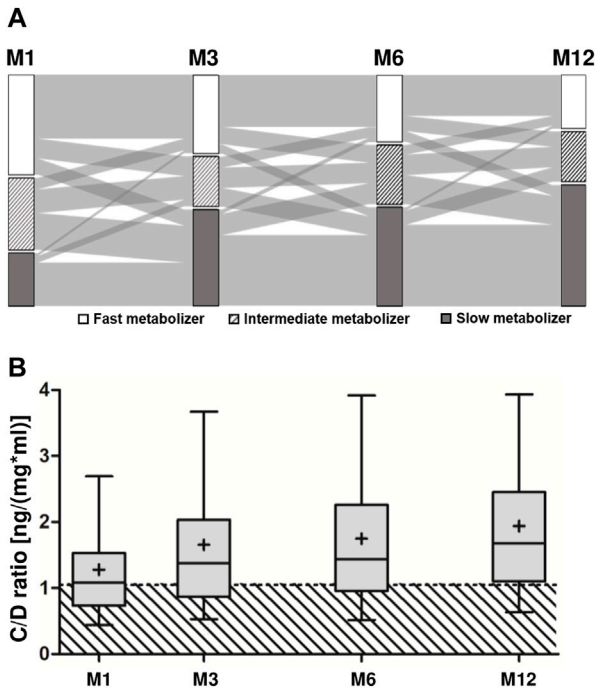


FIGURE 2 | Dynamics of the C/D ratio in the first year post-transplantation. **(A)** Patients were stratified as fast (<1.05 ng/[mg*ml]), intermediate (1.05–1.54 ng/[mg*ml]), or slow (≥1.55 ng/[mg*ml]) metabolizers according to the C/D ratio at M1 (n = 543), M3 (n = 485), M6 (n = 458), and M12 (n = 540). **(B)** A box plot with interquartile ranges and 5%–95% whiskers at M1 to M12 (mean indicated by +) shows the median C/D ratio. The hatched area indicates C/D ratios below 1.05 ng/(mg*ml). C/D, concentration/dose; FM, fast metabolizer; M1/3/6/12, 1/3/6/12 months post-transplantation.

was more frequent than BKV viremia, affecting 25.2% of the full cohort, but severe infection with impaired organ function occurred only in 2.2% (**Table 2**). Within the first 3 months post-transplantation, 35.1% of all BKV viremias and 43.9% of all CMV viremias had already been detected (**Table 2** and **Supplementary Figure S1**). We determined the metabolizer status at M1 or M3 and disregarded patients with infections prior to these points, respectively, to assess a potential utility of the C/D ratio in predicting viral infections. Neither determination of fast metabolism at M1 (HR 1.2 [95% CI 0.8–1.8], p = 0.290) nor at M3 (HR 0.7 [95% CI 0.4–1.3], p = 0.246) was associated with a successively increased occurrence of BKV viremia when compared with the rest of the cohort (**Figures 3A,D**). Similarly, subsequent CMV viremia was neither associated with fast metabolism at M1 (HR 1.1 [95% CI 0.8–1.6], p = 0.549) nor at M3 (HR 0.8 [95% CI 0.5–1.3], p = 0.329; **Supplementary Figures S2A,D**). Metabolism status was not significantly associated with BKV or CMV plasma copy loads in patients who developed viremia (**Figures 3B,E** and **Supplementary Figures S2B,E**). Patients with severe infection such as BKVN or CMV organ infection did not have significantly different C/D ratios than patients without severe infection (**Figures 3C,F** and **Supplementary Figures S2C,F**), and neither BKVN nor CMV organ infection was associated with fast metabolism (**Table 2**). In a further analysis independent of the pre-specified time points, we observed the C/D ratio and the time-to-maximum-copy-load for cases with severe BKV infection (maximum copy load exceeding 100,000 U/mL). Since the time-to-maximum-copy-load was approximately 6 months (mean 168 days) in that group, we chose the C/D ratios of all patients without BKV viremia at M6 for comparison. Patients with severe BKV infection did not have significantly lower C/D ratios than patients without BKV infection (1.55 vs. 1.46; p = 0.801; **Table 3**).

Very Early Determination of the C/D Ratio Cannot Predict Graft Rejections

We subsequently analyzed the association of acute graft rejections and kidney function with the C/D ratio. In the first-year post-transplantation, acute biopsy-proven graft rejections that required treatment ensued in 31.2%, 76.2% of which occurred during the first 3 months post-transplantation, but only few patients permanently lost their grafts (1.8%) or died (0.9%; **Table 2**). The kidney function in the full cohort, as indicated by the eGFR, was similar at M1 and M3, but had improved by M6 and M12 (**Table 2**). To assess the utility of the C/D ratio in predicting acute rejections, we determined fast metabolizer status at M1 and excluded all cases with rejections earlier than M1 (**Figure 4A**). Fast metabolism at M1 was not associated with an increased risk of subsequent episodes of graft rejection (HR 1.2 [95% CI 0.8–1.9], p = 0.460). Additionally, the eGFR of these fast metabolizers neither differed significantly from the rest of the cohort, nor did fast metabolizers show impaired graft development, as the eGFR in both groups improved significantly between M1 and M12 (46.2–52.4 mL/min/1.73 m^2 and 45.4–49.7 mL/min/1.73 m^2 in fast metabolizers and the rest of the cohort, respectively; **Figure 4B**). Contrarily, when patients were stratified by tacrolimus metabolism status at M3, fast metabolizers had a hazard ratio of 2.3 (95% CI 1.1–4.8, p = 0.026) for acute graft rejection between M3 and M12 as compared to patients with a slower tacrolimus metabolism (**Figure 4C**). In these fast-metabolizing patients, the eGFR increased slightly but non-significantly between M3 and M12 (45.4 and 47.4 mL/min/1.73 m^2, respectively), whereas the eGFR of the more slowly metabolizing patients improved considerably from 45.4 at M3 to 52.4 mL/min/m^2 at M12 (**Figure 4D**).

DISCUSSION

In this retrospective multicenter cohort study, we evaluated the tacrolimus C/D ratio of kidney graft recipients throughout the first-year post-transplantation and assessed whether earlier estimation of their metabolizer status than previously established could identify patients at risk for viral infection and graft rejection. We found that particularly during the early months after transplantation, the C/D ratio was unstable, and that it was not associated with opportunistic viral infections.

TABLE 2 | One-year outcomes for patients stratified at M1 or M3.

Characteristic	Full cohort *n* = 551	M1			M3		
		FM *n* = 250	IM & SM *n* = 293	*p*	FM *n* = 164	IM & SM *n* = 321	*p*
Graft failure	10 (1.8%)	4 (1.6%)	6 (2.0%)	ns	1 (0.6%)	5 (1.6%)	ns
Death	5 (0.9%)	2 (0.8%)	2 (0.7%)	ns	2 (1.2%)	3 (0.9%)	ns
BKVN	27 (4.9%)	10 (4.0%)	17 (5.8%)	ns	5 (3.0%)	20 (6.2%)	ns
BKV viremia	111 (20.1%)	56 (22.4%)	55 (18.8%)	ns	25 (15.2%)	64 (19.9%)	ns
Before M1	6 (5.4%)	3 (5.4%)	3 (5.5%)		0 (0.0%)	5 (7.8%)	
M3	39 (35.1%)	21 (37.5%)	18 (32.7%)		10 (40.0%)	24 (37.5%)	
M6	77 (69.4%)	38 (67.9%)	39 (70.1%)		15 (60.0%)	42 (65.6%)	
M12	111 (100.0%)	56 (100.0%)	55 (100.0%)		25 (100.0%)	64 (100.0%)	
CMV organ infection	12 (2.2%)	5 (2.0%)	7 (2.4%)	ns	2 (1.2%)	8 (2.5%)	ns
CMV viremia	139 (25.2%)	66 (26.4%)	73 (24.9%)	ns	41 (25.0%)	83 (25.9%)	ns
Before M1	24 (17.3%)	10 (15.2%)	14 (19.2%)		7 (17.1%)	14 (16.9%)	
M3	61 (43.9%)	29 (44.0%)	32 (43.8%)		21 (51.2%)	32 (38.6%)	
M6	89 (64.0%)	43 (65.2%)	46 (63.0%)		28 (68.3%)	50 (60.2%)	
M12	139 (100.0%)	66 (100.0%)	73 (100.0%)		41 (100.0%)	83 (100.0%)	
BPAR	172 (31.2%)	91 (36.4%)	79 (27.0%)	*	72 (43.9%)	83 (25.9%)	****
Before M1	96 (55.8%)	55 (60.4%)	40 (50.6%)		44 (61.1%)	43 (51.8%)	
M3	131 (76.2%)	68 (74.7%)	61 (77.2%)		56 (77.8%)	64 (77.1%)	
M6	156 (90.7%)	83 (91.2%)	71 (89.9%)		66 (91.7%)	75 (90.3%)	
M12	172 (100.0%)	91 (100.0%)	79 (100.0%)		72 (100.0%)	83 (100.0%)	
eGFR (mean ± SD)							
M1	44.5 (±16.8)	44.9 (±17.6)	44.2 (±16.0)	ns	43.3 (±16.7)	44.8 (±17.0)	ns
M3	44.4 (±16.4)	45.1 (±17.4)	43.8 (±15.6)	ns	44.2 (±16.9)	44.5 (±16.2)	ns
M6	46.1 (±16.4)	44.8 (±16.5)	47.2 (±16.4)	ns	43.1 (±16.4)	47.3 (±16.0)	*
M12	48.7 (±19.1)	47.8 (±22.1)	49.2 (±16.1)	ns	45.4 (±18.9)	50.6 (±19.3)	**

*All patients with calculable C/D ratios at the respective times were included in this analysis. Significance as indicated: ns p-value ≥0.05, * p-value <0.05, ** p-value <0.01, *** p-value <0.001, **** p-value <0.0001. BKV, BK virus; BKVN, BKV nephritis; BPAR, biopsy-proven acute graft rejection; CMV, cytomegalovirus; eGFR, estimated glomerular filtration rate (in mL/min/1.73 m^2); FM, fast metabolizer; IM, intermediate metabolizer; M1/3/6/12, 1/3/6/12 months post-transplantation; ns, not significant; SD, standard deviation; SM, slow metabolizer.*

Using the C/D ratio as a surrogate of metabolizer status 1 month post-transplantation did not identify patients at risk, but a low C/D ratio after 3 months predicted subsequent acute rejections and impaired kidney function.

The clinical purpose of determining the C/D ratio is to predict harmful events such as opportunistic viral infections or graft rejections. This could potentially enable transplant nephrologists to pre-emptively adjust immunosuppressive medication and more tightly monitor patients at risk. However, for such a preventative approach, the C/D ratio needs to be determined at the earliest opportunity, because most infections and rejections occur early after transplantation (5–7). Since tacrolimus metabolism is affected by co-medications such as prednisone, the dosing of which is subject to extensive changes during the first weeks post-transplantation, the C/D ratio may not be reliably estimated immediately after transplantation. Prednisone is known to induce metabolism of tacrolimus, and an increase of the C/D ratio would be expected as prednisone is tapered post-transplantation (16). Data from our cohort support this assumption, as only half of the fast metabolizers at M1 maintained their metabolism rate until M3. Thereafter, metabolism continued to reduce subsequently, but much less dynamically, and therefore, M3 appears to be the earliest reliable point at which metabolism rates should be determined.

However, our data also show that at M3, a substantial number of viral infections and acute graft rejections had already occurred, which severely limits the utility of the C/D ratio at M3. The evidence from other studies regarding the value of the C/D ratio earlier than at M3 is scarce, and previous large trials have not evaluated whether stratification of patients at a much earlier point would predict outcomes (9–12, 17). These questions were quite clearly answered in our cohort of patients: A low C/D ratio at M1 did not identify patients at risk for viral infection, acute rejection, or impaired graft function. Based on our data, the substantive increase in the C/D ratio between M1 and M3 in many patients rendered stratification at M1 unfeasible for risk prediction.

Fast metabolizers at M3 had significantly lower tacrolimus levels than slower metabolizers, which implies that fast metabolizers were consequently exposed to less immunosuppression. This might explain why we could confirm the previously reported association of fast metabolism at M3 with graft rejections and impaired kidney function (11, 12, 17), but not that with increased susceptibility to viral infection despite some studies reporting the opposite (9, 10).

Our study has some limitations. It should be kept in mind that we did not directly measure the tacrolimus metabolism, but rather used the C/D ratio as a surrogate marker. While this limits our ability to correlate the actual tacrolimus metabolism with clinical outcomes, it is a pragmatic approach because of its simplicity and clinical transferability. As this is a retrospective study, we were unable to draw causal assumptions from our findings and could only generate hypotheses. The study was underpowered to detect small differences of rarely occurring outcomes such as BKVN or CMV organ infections. However, the cohort size of nearly 600 patients from three transplant

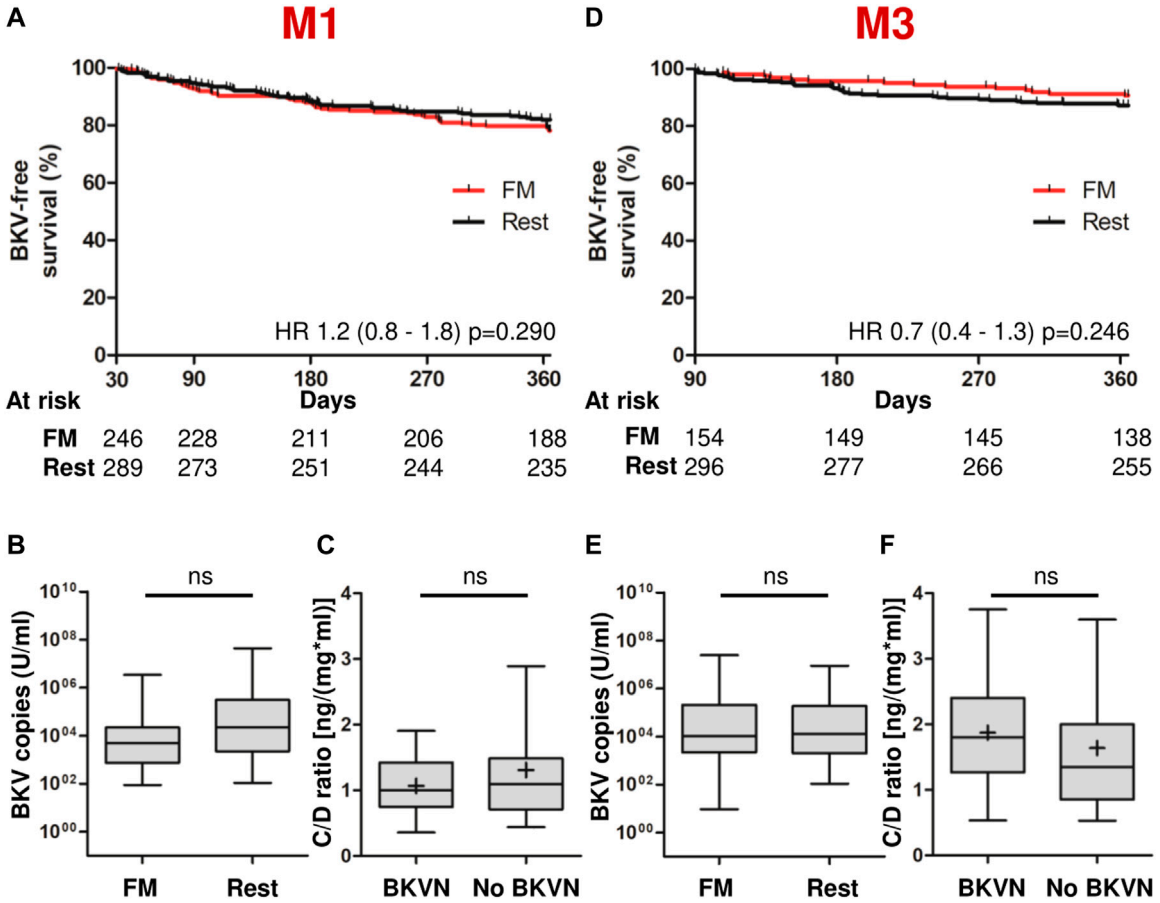


FIGURE 3 | Fast tacrolimus metabolism does not predict BKV infections. Patients with infections before the respective stratification points (M1 or M3) were not included in the analyses. **(A)** Kaplan-Meier-viremia-free survival (death censored) of FMs and the rest of the cohort, as determined at M1. **(B)** A box plot with interquartile ranges and 5%–95% whiskers shows the viral BKV copy loads in the plasma in cases of viremia, as quantified by PCR. $n = 53$ for FM, $n = 52$ for the rest of the cohort. **(C)** A box plot with interquartile ranges and 5%–95% whiskers (mean indicated by +) shows median C/D ratios of patients with BKVN at M1 ($n = 27$) compared to those of patients without infection ($n = 516$). **(D)** Kaplan-Meier-viremia-free survival (death censored) of FMs and the rest of the cohort, as determined at M3. **(E)** Identical analysis as **(B)** in patients stratified at M3. $n = 15$ for FMs, $n = 40$ for the rest of the cohort. **(F)** Identical analysis as **(C)** was done to evaluate the C/D ratios at M3. $n = 25$ for BKVN and $n = 458$ for patients without BKVN. ns, not significant, *p-value <0.05, **p-value <0.01, ***p-value <0.001, ****p-value <0.0001. BKV, BK virus; BKVN, BKV nephritis; C/D, concentration/dose; FM, fast metabolizer; M1/3/6/12, 1/3/6/12 months post-transplantation.

TABLE 3 | C/D ratios in patients with severe vs. no BKV infection.

BKV status	*n*	Time-to-maximum-copy-load in days (mean ± SD)	C/D-ratio (median [IQR])	Mann-Whitney test (*p*-value)
Severe BKV infection	32	168 ± 101	1.55 (1.19–2.08)	0.801
BKV negative	345	190 ± 36	1.46 (0.97–2.23)	

Severe BKV infection was defined as a copy load exceeding 100,000 U/mL. BKV negative patients with available C/D ratios 6 months post-transplantation served as controls. BKV, BK virus; IQR, interquartile range; SD, standard deviation.

centers is one of the largest to evaluate the impact of tacrolimus metabolism on clinical outcomes, and sufficient power was present to detect differences in the primary outcomes. Additionally, as we were primarily interested in short-term complications, the follow-up period of 1 year was relatively short.

In conclusion, our study confirmed the C/D ratio as a pragmatic tool to identify patients at risk for graft rejections and sub-optimal development of kidney function after transplantation. Unfortunately, the C/D ratio only appears useful from M3 onwards, and many early complications can therefore not be addressed. Investigating whether fast metabolizers could benefit from switching an immediate-release to a prolonged-release formulation of tacrolimus, which allows more steady tacrolimus levels throughout the day, remains reasonable. In this respect, in a recent *post hoc* analysis of a randomized-controlled phase III trial, Suwelack et al. assessed if fast metabolizers, as determined at M1, had better outcomes when administered prolonged-release tacrolimus (18). Although rejections were less frequent in the prolonged-release

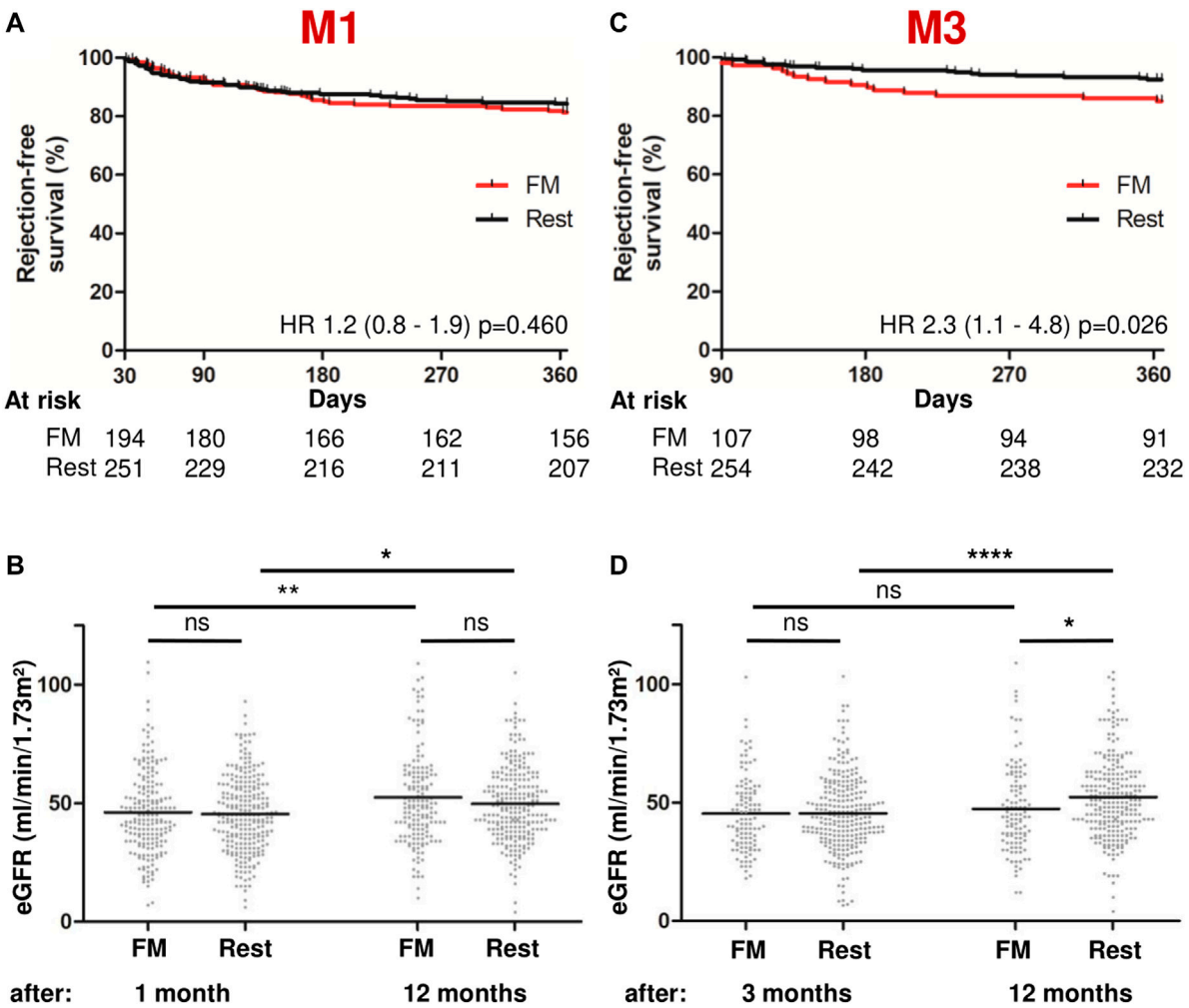


FIGURE 4 | Fast metabolism at M3, but not at M1, predicts acute graft rejections and impaired graft function. Patients with rejections before the respective points of stratification (M1 or M3) were not included in the analyses. **(A)** Kaplan-Meier-rejection-free survival (death-censored) between the FM group and the rest of the cohort at M1. **(B)** Scatter plots with means showing eGFR at M1 and M12. **(C)** Kaplan-Meier-rejection-free survival (death-censored) between the FM group and the rest of the cohort at M3. **(D)** Scatter plots with means showing eGFR at M3 and M12. ns, not significant, *p-value <0.05, **p-value <0.01, ***p-value <0.001, ****p-value <0.0001. C/D, concentration/dose; eGFR, estimated glomerular filtration rate; FM, fast metabolizer; M1/3/6/12, 1/3/6/12 months post-transplantation.

group than in the immediate-release group, this difference was not statistically significant. Based on the findings of our study, we hypothesize that the authors' timing of stratification at M1 was premature, and that stratification at M3 might have produced different results. Therefore, our findings provide a ground for the re-evaluation of efficacy of prolonged-release tacrolimus to prevent graft rejections in patients with fast metabolism. An alternative to tacrolimus maintenance was introduced with the co-stimulation inhibitor belatacept, which, compared to calcineurin inhibitors, improves long-term graft function (19–21). This benefit is limited by increased rejection rates in patients treated with belatacept compared to tacrolimus (21, 22). However, considering the results of this study and previous reports, patients with fast tacrolimus metabolism at M3 may have a fairly similar rejection risk as patients treated with belatacept. Consequently, the current restraints that prevent nephrologists from broadly prescribing belatacept to more patients might not apply to this patient group. Thus, a switch from tacrolimus to belatacept in fast metabolizers at M3 could be explored as a novel therapeutic concept.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics committee of the Christian-Albrechts University of Kiel. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

FvS-H, JK, TF, UK, AH, and KS designed the study. FvS-H, MM, UE, JK, UL, LA, TS, and BK collected the data. FvS-H, MM, and KS wrote the manuscript. FvS-H, MM, NK, BK, and KS designed the figures. All authors have read, edited, and approved the manuscript. Each author contributed substantially in conducting the study and submitting the manuscript.

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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.2023.11027/full#supplementary-material

Supplementary Figure S1 | BKV viremia, CMV viremia, and acute graft rejections throughout the first year post-transplantation. This figure includes all patients from the cohort (n = 551). **(A–C)** show the Kaplan-Meier-event-free survival (death censored). Dashed lines indicate follow-ups at M1 after 30 days, M3 after 90 days, and M6 after 180 days. Follow-up ended at M12 after 365 days. Abbreviations: BKV, BK virus; CMV, cytomegalovirus; M1/3/6/12 = 1/3/6/12 months post-transplantation.

Supplementary Figure S2 | Fast tacrolimus metabolism does not predict CMV infections. Patients with infections before the respective stratification points (M1 or M3) were not included in the analyses. **(A)** Kaplan-Meier-viremia-free survival (death censored) of FM and the rest of the cohort at M1. **(B)** A box plot with interquartile ranges and 5%–95% whiskers shows the viral CMV copy loads in the plasma in cases of viremia, as quantified by PCR. n = 56 for FM, n = 59 for rest. **(C)** A box plot with interquartile ranges and 5%–95% whiskers (mean indicated by +) shows median C/D ratios at M1 in patients with CMV organ infection (n = 12) compared to those of patients without infection (n = 531). **(D)** Kaplan-Meier-viremia-free survival (death censored) of FMs and the rest of the cohort, as determined at M3. **(E)** Identical analysis as **(B)** in patients stratified at M3. n = 20 for FM, n = 51 for rest of the cohort. **(F)** Identical analysis as **(C)** evaluating the C/D ratios at M3. n = 10 for CMV organ infection and n = 473 for no CMV organ infection. ns, not significant, *p value < 0.05, **p-value < 0.01, ***p-value < 0.001, ****p-value < 0.0001. CMV, cytomegalovirus; C/D, concentration/dose; FM, fast metabolizer; M1/3/6/12, 1/3/6/12 months post-transplantation.

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Donating a Kidney to a Stranger: Are Healthcare Professionals Facilitating the Journey? Results From the BOUnD Study

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Unspecified kidney donors (UKDs) are approached cautiously by some transplant professionals. The aim of this study was to interrogate the views of UK transplant professionals towards UKDs and identify potential barriers. A purposely designed questionnaire was validated, piloted and distributed amongst transplant professionals at each of the 23 UK transplant centres. Data captured included personal experiences, attitudes towards organ donation, and specific concerns about UKD. 153 responses were obtained, with representation from all UK centres and professional groups. The majority reported a positive experience with UKDs (81.7%; $p < 0.001$) and were comfortable with UKDs undergoing major surgery (85.7%; $p < 0.001$). 43.8% reported UKDs to be more time consuming and 52% felt that a mental health assessment should take place before any medical tests. 77% indicated the need for a lower age limit. The suggested age range was broad (16–50 years). Adjusted mean acceptance scores did not differ by profession ($p = 0.68$) but higher volume centres were more accepting (46.2 vs. 52.9; $p < 0.001$). This is the first quantitative study of acceptance by transplant professionals to a large national UKD programme. Support is broad, however potential barriers to donation have been identified, including lack of training. Unified national guidance is needed to address these.

Keywords: transplantation, kidney donation, living, unspecified, altruistic

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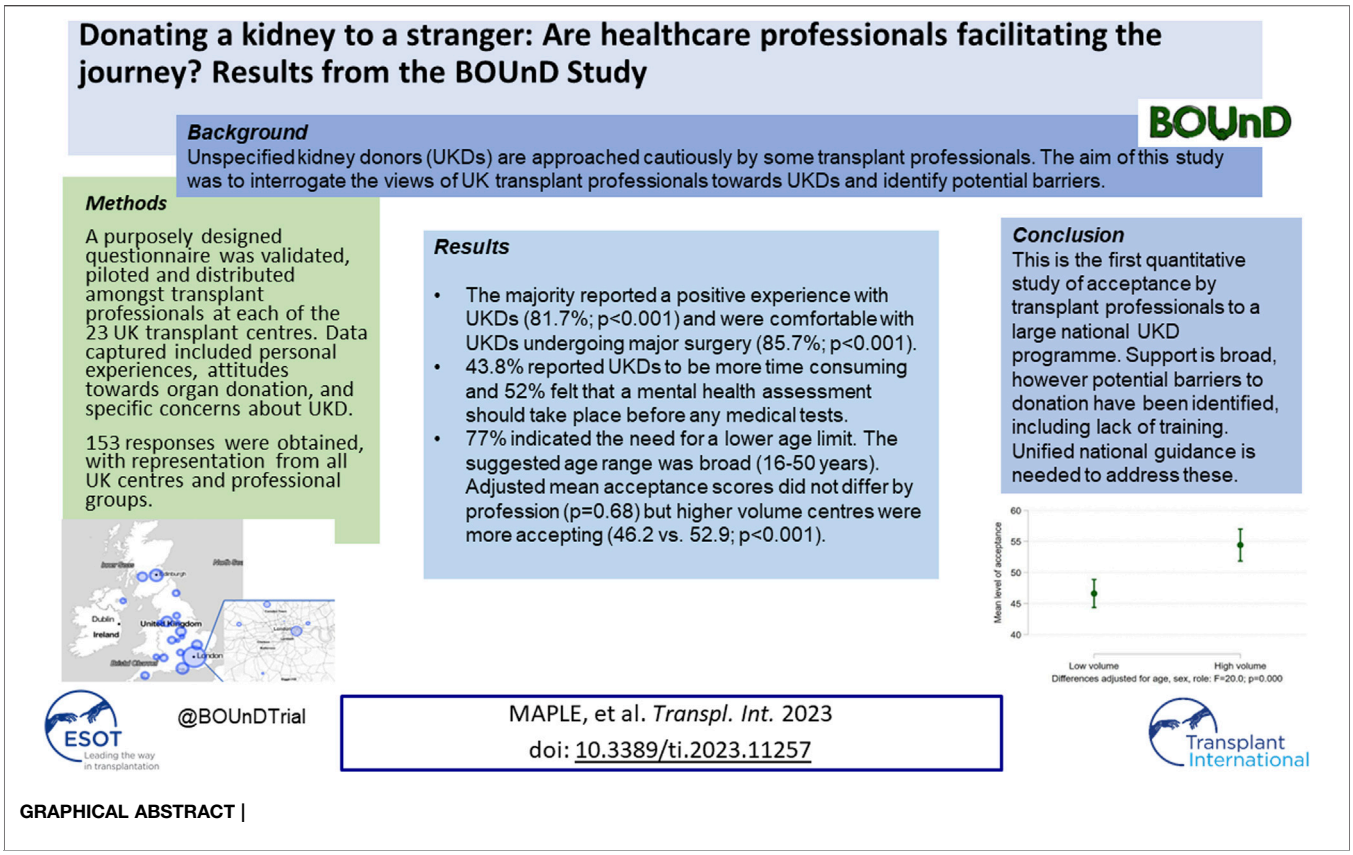
INTRODUCTION

Unspecified kidney donation (UKD), also known as altruistic or non-directed altruistic donation, describes the intention of an individual to donate a kidney to a stranger during their lifetime [1]. UKD has significant potential for patient benefit by contributing to organ sharing schemes which facilitate transplants between blood group and human leucocyte antigen (HLA) incompatible pairs. In the 2019/20 financial year, 95 unspecified kidney donors (UKDs) in the United Kingdom (UK) (10% of living

Abbreviations: ADCs, Altruistic Donor Chains; BOUnD, Barriers and Outcomes in Unspecified Donation; EAPM, European Association of Psychosomatic Medicine; HLA, Human Leucocyte Antigen; SKD, Specified Kidney Donation; SKDs, Specified Kidney Donors; UK, United Kingdom; UKD, Unspecified Kidney Donation; UKDs, Unspecified Kidney Donors; UKLKSS, UK Living Kidney Sharing Scheme.



GRAPHICAL ABSTRACT |

donors) facilitated 146 living donor kidney transplants. Critically, 48 UKDs donated directly to high-priority candidates on the waiting list and 47 initiated Altruistic Donor Chains (ADCs) as part of the UK Living Kidney Sharing Scheme (UKLKSS) [2, 3].

Despite a measurable benefit to the UK kidney transplant programme, public endorsement [4, 5], and comparable clinical outcomes to specified kidney donors (SKDs) [6, 7], there is historic evidence of UKDs being approached with a degree of caution and suspicion by some transplant professionals [8]. Qualitative studies have shown that UKDs detect these negative attitudes during clinical encounters, and these manifest as attempts to discourage donation and the presentation of inconsistent information. Donors have also reported feeling distressed at the mandatory requirement for a mental health assessment [9, 10], which is partly based upon the desire to exclude underlying psychopathological motives. This makes donors feel overly scrutinised and as though they must prove their sanity [11].

Despite the issues mentioned above, there is no existing evidence that quantifies the attitudes of UK transplant professionals towards UKD and whether these are consistent with what has been reported by UKDs. Additionally, since the beginning of the UKD programme there have been large centre variations across the UK. Currently 45% of UKD donations take place in just six out of 23 transplant centres [6]. Of the six, three are in the south of England. There is no robust explanation for these variations, nor whether this is a manifestation of the professional attitudes and values of the transplant professionals working within these centres.

The Barriers and Outcomes in Unspecified Donation (BOUnD) study was devised to conduct a comprehensive assessment of the UK UKD programme. BOUnD is a mixed methods study aiming to capture clinical and psychosocial data on outcomes following UKD (and how these compare with Specified Kidney Donation (SKD)), as well as data on the attitudes of transplant professionals towards UKD [12]. The study arm investigating transplant professionals' attitudes consists of two components. The qualitative arm involved interviews with transplant professionals from high and low volume UKD centres. The quantitative arm captured data from professionals across the country using a validated questionnaire. These were both informed by focus groups held with both service users and transplant professionals. This paper presents the results from the quantitative study, the aim of which was to elicit the views of UK transplant professionals towards different aspects of UKD, and whether any of these could be identified as potential barriers to donation. We hypothesised that:

1. A minority of transplant professionals would express negative views toward unspecified kidney donation and unspecified kidney donors
2. Surgeons and nephrologists working with unspecified kidney donors would hold more negative views than nursing and other clinical staff

TABLE 1 | Sample demographics and involvement in Unspecified Kidney Donation.

	n (%)
Gender	
Male	57 (37.2)
Female	96 (62.7)
Age	
25–34	12 (7.8)
35–44	48 (31.4)
45–54	64 (41.8)
>55	29 (19)
Role	
Administrative staff	3 (2.0)
Inpatient nurse	11 (7.2)
Outpatient nurse	3 (2.0)
Co-ordinator	42(27.5)
Consultant Physician	28 (18.3)
Trainee Physician	5 (3.3)
Consultant Surgeon	28 (18.3)
Trainee Surgeon	8 (5.2)
Other Healthcare Professional	25 (16.2)
Member of minority ethnic group	
Yes	21 (13.7)
No	136 (82.4)
Prefer not to answer	6 (3.9)
Consider themselves religious	
Yes	39 (25.5)
No	109 (71.2)
Prefer not to answer	5 (3.3)
Consider themselves spiritual	
Yes	62 (40.5)
No	82 (53.6)
Prefer not to answer	9 (5.9)

3. Individuals working in low volume centres would hold more negative views than those working in high volume centres; potentially providing a contributory reason for why donation rates are lower in these centres

MATERIALS AND METHODS

Study Design

We undertook a cross-sectional survey of transplant professionals from across the UK (**Supplementary Digital Content File (SDC) S1**). A focus group was held with former service users and transplant professionals to inform the themes to be captured. The questionnaire was subsequently written, undergoing multiple iterations which were circulated amongst the research team. Once this was finalised the questionnaire underwent further refinement and reliability testing, as well as robust validation and piloting. The details of this are documented in **Supplementary Digital Content File S2**.

Transplant professionals were defined as any patient-facing healthcare worker involved in the care of a potential unspecified kidney donor. This included surgeons and nephrologists, ward and outpatient nurses, donor co-ordinators, independent assessors, psychiatrists, and clinical administrative staff. Physicians and surgeons were only recruited if they were at consultant or senior trainee level, having declared transplantation as a specialist interest. The rationale for this was to reduce the potential for bias within the sample by only including those with sufficient clinical experience in living donation.

The principal investigator and nominated transplant co-ordinator at all of the 23 UK transplant centres were charged with distributing electronic or paper-based questionnaires. A subsequent snowball sampling approach was encouraged to optimise recruitment of relevant individuals both within and outside their organisation. Relevant professionals outside the transplant centre include those working within non-transplant renal centres who undertake their own UKD workup locally before referring them to the transplant centre for surgery. Due to the large variation in transplant centre size we aimed to have at least one clinician from each professional group from each centre. Interim analysis of the results at 6 months identified low-responding centres and professional subgroups, leading to recruitment drives targeting these groups. Adequate representation was agreed to have been achieved once one clinician from each professional group in each transplant centre had completed the questionnaire.

Statistical Analysis

Categorical variables were described as the number of non-missing values and percent. Continuous variables were described using means and standard deviations, or medians and quartiles where high levels of skew were observed. Differences between variables across groups for continuous variables were assessed using mixed effects models, including a random intercept to account for the nesting of individuals within centres. Robust standard errors were estimated, and the 5% alpha level used for interpreting *p*-values.

Responses to some items were combined to form scales indicating the level of acceptance of UKD. A pool of 13 items potentially indicating acceptance of UKD were selected by the research team and the suitability for generating an acceptance score was confirmed by exploratory factor analysis. Specifically, 7 items loaded onto an acceptance factor were retained as an acceptance score (Cronbach's alpha of 0.77). To account for differing response categories across items the scale of the score was standardised with the mean for the sample set at 50 and the standard deviation of 10. A higher score indicated greater acceptance of UKD. Whilst arbitrary, it allowed for comparisons across groups within the sample. Analyses were conducted in Stata 15.1 and IBM SPSS version 24. Full details, along with the psychometrics for the score, is provided in **Supplementary Digital Content File S3**.

RESULTS

Demographics and involvement with UKDs (**Table 1**).

The study provided a comprehensive coverage of the UK transplant community, covering 153 individuals from all 23 UK centres (**Figure 1**). The majority of participants were women (63%), and the most represented professional role was transplant coordinator (28%). Most participants were aged between 45 and 54 years and did not consider themselves to be from a minority

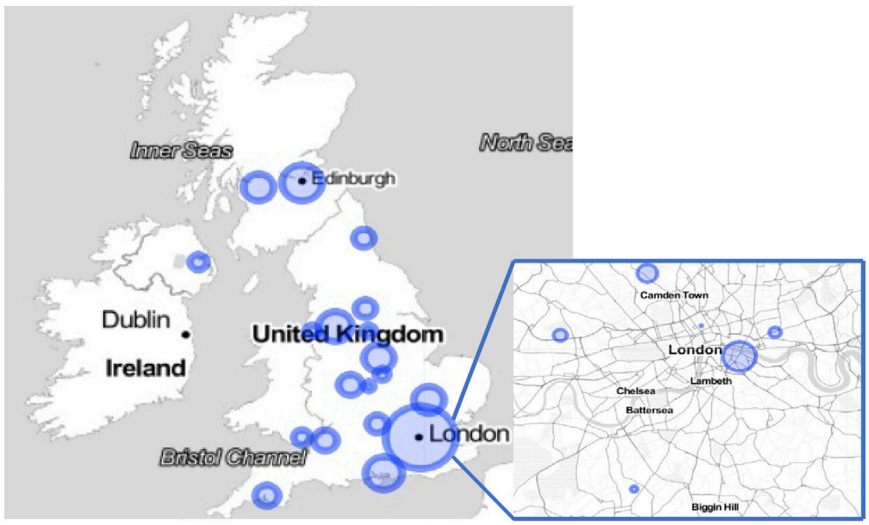


FIGURE 1 | Map demonstrating distribution of participation across the UK.

TABLE 2 | Professionals and UKDs.

	n (%)					
	Strongly agree	**Agree**	**Neither agree nor disagree**	**Disagree**	**Strongly Disagree**	
"I am confident dealing with people wishing to become UKDs"	65 (42.5)	69 (45.1)	15 (9.8)	3 (2.0)	1 (0.7)	
"My experience with people wishing to become unspecified (non-directed altruistic) donors has been generally positive"	48 (31.4)	77 (50.3)	25 (16.3)	3 (2.0)	0 (0)	
"I am comfortable with UKDs undergoing major surgery"	42 (27.5)	89 (58.2)	16 (10.5)	5 (3.3)	1 (0.7)	
Compared to SKDs BEFORE donation, potential UKDs:						
Have a higher dropout rate	7 (4.6)	62 (40.5)	57 (37.3)	27 (17.6)	0 (0.0)	
Are more time consuming for transplant professionals	14 (9.2)	53 (34.6)	42 (27.5)	40 (26.1)	4 (2.6)	
Need a greater number of assessments or investigations compared with specified living donors	2 (1.3)	44 (28.8)	45 (29.4)	59 (38.6)	3 (2.0)	
Compared to SKDs AFTER donation, potential UKDs:						
"UKDs receive less support after donation"	2 (1.3)	6 (3.9)	37 (24.2)	73 (47.7)	35 (22.9)	
More likely to seek medical help from transplant units regarding donation related issues	6 (3.9)	22 (14.4)	62 (40.5)	61 (39.9)	2 (1.3)	
More likely to seek mental health help regarding donation related issues compared to SKDs	2 (1.3)	15 (9.8)	72 (47.1)	62 (40.5)	2 (1.3)	
More likely to seek medical help from transplant units regarding non-donation related issues compared to specified donors	5 (3.3)	18 (11.8)	69 (45.1)	59 (38.6)	2 (1.3)	
	Much better	Better	Same	Worse	A lot worse	Unsure
"How are UKDs treated during the donation process compared with SKDs"	1 (0.7)	9 (5.9)	133 (86.9)	2 (1.3)	0 (0.0)	8 (5.2)

ethnic group. A quarter considered themselves to be religious, with a slightly higher proportion identified as being spiritual (41%). Most respondents (77%) had between 2 and 10 years of experience in the field (77%) and 96 (64%) stated they have been involved with five or more UKDs.

Due to the snowball recruitment strategy, it was not possible to calculate a denominator of our sample size, as it is impossible to account for how many individuals received the questionnaire, nor how many individuals worked within the transplant programme within each centre. A surrogate marker was calculated based on the number of nephrologists, surgeons and co-ordinators responding to the questionnaire per centre; data obtained from the principal investigators at each site. This demonstrated a 73% response rate to the questionnaire amongst clinicians and a 68% response rate amongst transplant co-ordinators.

The questionnaire covered a range of topics pertinent to UKDs, the full range of which cannot be discussed at length as part of this manuscript. Those questions directly relevant to the hypotheses are provided below.

TABLE 3 | Concerns about donation and donor motivations.

	n (%)				
	Strongly agree	**Agree**	**Neither agree nor disagree**	**Disagree**	**Strongly disagree**
"I am worried about the potential long-term effects of UKD on the donor's. . ."					
Physical health	10 (6.5)	55 (35.9)	29 (19.0)	49 (32.0)	10 (6.5)
Psychological health	7 (4.6)	51 (33.3)	40 (26.1)	48 (31.4)	7 (4.6)
"I am worried UKDs may regret their decision to donate in the future"	3 (2.0)	47 (30.7)	46 (30.1)	51 (33.3)	6 (3.9)
"I am worried that UKD is potentially a burden for the donor's family"	10 (6.5)	55 (35.9)	29 (19.0)	49 (32.0)	10 (6.5)
"I believe unspecified (non-directed altruistic) living kidney donors make balanced decisions when choosing/deciding whether to donate or not"	28 (18.3)	76 (49.7)	43 (28.1)	5 (3.3)	1 (0.7)
"I think many people wishing to be UKDs are more likely to be risk-takers who do not fully consider the consequences of their actions"	1 (0.7)	16 (10.5)	48 (31.4)	74 (48.4)	14 (9.2)
"I think many people wishing to be unspecified (non-directed altruistic) kidney donors are likely to have a history of mental health problems"	3 (2.0)	25 (16.3)	40 (26.1)	66 (43.1)	19 (12.4)
"I believe it is possible for unspecified (non-directed altruistic) donors to be motivated purely by the desire to help others"	61 (39.9)	80 (52.3)	9 (5.9)	3 (2.0)	0 (0)
How often do you think that altruistic donors are motivated by. . .					
"Personal psychological benefit"	32 (20.9)	101 (66.0)	14 (9.2)	5 (3.3)	1 (0.7)
"Desire to improve social status"	3 (2.0)	30 (19.6)	55 (35.9)	59 (38.6)	6 (3.9)
"Religious or spiritual beliefs"	18 (11.8)	82 (53.6)	41 (26.8)	11 (7.2)	1 (0.7)
"Civic duty and social responsibility"	15 (9.8)	91 (59.5)	31 (20.3)	16 (10.5)	0 (0)
"Personal psychological ill-health"	6 (3.9)	24 (15.7)	60 (39.2)	57 (37.3)	6 (3.9)

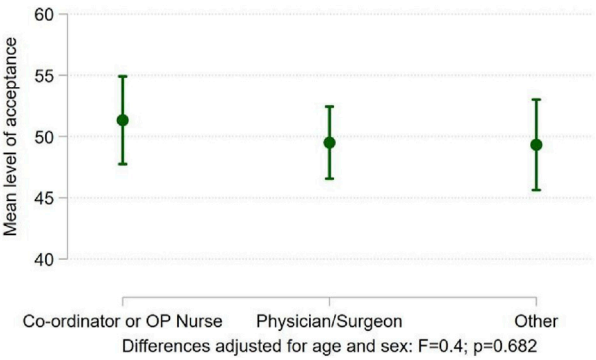


FIGURE 2 | Differences in acceptance of UKD score between transplant professionals.

Hypothesis 1. A minority of transplant professionals would express negative views toward UKD / UKDs.

The majority of participants (81.7%) stated that their experience with UKDs had been generally positive, where a significance test against a null hypothesis of 50% was $p < 0.001$; CI 75.4%–87.7%. Similarly, the majority of participants (85.7%) said they were comfortable with UKDs undergoing major surgery, where a significance test against a null hypothesis of 50% was $p < 0.001$; CI 80.2%–91.2%. A considerable proportion of individuals did hold some negative views, including UKDs being more time consuming (43.8%; CI 35.9%–51.7%) and having a higher dropout rate (45.1%; CI 37.2%–53.0%) (**Table 2**).

Participants were specifically asked about their concerns about outcomes and motivations in UKDs (**Table 3**). High numbers of professionals felt that UKDs were more likely to have a history of mental health problems and expressed concerns for donors' long-term mental and physical health, regret, and the potential for burden upon the donor's family. This view was supported by the large numbers (83%; CI 77.0%–89.0%) stating that a formal mental health assessment should remain mandatory as part of the workup process. Of these, a small majority (52%; CI 43.3%–60.7%) felt this should take place before any medical tests.

Whilst UKD was broadly viewed positively, one area of significant contention was donor age. There was little consensus between participants about official upper and lower age limits for donation within their centre, with significant numbers unsure. Participants were asked separately whether they felt there ought to be age limits for UKD. Only 15% (CI 9.3%–20.7%) thought that an upper age limit should apply; and where this was indicated, this should be 70 years (range 50–80 years). More participants (77%; CI 70.3%–83.7%) thought a lower age limit should apply; and where this was indicated, should be 25 years (range 16–50 years).

A separate indication of negative feelings towards UKD was demonstrated in the responses to questions relating to whether the individual would consider being a living kidney donor themselves. A significantly higher proportion were comfortable with the idea of being a specified donor (86.9%), compared with only 21.6% comfortable with the idea of being an unspecified donor ($p = 0.006$).

Hypothesis 2. Surgeons and nephrologists working with UKDs would hold more negative views than nursing and other clinical staff.

As described in the methods section, responses to some items were combined to form a scale indicating the level of acceptance of UKD. **Figure 2** displays the mean acceptance scores for different categories of transplant professional.

TABLE 4 | Acceptance of UKD. Correlations between the acceptance score were calculated against a selection of variables from the questionnaire. Where items were both continuous, the correlation coefficient was estimated by the Pearson method. For ordinal and binary items the correlation coefficient was estimated by the polyserial method. Note that the Bonferroni adjusted critical value for p is reduced from $p < 0.05$ to $p < 0.003$. Acceptance scores were not related to demographic variables. They were, however, related to some variables relating to perceived resource use and more negative views regarding psychological motivations for wanting to donate.

	Acceptance score	
	Correlation	***p*-value**
Age	–0.07	0.425
Female	0.03	0.749
Ethnic minority	0.07	0.551
Spiritual	–0.03	0.746
Religious	0.03	0.746
Altruism score	0.14	0.086
Direct experience with UKD	–0.51	**0.000***
Years experience UKD	–0.41	**0.000***
UKDs are likely to have mental health problems	–0.25	0.003
UKDs are more likely to be risk-takers	–0.26	**0.002***
UKDs have a higher dropout rate	–0.07	0.434
UKDS are more time consuming	–0.14	0.118
UKDS need a greater number of assessments or investigations	–0.2	**0.024***
UKDs more likely to seek medical help regarding donation issues	–0.19	**0.029***
UKDs more likely to seek mental health help	–0.31	**0.000***
UKDs more likely to seek medical help regarding non-donation issues	–0.26	**0.003***
UKDs make balanced decisions when choosing to donate	0.53	**0.000***
Personal psychological benefit	–0.03	0.744
Medical fitness	0.11	0.217

*p < *0.003*.

Adjusted means across groups were not statistically significantly different ($p = 0.682$), suggesting that professional background did not impact on UKD support or opposition. Levels of acceptance around UKD was unrelated to demographic variables. There were negative correlations between the score and more negative attitudes towards UKDs, including perceived resource use and decision making.

Hypothesis 3. Individuals working in low volume centres held more negative views than those working in high volume centres.

The sample were divided into high and low volume centres. Six out of 17 centres were found to contribute to 50% of the total number of UKDs and these units were classed as high volume. Across the majority of questions there was no significant difference between the two groups in the way the questions were answered. Negative correlations were found with level of direct experience with UKDs, with those with less experience being less comfortable with UKD as a practice ($p < 0.003$) (**Table 4**). Conversely, fewer professionals in high volume centres felt that those making enquiries about UKD received a positive response ($p < 0.001$). They did not feel that staff at their centre had been adequately trained ($p = 0.025$), and nor did they feel that the facilities available were sufficient to support the UKD programme ($p = 0.012$). Fewer professionals at high volume centres reported positive experiences with UKD candidates ($p < 0.001$). Despite this, acceptance of UKD was significantly higher in high volume centres (46.2 vs. 52.9; $p < 0.001$) (**Figure 3**).

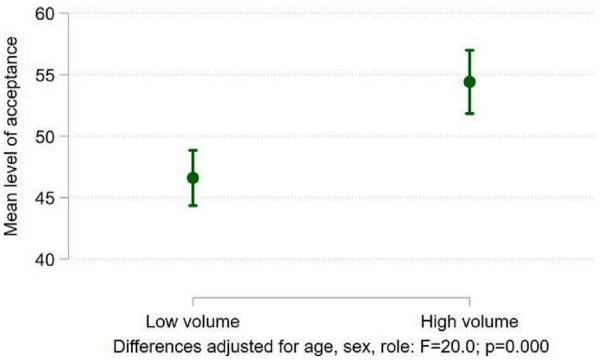


FIGURE 3 | Differences in acceptance of UKD score between high and low volume centres.

DISCUSSION

This is the first quantitative study to report systematically on acceptance by transplant professionals to a large national UKD programme. We received responses from a broad range of different professionals involved in transplantation, with representation from each UK transplant centre. We hypothesised that negative views towards UKD would be held by a minority of transplant professionals, that surgeons and nephrologists would hold more negative views than nursing and other clinical staff, and that those working in low volume centres would have more negative views than those in high volume centres.

The study has shown that a large majority of transplant professionals are in support of UKD and that whilst levels of acceptance did not differ between professional groups, those from higher volume centres were more accepting. Whilst the majority of transplant professionals had positive experiences with UKDs, a considerable proportion perceived UKDs to be more time consuming with higher dropout rates. One of the aims of the prospective donor study being conducted as part of BOUnD [12] is to either confirm or deny these claims by providing prospective data on donor workup and donation times. Whilst formal analyses are ongoing, preliminary data has indicated that UKDs take longer to donate, but this is primarily due to their inclusion in the UKLKSS, which is conducted quarterly [13]. On occasions where UKDs donate to a high priority recipient on the waiting list, there is no significant difference in workup and donation times.

This study has confirmed long-held anecdotal views within the transplant community regarding donor motivations and concerns regarding mental health, both before and after

donation. It is therefore understandable that the majority of participants wanted a formal, mandatory mental health assessment of UKDs to remain part of the workup process. This is in keeping with guidance from the British Transplantation Society, which considers it prudent for mental health assessments, conducted by a trained mental health professional, to remain best practice until more specific and sensitive evidence about the impact on mental health is available [9]. These guidelines are heavily influenced by a consensus statement written by transplant psychologists and psychiatrists on behalf of the European Association of Psychosomatic Medicine (EAPM) [14]. Data from this study has shown that a small majority of professionals felt that this assessment should take place prior to any medical tests being conducted. We believe this links two of the findings outlined above: the assumption of a higher incidence of mental health history within potential UKDs and the feeling that they are more likely to withdraw from the process. Should a transplant professional hold either or both of these views, it follows that by conducting mental health assessments early in the process, fewer individuals are subjected to further medical assessment, which is both costly and time consuming [15]. The EAPM recommend mental health screening "after initial medical screening, clinical assessment, and provision of information by the transplant team, but before any invasive investigations which carry physical risks," in order to avoid subjecting potential donors to a risk of harm.

Transplant professionals with specific concerns related to potential UKDs withdrawing for mental health reasons may be reassured by findings from a qualitative study conducted as part of the BOUnD, which specifically investigated the experiences of UKD candidates who both completed and withdrew from the process [16]. In this study only very few participants not proceeding with UKD did so as a direct consequence of a mental health issues. Given that so many UKDs report difficulties with the experience of a mental health assessment [11] and that supply of adequately trained mental health professionals often leads to delays in the workup, concerns amongst about donors undertaking this assessment when they are towards the end of their work-up, may be misplaced.

A broader understanding of the attitudes of transplant professionals towards UKD can be gleaned from their own preferences regarding organ donation, with significantly more being comfortable with SKD compared to UKD. We postulate that this is due to an awareness and negative experiences of the risks of surgery which may only be deemed acceptable for a specified recipient. This is supported by previous research demonstrating that living donors are willing to accept significantly higher risks than transplant surgeons [17]. A qualitative interview study conducted in addition to this survey further probed some of these attitudes and the manuscript is currently under peer review.

An area of longstanding interest and controversy within UKD, and one which anecdotally has generated a huge amount of discussion amongst transplant professionals, is that of donor age. This study is the first to provide a quantitative assessment of transplant professionals' views on this topic. As evident from the findings of this study, transplant professionals feel more strongly about a lower age limit than an upper age limit. Whilst there is no official lower limit for living donation in the UK, the BTS living donor guidelines [9] state that most programmes do not consider SKDs or UKDs aged under 18 years and view an age of 18–21 to be a relative contraindication to donation. The range of responses to what the lower age limit for UKD should be demonstrates the breadth of feeling within the transplant community. Proponents of donation later in life rationalise this viewpoint on the basis that time allows UKDs to live the majority of their lives with two kidneys (thereby reducing the long-term medical complications associated with donation) and to achieve an undefined degree of psychosocial maturity, which should in turn lead to lower levels of regret. Counterarguments against lower age limits are mainly focused on paternalism and whether this ought to override the autonomy of young people with capacity. There is no current evidence to prove that young people are more or less likely to regret their decision to donate, however there is evidence to show that younger donors (aged between 18 and 34) are more likely to develop end-stage renal disease and themselves require a transplant [9].

This study has highlighted a large gap in the literature which potentially fuels negative views and creates barriers towards UKD; a practice which has been proven to be of huge benefit patients with end-stage renal disease in the UK. In the only previous study we are aware of, 78% of French physicians were opposed to the practice of UKD [18]. UKDs make an important contribution to the UK living donor programme via the UKLKSS and facilitate transplants for some of the most difficult to transplant patients on the waiting list. However, transplant professionals remain concerned about donor motivations, mental health issues and outcomes following UKD. It is crucial that robust data are provided to address this gap to either confirm or deny the apprehensions held by the transplant community. The longitudinal prospective study into UKDs' outcomes will invariably help to fill this gap in due course.

Professional groups were not found to differ in their acceptance of UKD, which provides some baseline reassurance that units are working harmoniously in their approach towards UKDs. With regard to centre volume, this study has demonstrated that whilst higher volume centres report higher levels of acceptance for UKD, there are ongoing practical issues and more negative personal experiences. These somewhat mixed findings may be explained by the increased workload that UKD places on the existing living donor programme, leading to individuals feeling inadequately resourced, underprepared and overwhelmed. Fewer positive experiences with UKD candidates in higher volume centres may also reflect the larger number and broader range of individuals presenting as potential UKDs who then do not proceed for a variety of different reasons. Whilst the number of UKDs at each centre are known, the number of potential UKDs approaching each centre and the drop-out rates remains unclear. This is another area in which BOUnD will hopefully provide detailed data for the transplant community.

Strengths and Limitations

The strengths of this study lie within its questionnaire tool which was devised and piloted with the specific research questions in

mind. The study also sampled a large number and range of transplant staff and included every transplant centre in the UK. One limitation is that the questionnaire was not designed explore why participants held the views they expressed. A separate qualitative study has addressed some of these issues and is currently under peer review. Due to the questionnaire being distributed broadly across transplant centres and their professionals it was not possible to calculate the denominator in the population contacted. This introduces the potential for responder bias and a theoretical limitation regarding how representative this view is of the transplant professional population as a whole. There was also a potential for bias as individuals interested in, or with experience of, UKD may have been more likely to respond than those with little interest or experience.

Conclusion

This study has demonstrated that whilst there is broad support of UKD as a practice, there are a number of potential barriers. These include a perception that UKDs are more time consuming and a need to exclude psychopathological motives prior to any medical tests being performed. There is ongoing uncertainty related to donor age and a feeling in higher volume centres that more training and resources are needed to facilitate UKD practices. The results from the prospective longitudinal study being conducted as part of BOUnD will provide a robust assessment of many of these factors and provide the transplant community with much needed data on this group of donors.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HM: participated in research design, data analysis and writing of the paper. PG: participated in research design, performance of the research and writing of the paper. RG: participated in performance of the research and writing of the paper. LB: participated in research design and writing of the paper. HD: participated in writing of the paper. JC: participated in research design and writing of the paper. SN: participated in research design, data analysis and writing of the paper. NM: participated in research design, analysis and writing of the paper.

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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.2023.11257/full#supplementary-material

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ORIGINAL RESEARCH
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Obesity is Associated With Delayed Graft Function in Kidney Transplant Recipients: A Paired Kidney Analysis

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Obesity is increasingly prevalent among candidates for kidney transplantation. Existing studies have shown conflicting post-transplant outcomes for obese patients which may relate to confounding bias from donor-related characteristics that were unaccounted for. We used ANZDATA Registry data to compare graft and patient survival between obese (BMI >27.5 kg/m^2 Asians; >30 kg/m^2 non-Asians) and non-obese kidney transplant recipients, while controlling for donor characteristics by comparing recipients of paired kidneys. We selected transplant pairs (2000–2020) where a deceased donor supplied one kidney to an obese candidate and the other to a non-obese candidate. We compared the incidence of delayed graft function (DGF), graft failure and death by multivariable models. We identified 1,522 pairs. Obesity was associated with an increased risk of DGF (aRR = 1.26, 95% CI 1.11–1.44, $p < 0.001$). Obese recipients were more likely to experience death-censored graft failure (aHR = 1.25, 95% CI 1.05–1.49, $p = 0.012$), and more likely to die with function (aHR = 1.32, 95% CI 1.15–1.56, $p = 0.001$), versus non-obese recipients. Long-term patient survival was significantly worse in obese patients with 10- and 15-year survival of 71% and 56% compared to 77% and 63% in non-obese patients. Addressing obesity is an unmet clinical need in kidney transplantation.

Keywords: kidney transplantation, patient survival, graft survival, obesity, DGF

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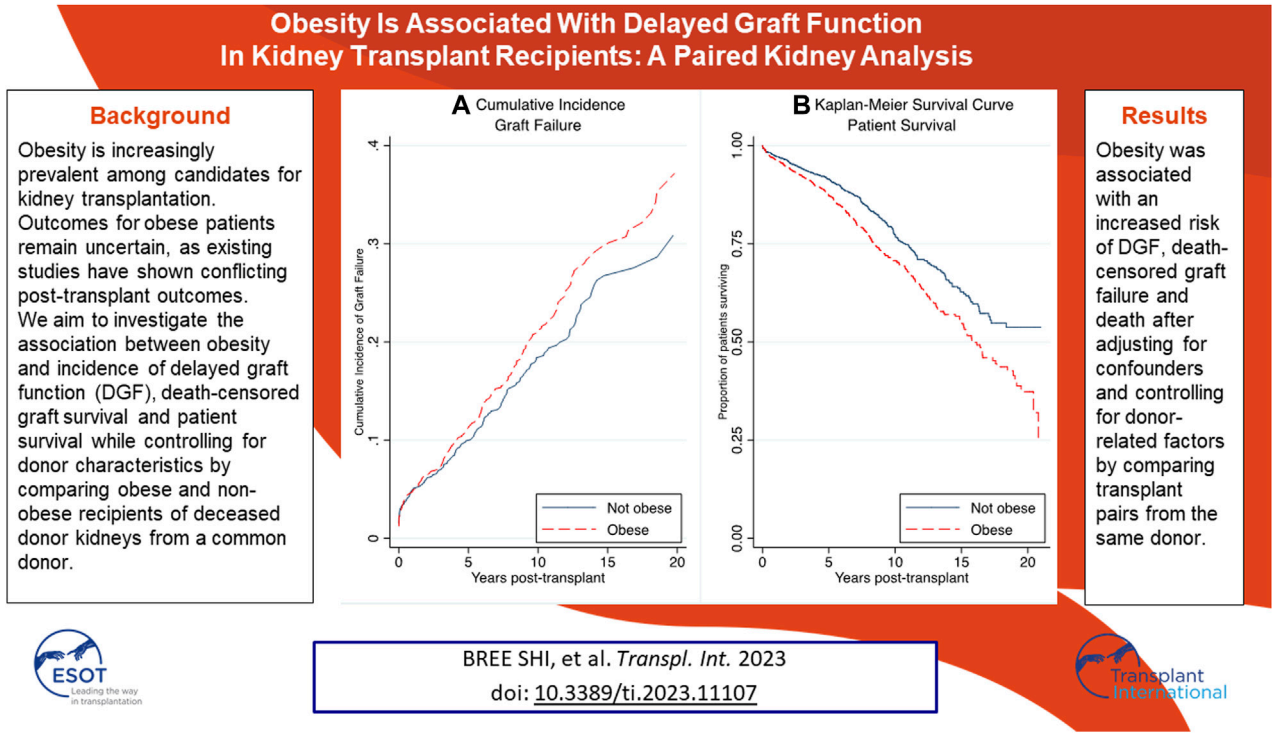
INTRODUCTION

Over the past four decades, the worldwide prevalence of obesity has tripled. In addition to the associations between obesity and hypertension, type 2 diabetes and coronary artery disease, obesity is clearly associated with premature mortality [1]. Obesity has therefore had a significant impact on community health as well as posing a major economic challenge to global healthcare systems. Obesity is increasingly prevalent in the end-stage kidney disease (ESKD) and kidney transplant populations

Abbreviations: aHR, adjusted hazard ratio; ANZDATA, the Australia and New Zealand Dialysis and Transplant registry; aRR, adjusted rate ratio; BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; DGF, delayed graft function; ESKD, end-stage kidney disease; GN, Glomerulonephritis; HD, hemodialysis; HLA, human leukocyte antigen; PD, peritoneal dialysis; RRT, renal replacement therapy; WHO, World Health Organization.



GRAPHICAL ABSTRACT |

[2,3]. In the US, the proportion of ESKD patients that were obese between 2008 and 2016 was nearly 40% [4].

Whilst kidney transplant recipients with high body mass index (BMI) are more like to develop post-transplant diabetes, congestive heart failure, atrial fibrillation, and cardiovascular death [5–7], transplantation offers a survival benefit for obese recipients compared to remaining on dialysis [8,9]. However, kidney transplant recipients with high BMI are at an increased risk of post-transplant complications, including prolonged wound healing, dehiscence, hernias, surgical site infections, deep vein thrombosis, and reintubation. These issues contribute to a longer hospital stay and higher hospital costs for transplantation in the obese [10–12].

The long-term graft and patient outcomes of obese recipients compared to non-obese recipients have remained controversial. When compared to non-obese recipients, some reports described an increased risk of graft failure and mortality for obese recipients whilst others have found no significant differences [12–15]. These disparate outcomes, may relate to the confounding bias of non-randomly distributed donor-related characteristics which were not accounted for [16–18]. Therefore, we sought to investigate the association between obesity and incidence of delayed graft function (DGF), graft survival and patient survival while controlling for donor characteristics by comparing obese and non-obese recipients of kidneys from a common donor, a matched-pair analysis. We hypothesized that obesity would increase the risk of DGF and lead to inferior graft and patient survival.

MATERIALS AND METHODS

We extracted data from the Australia and New Zealand Dialysis and Transplant Registry (ANZDATA). The ANZDATA Registry is a clinical quality registry that collects comprehensive data from all patients with ESKD in Australia and New Zealand. Details of the structure and method of ANZDATA Registry data collection can be found on the Registry website (https://www.anzdata.org.au/anzdata/). We included all deceased donor kidney-only transplant pairs between 1 January 2000 and 31 December 2020, where a deceased donor supplied one kidney to an obese recipient and the other to a non-obese recipient. We excluded recipients under the age of 18, recipients of a deceased donor kidney retrieved outside Australia or New Zealand, and recipients of a second or subsequent transplant. We used the World Health Organization (WHO) classification of obesity as BMI greater than 30 kg/m^2 for non-Asians, and greater than 27.5 kg/m^2 for Asians due to differences in body habitus compared to the Western population [19–21]. Follow-up was until loss to follow-up, or 31 December 2020. The primary outcome was DGF which was defined as receipt of hemodialysis within 72 h after transplant prior to 2017, and receipt of hemodialysis within 7 days of transplantation after 2017 [22]. This modification to the definition of delayed graft function was due to a policy change

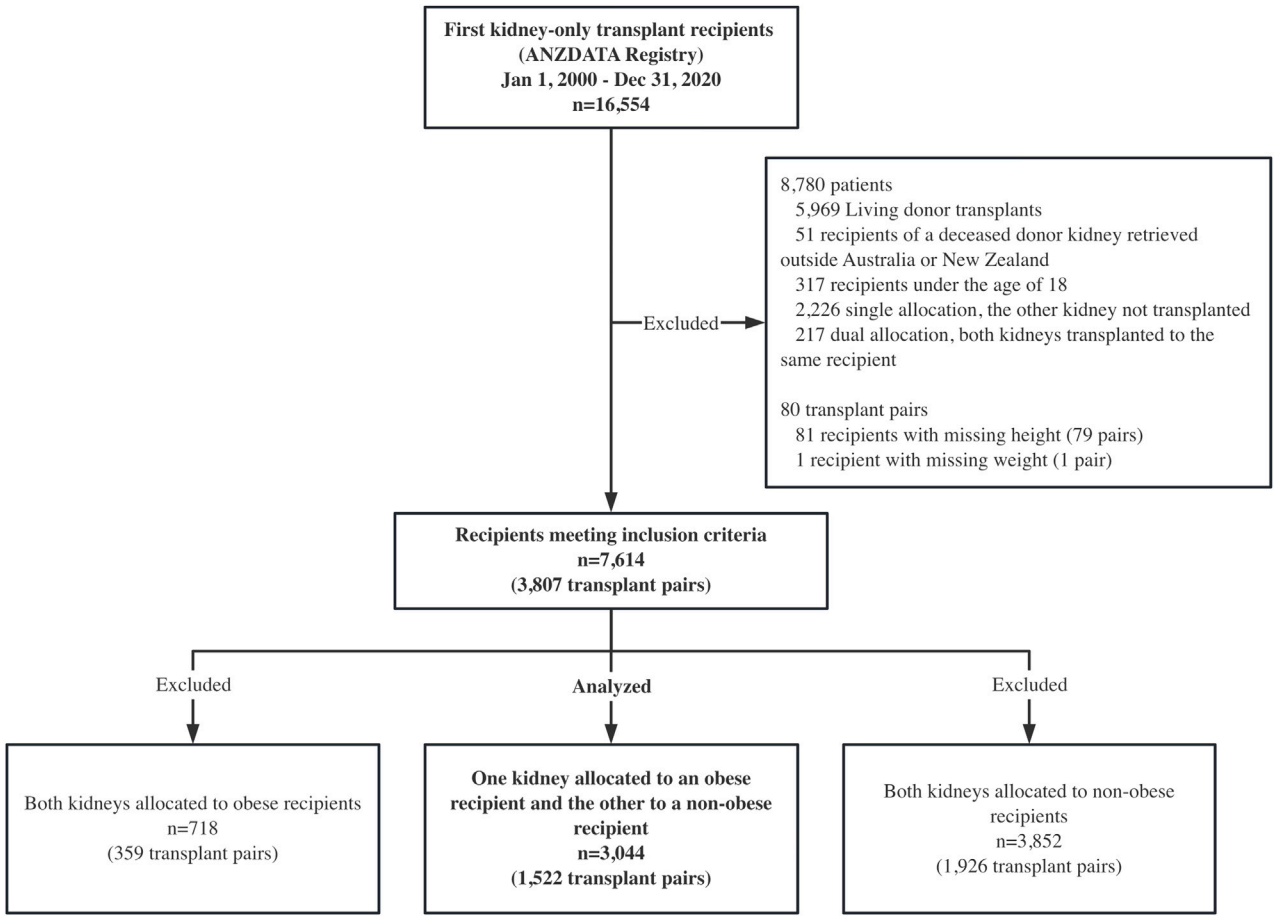


FIGURE 1 | Study design: a paired kidney analysis where one kidney was allocated to an obese recipient and the other to a non-obese recipient. ANZDATA, the Australia and New Zealand Dialysis and Transplant registry.

made by ANZDATA in 2017. The secondary outcomes were death and death-censored graft failure.

We compared baseline characteristics of paired recipients using paired t-test or Wilcoxon's signed rank test for continuous variables and McNemar's test for dichotomous variables. We estimated the cumulative incidence of graft failure using Aalen-Johansen estimator to account for death as a competing event. We used Gray's test to compare the cumulative incidence of graft failure in the presence of the competing risk of death. We used Kaplan-Meier curves to compare unadjusted patient survival. We used a logrank test to compare the probability of patient survival at different time points. We estimated the rate ratio of DGF for obese patients compared with non-obese patients, using conditional Poisson regression, adjusting for potential confounders [23–25]. As a sensitivity analysis, we repeated this analysis excluding patients who experienced graft failure within 90 days of transplantation. Time to graft failure and time to death were analyzed using Cox regression stratified by donor [24,25].

A dose-response analysis was performed to examine the association between the degree of obesity (i.e., class I, class II and class III) and clinical outcomes. Obesity was categorized as class I, class II and class III according to WHO guidelines (**Table 3**). We estimated the rate ratio of DGF using conditional Poisson regression and the hazard ratio of graft failure and death using Cox regression, adjusting for potential confounders.

The potential confounders considered were age at transplantation, sex, ethnicity, cause of kidney disease, duration of dialysis, dialysis modality prior to transplant, human leukocyte antigen (HLA) mismatch, ischemia time, maximum panel reactive antibodies, donor kidney side, pre-existing comorbidities including diabetes, chronic lung disease, cardiovascular disease (any of coronary artery, cerebrovascular or peripheral vascular), and non-skin cancer, acute rejection within 6 months of transplantation (for graft failure and death only), DGF (as a categorical variable, for graft failure and death only), and graft failure

(as a time-varying covariate, for death only). We used stepwise selection methods where variables with a significance level of 0.20 were considered and included in the base multivariable model. We used backward selection method to remove variables that were not significant at the 0.05 level [26]. We used complete case analysis because the number of missing values was less than 5%. All analyses were performed using Stata Statistical Software: Release 14.2 (StataCorp., College Station, TX) This study was approved by the Ethics Review Committee of the Sydney Local Health District, Royal Prince Alfred Hospital Zone.

RESULTS

Study Cohort

Between 1 January 2000, and 31 December 2020, 16,554 patients received their first kidney transplant in Australia and New Zealand. After inclusion and exclusion criteria were applied, 1,522 pairs were identified where a deceased donor supplied one kidney to an obese recipient and the other to a non-obese recipient (**Figure 1**). Follow-up time was 19,768 person-years in total, with a median follow-up time of 5.3 years (interquartile range 2.5–9.5 years). Nine of the obese recipients and seven of the non-obese recipients were lost to follow-up.

Donor and recipient baseline characteristics are summarized in **Tables 1**, **2**. Baseline characteristics indicate that obese and non-obese recipients were comparable in terms of sex, time on dialysis, ischemia time, HLA mismatch and maximum panel reactive antibody percentage. The obese group included a higher proportion of recipients aged 50–65 (48% vs. 44%), $p < 0.001$), fewer people of Asian ancestry (12% vs. 15%, $p < 0.001$), more Indigenous people (17% vs. 11%, $p < 0.001$), more people with pre-existing diabetes (33% vs. 21%, $p < 0.001$) and comorbid cardiovascular disease (33% vs. 27%, $p = 0.001$) and more right-sided kidneys (55% vs. 45%, $p < 0.001$).

TABLE 1 | Donor characteristics.

Factor	N = 1,522 n (%)
Age	
<18	79 (5)
18–34	254 (17)
35–49	426 (28)
50–65	556 (37)
65+	207 (14)
Male	870 (57)
Body mass index (BMI)	
Underweight	47 (3)
Normal	556 (37)
Overweight	534 (35)
Obese	383 (25)
Terminal serum creatinine concentration, μmol/L	96.4 ± 83.2
Diabetes	96 (6)
Hypertension	388 (25)
Neurological determination of death (NDD)	1,167 (77)
Cause of death	
Intracranial hemorrhage	640 (44)
Traumatic brain injury	285 (19)
Cerebral infarct	94 (6)
Cerebral hypoxia/ischemia	380 (26)
Other neurological condition	12 (1)
Non-neurological condition	59 (4)

Outcomes

Delayed Graft Function

A greater proportion of obese recipients experienced DGF compared to non-obese recipients (39% vs. 30%, $p < 0.001$). Conditional Poisson regression demonstrated an increased risk of DGF for obese recipients versus their non-obese pair (aRR = 1.27, 95% CI 1.12–1.44, $p < 0.001$), after adjusting for dialysis modality prior to transplant, ischemia time and pre-existing cardiovascular disease and accounting for donor-related factors (**Supplementary Table S1**).

Sensitivity analysis, excluding those patients who experienced graft failure within 90 days of transplantation, showed a similar effect of obesity on DGF to the primary analysis (aRR = 1.29, 95% CI 1.12–1.48, $p < 0.001$).

Graft Failure

Unadjusted graft failure was more common amongst obese recipients (**Figure 2**). Cumulative incidence of graft failure at 5 years was not affected by obesity status (11% obese vs. 10% non-obese), however, obese recipients were found to have a higher incidence of long-term graft failure with 10- and 15-year cumulative incidence of 21% and 30% compared to 18% and 27% in non-obese patients. The Gray's test confirmed a significant difference on the overall incidence of graft failure between obese and non-obese recipients ($p = 0.044$). On multivariable analysis, obesity was confirmed as an independent risk factor for death-censored graft failure. Obesity was associated with a higher risk of death-censored graft failure after adjusting for DGF, donor kidney side, age, ethnicity and HLA mismatch (aHR = 1.25, 95% CI 1.05–1.49, $p = 0.012$) (**Supplementary Table S2**). Recipients who experienced delayed graft function were more likely to experience death-censored graft failure (aHR = 1.84, 95% CI 1.39–2.44, $p < 0.001$).

Patient Survival

There were 342 (22%) deaths in the obese group compared to 260 (17%) ($p < 0.001$). Death from cardiovascular disease was the most prominent cause of death amongst the obese recipients, with 105 cardiovascular deaths (31%) compared to 65 (25%) among the non-obese recipients. Obesity was strongly associated with inferior survival in both the short and long-term ($p < 0.001$) (**Figure 3**). Short and long-term patient survival was significantly worse in obese recipients with 5-, 10- and 15-year survival of 87%, 71% and 56% compared to 91%, 77% and 63% in non-obese patients ($p = 0.017$, $p < 0.001$, $p < 0.001$). In the multivariable model, obesity was found to be strongly associated with worse patient survival. Obese recipients had an increased risk of death compared to non-obese recipients (aHR = 1.32, 95% CI 1.15–1.56, $p = 0.001$) (**Supplementary Table S3**). Significant determinants of death that were included in the final model were graft failure, older age, Indigenous ethnicity, diabetes as

TABLE 2 | Recipient and transplantation characteristics for obese and non-obese recipients.

Factor	Obese	Not obese	*p*-value
	N = 1,522	N = 1,522	
Age at transplant	n (%)	n (%)	<0.001
18–34	113 (7)	193 (13)	
35–49	430 (28)	414 (27)	
50–65	728 (48)	667 (44)	
65+	251 (16)	248 (16)	
Male	985 (65)	991 (65)	0.82
Ethnicity			<0.001
Caucasian	1,001 (66)	1,006 (66)	
Indigenous	257 (17)	162 (11)	
Asian	185 (12)	232 (15)	
Other	79 (5)	122 (8)	
Primary renal disease			<0.001
GN	575 (38)	628 (41)	
Renovascular	123 (8)	112 (7)	
Diabetes	351 (23)	231 (15)	
Other	473 (31)	551 (36)	
Time since first RRT			0.14
0–1 year	173 (11)	209 (14)	
1–3 years	594 (39)	575 (38)	
Over 3 years	755 (50)	738 (48)	
Dialysis modality prior to transplant			0.008
Pre-emptive transplant	11 (1)	17 (1)	
HD	1,106 (73)	1,030 (68)	
PD	405 (27)	475 (31)	
Ischemia time [mean (sd)]	12.1 (4.9)	12.0 (5.0)	0.52
HLA mismatches			0.58
0	46 (3)	38 (2)	
1–2	408 (27)	427 (28)	
3–4	483 (32)	460 (30)	
5–6	580 (38)	596 (39)	
Maximum panel reactive antibodies			0.50
0	918 (60)	935 (61)	
1–50	491 (32)	465 (31)	
>50	110 (7)	121 (8)	
Pre-existing comorbidities			
Chronic lung disease	130 (9)	124 (8)	0.69
Cardiovascular disease	501 (33)	418 (27)	0.001
Diabetes	504 (33)	316 (21)	<0.001
Right kidney	832 (55)	690 (45)	<0.001

GN, Glomerulonephritis; HD, hemodialysis; PD, peritoneal dialysis; HLA, Human Leukocyte Antigen; RRT, renal replacement therapy.

primary renal disease, length of time on dialysis and pre-existing cardiovascular disease. Graft failure was adjusted as a time-varying covariate in the model. Recipients with graft failure had a much higher risk of death (aHR = 2.84, 95% CI 2.00–4.03, $p < 0.001$).

Degree of Obesity and Clinical Outcomes

We performed a dose-response analysis to examine the association between the degree of obesity and clinical outcomes. The 1,522 obese recipients were classified as 1,173 (77%) class I; 304 (20%) class II and 45 (3%) class III (**Table 3**). We combined obesity classes II and III due to insufficient patient numbers in obesity class III.

When comparing with non-obese recipients, class II/III obese recipients had a 1.44 higher rate of DGF whilst class I obese recipients had a 1.20 higher rate. This trend was not statistically significant when comparing class I obese recipients to class II/III obese recipients (**Figure 4**. aRR 1.20, 95% CI 0.88–1.62, $p = 0.25$). A similar non-significant trend was found for death-censored graft failure and death. Class II/III obese recipients had a 1.67 higher rate of death-censored graft failure compared to a 1.16 higher rate for class I obese recipients (aHR 1.45, 95% CI 0.95–2.21, $p = 0.085$). Class II/III obese recipients had a 1.42 higher rate of death compared to a 1.26 higher rate for class I obese recipients (aHR 1.10, 95% CI 0.71–1.71, $p = 0.66$).

DISCUSSION

In this paired analysis, we controlled for unmeasured donor-related characteristics by comparing outcomes of kidneys from the same donor and demonstrated that obese recipients were more likely to experience DGF, death-censored graft failure and death after deceased donor kidney transplantation when comparing with non-obese recipients.

Studies examining the impact of obesity on kidney transplant outcomes have shown conflicting results, but may be confounded by unmeasured donor-related characteristics. These may include donor kidney function and proteinuria, pre-renal insults to the donor kidney during terminal illness, use of inotropic medications and nephrotoxin exposure, many of which are not adequately captured nor accounted for in existing studies, The majority of published studies have reported an increased risk of delayed graft function for obese recipients [12,13,15]. However, the impact of DGF on long-term transplant outcomes including graft and patient survival remains contentious. Our results are consistent with two systematic review and meta-analyses which showed an increased risk of graft failure for obese recipients compared to non-obese recipients [14,15]. In terms of overall mortality, two meta-analyses reported an increased risk of death for obese recipients, in line with our results [12,14]. One

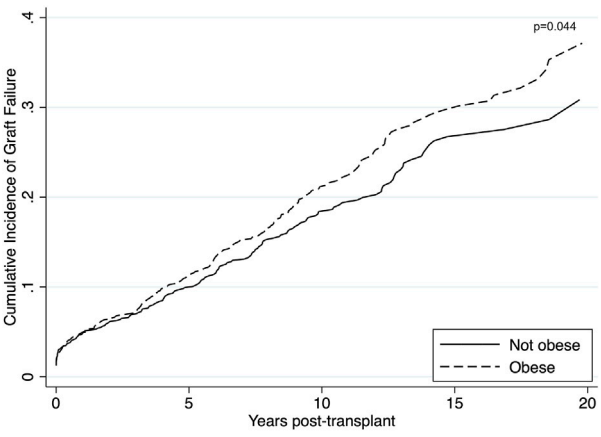


FIGURE 2 | Unadjusted cumulative incidence of graft failure using Aalen-Johansen estimator to account for death as a competing event among obese (BMI >27.5 kg/m^2 for Asians; >30 kg/m^2 for non-Asians) and non-obese recipients. Obese recipients had a higher incidence of graft failure compared to non-obese recipients ($p = 0.044$).

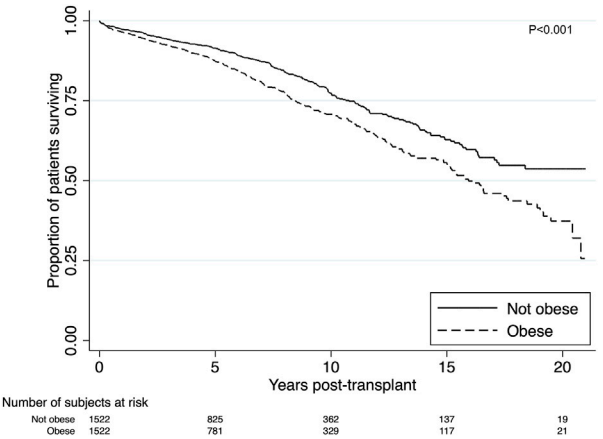


FIGURE 3 | Kaplan-Meier estimates of patient survival after renal transplant among obese (BMI >27.5 kg/m^2 for Asians; >30 kg/m^2 for non-Asians) and non-obese recipients. Unadjusted patient survival was superior for non-obese recipients ($p < 0.001$).

systematic review and meta-analysis reported no association between obesity and overall mortality [27], however, this analysis included only six studies that reported hard transplant outcomes. Another systematic review and meta-analysis reported that there was an increased risk of graft failure and death only for studies that included obese patients who were transplanted before 2000, but no association for those transplanted after 2000 [13]. This contradicts our study result which included patients transplanted after 2000 only.

We found a trend towards increasing risks of DGF, graft failure and death with increasing degrees of obesity, however, this increase was not statistically significant. The number of recipients with class II/III obesity in our study was small and likely inadequately powered to provide certainty. A recent US registry study reported 27% lower odds of DGF ($p < 0.001$) for recipients with BMI >30–35 versus BMI >35 kg/m^2, though no difference in graft or patient survival at a median follow-up of 3.9 years [28].

Our study provides detailed insights from a large, bi-national kidney transplant registry over a 20-year period. We examined a different BMI cut-off for the Asian population that has significant structural variations compared to the Western population. Donor-related factors, which could potentially impact outcomes such as DGF, were carefully accounted and unmeasured confounders were evenly matched by the use of a matched-pair analysis. As randomized controlled trials to compare outcomes for obese versus non-obese recipients are not feasible, we believe the paired analysis we have performed provides the most rigorous assessment of the impact of obesity on hard outcomes following kidney transplantation.

Obesity has more than doubled worldwide in the past 20 years. Although our study has demonstrated that obesity was strongly associated with an increased risk of DGF and inferior long-term outcomes, previous work has clearly indicated that transplantation yields superior outcomes compared to remaining on dialysis for the majority of obese candidates for transplantation [8,9,29]. Our findings should be used to inform patients and providers of the increased risks associated with transplantation for obese recipients. Rather than avoiding transplantation for the obese, these data should encourage the pursuit of strategies to improve outcomes, such as weight-loss management prior to transplantation and improvements in peri-operative management to reduce the incidence of DGF and other complications associated with obesity. This poses two key questions: (1) can transplant management be optimized for obese recipients; and (2) can weight loss before or post-transplant improve transplant outcomes for obese candidates. Some studies have reported an "obesity paradox" where a decrease in BMI for dialysis patients was associated with worse graft and patient survival [30–33]. However, in these studies there was no clear indication of whether the weight loss was intentional, or unintentional due to disease progression or comorbidities. The reason behind the paradox remains unknown. Hypotheses include that obese patients may be less prone to protein energy wasting [34], have a better appetite and well-preserved energy stores, have better hemodynamic tolerance, stem cell mobilization, hemodynamic tolerance, and more efficient disposal of lipophilic uremic toxins [35,36]. A healthy lifestyle that is beneficial to the general public has been shown to improve mortality in chronic kidney disease (CKD) patients [37]. Intentional weight loss in the pre-transplant population may reduce the risk of wound infection, DGF, death-censored failure and reduce the length of hospitalization and alleviates the financial burden on transplant programs [38]. Weight-management programs for CKD patients that include a renal-specific diet, regular exercise combined with anti-obesity medication have been reported to be effective in weight reduction, with improved functional ability, graft function and significantly longer adverse event-free period for the combined outcome of all-cause mortality, myocardial infarction, stroke, and hospitalization for congestive heart failure [39–41]. Another possible intervention is bariatric surgery. A recent study reported a lowering of 7 kg/m^2 in BMI in the long-term and a median of 2.4 years longer life expectancy in the bariatric surgery cohort compared to usual obesity care [42]. However, there is very limited data on the outcomes of bariatric surgery on dialysis and kidney transplant patients. In a retrospective cohort study, researchers demonstrated lower all-cause mortality at 5 years for

TABLE 3 | Degree of obesity was categorized into obese class I, obese class II, and obese class III according to World Health Organization guidelines.

Classification	BMI, kg/m^2, non-Asians	BMI, kg/m^2, Asians	n (%)
Obese class I	30–34.9	27.5–32.4	1,173 (77)
Obese class II	35–39.9	32.5–37.4	304 (20)
Obese class III	40+	37.5+	45 (3)

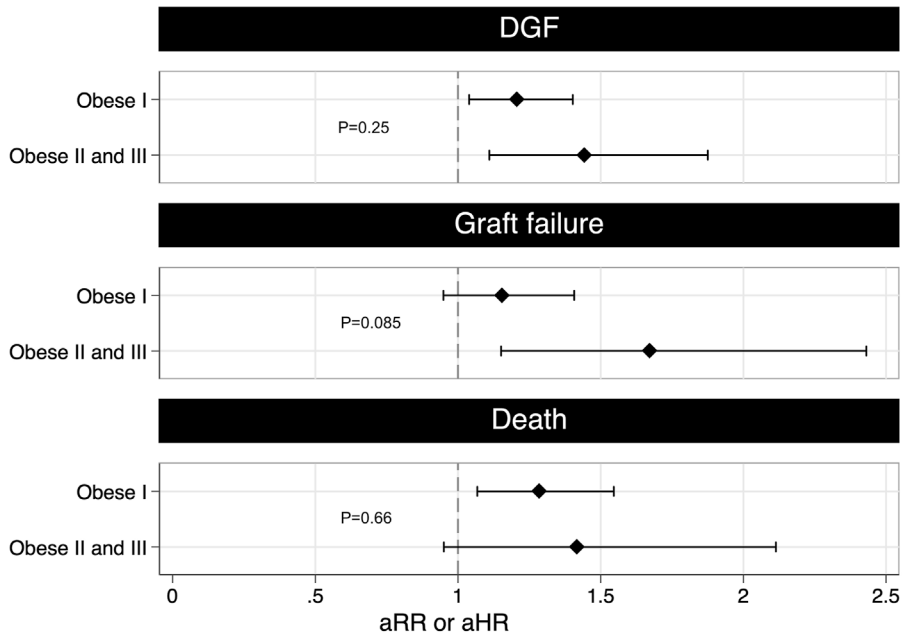


FIGURE 4 | The figure shows the multivariable adjusted risk ratio for DGF and adjusted hazard ratio for graft failure and death grouped by class I obese and class II and III obese. A higher risk of DGF and graft failure was found for greater vs. lesser degrees of obesity, suggesting a possible dose-response relationship (DGF: aRR 1.20, 95% CI 0.88–1.62, $p = 0.25$; graft failure: aHR 1.45, 95% CI 0.95–2.21, $p = 0.085$). The adjusted rate ratio for DGF was estimated using conditional Poisson regression after adjusting for dialysis modality prior to transplant, ischemia time and pre-existing cardiovascular disease. The adjusted hazard ratio for graft failure was estimated using stratified Cox regression after adjusting for DGF, age at transplantation, ethnicity, HLA mismatches and donor kidney side. The adjusted hazard ratio for death was estimated using stratified Cox regression after adjusting for graft failure, age at transplantation, time since first renal replacement therapy, HLA mismatches, pre-existing cardiovascular disease and pre-existing diabetes. aHR, adjusted hazard ratio; aRR, adjusted rate ratio; DGF, delayed graft function; HLA, Human Leukocyte Antigen.

obese ESKD patients who had undergone bariatric surgery [43]. In another retrospective study, bariatric surgery before or after kidney transplantation was reported to be associated with reduced risk of graft failure and mortality compared to control with no bariatric surgery [44]. More data are required to determine if bariatric surgery does improve long-term outcomes from kidney transplantation.

Several limitations should be noted in considering our analysis. First, it is a retrospective registry study that depends on the quality of data captured. Second, the analysis used BMI as the only indicator for categorizing obesity, which does not differentiate between fat and muscle mass, nor between visceral and subcutaneous fat. Other methods such as waist circumference, waist-to-hip ratio, *in vivo* neutron activation analysis (IVNAA), densitometry, deuterium oxide dilution, and dual energy X-ray absorptiometry (DXA) are also available and may enhance specificity. However, such measures are not routinely used in candidate assessment and are not reported to ANZDATA. Third, there may be other potential confounders that are unaccounted for, such as social status, genetic factors, immunosuppression and drug dosing. Fourth, even though significant confounders were adjusted for in the model, residual confounding is still possible. Five, indication of whether dialysis is required after transplantation may vary between centers resulting in potential center effect for DGF which was not accounted for. Six, there may be a loss of statistical power due to pairing. However, we believe that it is important to utilize a matched pair analysis to minimize bias due to donor-related characteristics, such as donor kidney function, hemodynamic instability during organ procurement, use of vasoactive medications and exposure to nephrotoxins, all of which are captured crudely or not at all in registry data. Finally, the study cohort was predominantly Caucasian. The remaining non-Caucasian patient group was heterogeneous, with 40% and 23% of the Indigenous group being Australian Aboriginal and New Zealand Mauri, and 25% and 23% of the Asian group being Indian and Chinese, respectively. Therefore, the comparison between Caucasian and non-Caucasian patients in our study is different from the same comparison in the US where around 70% of non-Caucasian patients were Black/African American [45].

In conclusion, our study demonstrates a relationship between obesity and post-transplant outcomes after carefully controlling for donor-related factors in a paired kidney analysis. Addressing obesity is an unmet clinical need in kidney transplantation. Transplantation is recommended for many obese candidates as it is acknowledged to yield superior outcomes to dialysis. However, design and evaluation of strategies to: (1) optimize transplant management for obese recipients; and (2) reduce the prevalence of obesity among transplant candidates are required.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: The data are not available due to privacy and ethical considerations. Requests to access these datasets should be directed to https://anzdata.org.au/.

AUTHOR CONTRIBUTIONS

BS—prepared the study protocol, acquired the data, designed and conducted the statistical analysis, interpreted the results, drafted the manuscript, edited the manuscript and approved the final version of the manuscript. TY—conceived the study, designed the statistical analysis, interpreted the results, edited the manuscript and approved the final version of the manuscript. JX—prepared the study protocol, acquired the data and approved the final version of the manuscript. KW—interpreted the results, edited the manuscript and approved the final version of the manuscript. JL—conceived the study, designed the statistical analysis, interpreted the results, edited the manuscript and approved the final version of the manuscript. SC—conceived the study, designed the statistical analysis, interpreted the results, edited the manuscript and approved the final version of the manuscript.

CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2023.11107/full#supplementary-material

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ORIGINAL RESEARCH
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Optimizing COVID-19 Vaccination Strategy in Pediatric Kidney Transplant Recipients: Humoral and Cellular Response to SARS-CoV-2 mRNA Vaccination

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In this retrospective cohort study, we analyze the early humoral and cellular response in 64 adolescents KTx recipients, after two or three doses of mRNA vaccine BNT162b2 against different variants of COVID-19. After 2 doses, 77.8% % of children with no history of infection had a positive humoral response with a median anti-S IgG level of 1107 (IQR, 593–2,658) BAU/mL. All the patients with a history of infection responded with a higher median IgG level (3,265 (IQR, 1,492–8,178) BAU/mL). In non-responders after 2 doses, 75% responded after a third dose with a median Ab titer at 355 (IQR, 140–3,865 BAU/mL). Neutralizing activity was significantly lower against the delta and the omicron variants compared to the wild-type strain and did not improve after a 3rd dose, while infection did provide higher levels of neutralizations against the variants. T cell specific response correlated with humoral response and no patient displayed a cellular response without a humoral response. Adolescent KTx recipients exhibit a high seroconversion rate after only two doses. A third injection, induces a response in the majority of the non-responders patients but did not counterbalance the strong decrease in neutralizing antibody activities against variants highlighting the need for boosters with specific vaccines.

Keywords: COVID-19, kidney transplantation, children, pediatric, immunology

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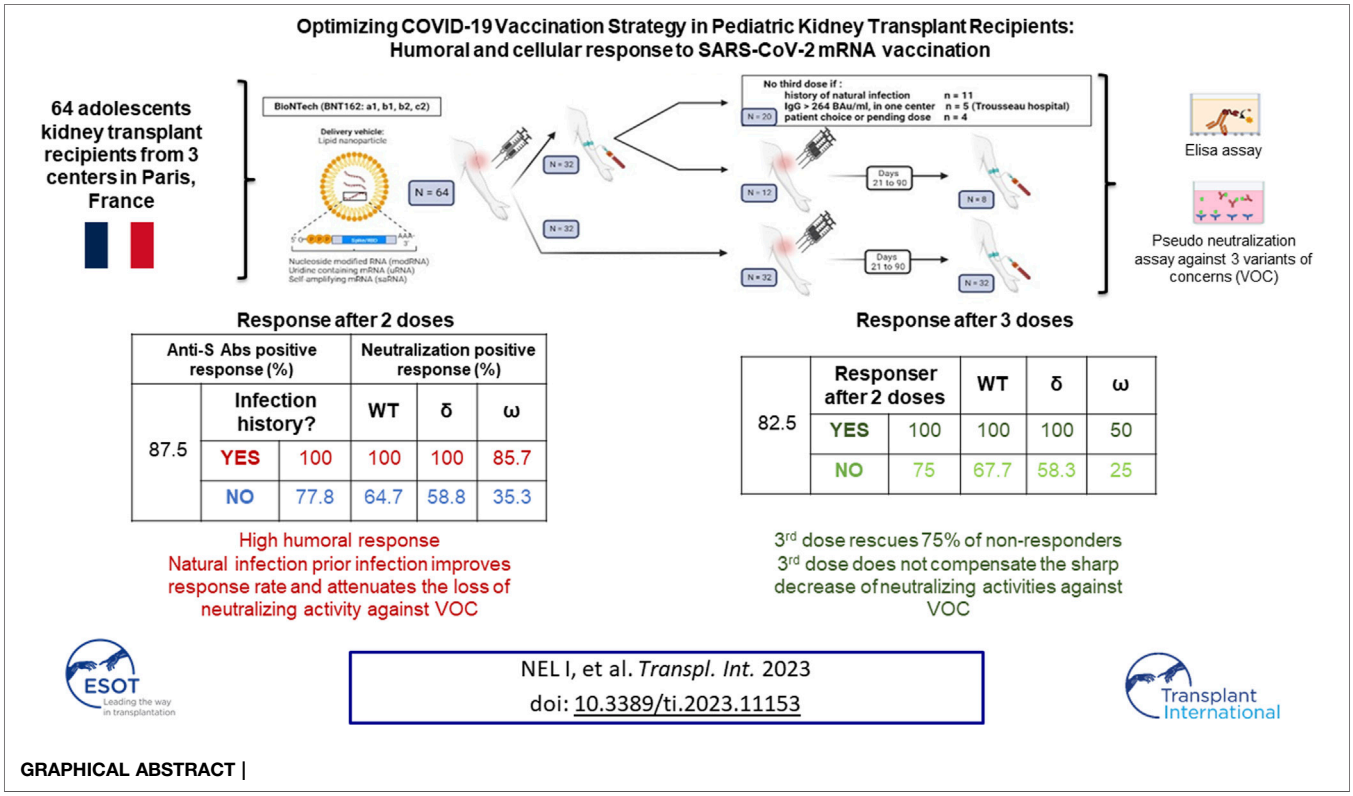
Abbreviations: Anti-S Abs, anti-spike antibodies; CAKUT, congenital anomalies of the kidney and the urinary tracts; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; IgG, immunoglobulin G; kTx, kidney transplant; MMF, mycophenolate mofetil; MPA, mycophenolic acid; PBMC, peripheral blood mono-nucleated cells; SOT, solid organ transplant.



GRAPHICAL ABSTRACT |

INTRODUCTION

Solid organ transplant (SOT) recipients are at risk of severe complication associated with SARS-COV2 infection [1, 2] and vaccination campaigns in many countries prioritized SOT recipients to receive vaccination. Although, the risk of severe SARS-COV2 infection in pediatric SOT recipients is much lower than in their adult counterparts [3–5] providing pediatric SOT with adequate immunization against SARS-COV2 remains essential.

Previous reports demonstrated poor immunogenicity of mRNA vaccines in adult SOT recipients and especially kidney transplant (kTx) recipients with around 50% of the patients developing anti-spike IgG after two injections [6]. Antibody response improved after a third dose with 60%–70% of the recipients developing anti-spike IgG [7, 8]. This prompted health authorities, in some countries, including France to recommend a third dose of vaccine in adult SOT recipients. T-cell response specific to SARS-COV2 was also studied in adults with conflicting results [7, 9].

The results from a phase 3 safety, immunogenicity, and efficacy data for the Pfizer-BioNTech BNT162b2 mRNA COVID-19 vaccine in healthy adolescents were published in May 2021 [10]. In this study including 2,260 participants aged 12–15 years, antibody titers measured after a 2-dose series met non-inferiority criteria compared with 16- to 25-year-old participant and the tolerance of the vaccine was good. Moreover, full vaccination with 2 doses of Pfizer-BioNTech vaccine was associated with a high efficacy of over 90% in healthy adolescents [11]. This led to the approval of this vaccine for children aged 12–15 in the United States and Europe in May 2021. Data on the immunogenicity of mRNA COVID-19 vaccine in pediatric kTx recipients are scarce and divergent. Sattler et al. reported data on 20 pediatric kTx recipients and found positive antibody titers in 90% of the patients after two doses of BNT162b2 mRNA COVID-19, with 75% developing neutralizing titers against vaccine variant [12]. Another report in older adolescent with kTx reported only 52% of anti-spike IgG after two injections, similar to the results in the adult population [13]. Moreover, there are currently few data on the response to a third dose of mRNA COVID-19 vaccine in pediatric SOT recipients or on SARS-COV2 T-cell specific response following vaccination. These data, but also the neutralizing antibody response against VOC, are needed to assess the optimal vaccination strategy in this population. In this study, we report the immunogenicity of BNT162b2 mRNA by studying humoral response and specific T cells following two or three injections of PfizerBioNTech BNT162b2 mRNA COVID-19 vaccine in pediatric kTx recipients.

MATERIAL AND METHODS

Patients

We included all kTx recipients aged over 12 years old followed in one of the three Pediatric Nephrology Departments in Paris

(Robert Debré Hospital, Necker Hospital and Trousseau Hospital) who were vaccinated against SARS-CoV-2 with the Pfizer SARS-CoV-2 mRNA BNT162b2 vaccine between 30 January 2021 and 21 December 2021. French health authorities approved vaccination in children with comorbidities more than 16 years old on 20 January 2021 and extended it to children aged 12–15 years old on 01 June 2021. Specific guidelines in adult patients with SOT recommended three injections of mRNA vaccine but no specific pediatric guidelines were available. Therefore, the vaccination strategy was left to the treating physician's decision with some performing three injections systematically and others only in patients with low anti-S IgG 1 month after the second injection. Patients with a proven (positive SARS-COV2 PCR or home-antigen test) natural infection prior to vaccination only received 2 doses of vaccine (**Figure 1A**). All centers evaluated patients' humoral and cellular responses. Blood samples were collected between 21 and 90 days after vaccine injection and processed immediately in a centralized laboratory (Immunology department, Robert Debré Hospital). Clinical and biological data were collected retrospectively. In order to analyze the effect of COVID-19 infection on vaccination, patients were considered as having had an infection if they had a positive PCR, a positive anti-N serology or a positive anti-N T-cell response. The study was approved by Robert Debré Hospital Ethics committee.

Measurement of Plasma Anti-Spike and Anti-Nucleocapsid SARS-CoV-2 Antibodies

Anti-Spike SARS-CoV-2 antibody levels were evaluated by chemiluminescent immunoassay in plasma using the LIAISON® SARS-CoV-2 TrimericS IgG kit according to manufacturer recommendations (Diasorin®). A serological positive response was defined as anti-S IgG response >33.8 BAU/mL. We also present results based on a higher cut-off 264 BAU/mL. This Ab level was found associated with 80% of vaccine efficacy against primary symptomatic COVID-19 (264 BAU/mL) in previous studies [14]. In some patients, serological response after the 2nd vaccine injection was tested in an outside laboratory. These results were collected and used to classify patients in responders and non-responders, but these patients were excluded from the comparison of Ab titers. Anti-Nucleocapsid SARS-CoV-2 IgG levels were evaluated by chemiluminescent immunoassay in plasma using the Alinity I® anti-N SARS-CoV-2 IgG kit according to manufacturer recommendations (Abbott®). A serological positive response was defined as anti-N IgG index response >1.4 (**Figure 1B**).

Measurement of Neutralizing Antibody Activity Against SARS-CoV-2 Strains

Neutralizing antibodies were quantified using the GenScript® SARS-CoV-2 surrogate Virus Neutralization Test (sVNT). Briefly, the kit detects the ability of antibodies in the plasma of patients to block the interaction between the HRP-conjugated SARS-CoV-2 RBD fragment (HRP-RBD) and the human ACE2 protein (hACE2). Circulating neutralizing antibodies form with HRP-RBD a complex that get removed during washing. Unbound HRP-RBD is captured on a hACE2 pre-coated plate and reacts with the added TMB by changing the color of the solution. The absorbance is inversely dependent on the level of cirulating neutralizing antibodies. HRB-RBD protein used in the kit is selected based on the strain of SARS-CoV-2 tested (wild-type, Delta or Omicron). A positive neutralizing antibody activity is defined as more than 30% according to manufacturer recommendations. Serum from one of the 32 kTx children followed after two vaccine doses was not available for measuring the neutralizing antibody response against SARS-CoV-2 strains (**Figure 1B**).

Quantification of Anti-Spike and Anti-Nucleocapsid SARS-CoV-2 T Cells

Human peripheral blood mono-nucleated cells (PBMCs) were isolated from fresh blood samples by density gradient centrifugation (Leucosep) to obtain a final cell concentration of 2.5 10^6 PBMCs/mL in AIM-V-Medium (Ficher Scientific, Suisse). Anti-Spike and anti-Nucleocapsid cellular responses were evaluated on fresh cells using the T-spot COVID ELIspot system from Oxford Immunotec® (United Kingdom). Briefly, 50 µL of PHA (positive control), AIM-V-Medium (negative control), Spike-antigen mix and Nucleocapsid-antigen mix were added in wells of anti-IFNγ Abs pre-coated plate. 100 µL of the diluted cell suspension were added in each well and the plate was incubated for 16–20 h at 37°C, 5% CO_2. After discarding supernatant, wells were washed three times with PBS. Afterwards, 50 µL of conjugate reagent (anti-IFNγ Abs, conjugated to alkaline phosphatase) were added to each well and incubated for 1 hour at 4°C in the dark. After washing, 50 µL of substrate solution was added to each well at room temperature for 7 min leading to form insoluble precipitate at the site of reaction. Then, the plate was washed and dried. T-spots were counted by an Elispot-reader (Bioreader® 6000-E Biosys, Germany) and results expressed as SFC (Spot Forming Cell)/ 250,000 PBMCs. Results were positive for a specific antigen if negative control was ≤10 spots, positive control ≥20 spots and the Antigen-Mix >4 spots per well (according to manufacturer recommendations) (**Figure 1B**).

Statistical Analysis

Patient characteristics and immunological response to vaccine are presented as medians and interquartile ranges for continuous variables, and counts and percent for categorical variables. Chi-square test and Mann-Whitney U test were used to compare characteristics between the groups for categorical and continuous variables, respectively. A significant statistical difference was assumed when the p-value was <0.05. All analyses were conducted using GraphPad PRISM version 5.00.288 (GraphPad Software Inc., San Diego, CA, United States) and SAS 9.1. The reporting of the data followed the STROBE statement.

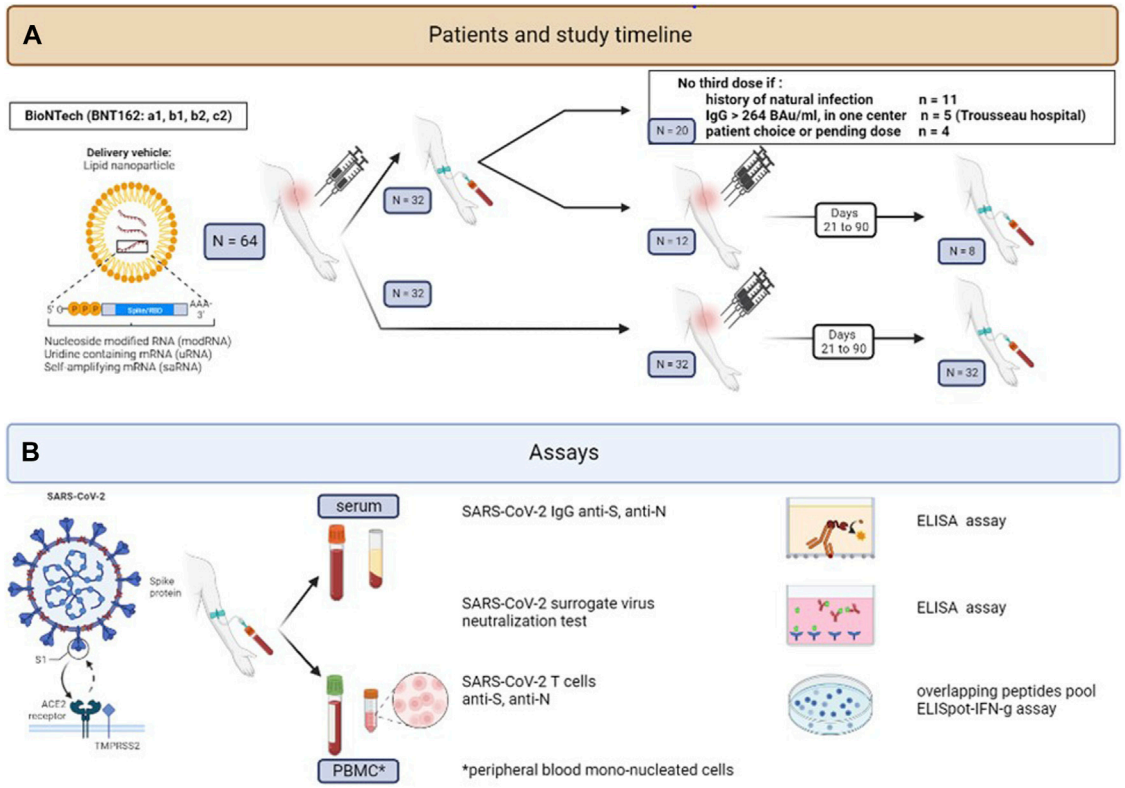


FIGURE 1 | Study design: Study population flow-chart **(A)** and immunological assays **(B)**.

RESULTS

Patients' Characteristics

Table 1 describes patients' and transplants' characteristics at the time of the first vaccine injection. 64 patients aged 16.9 years (14.9; 17.6) were included, 49 of whom received their transplant from a deceased donor (76.5%). Patients were 56% male; the first causes of ESKD were urological abnormalities in 31.2%, hereditary nephropathies in 37% and glomerular diseases in 19% of the patients. Fifteen patients were transplanted preemptively and 49 patients were transplanted after a median time on dialysis of 1.56 years (1.11; 2.69) (37 in hemodialysis and 12 in peritoneal dialysis). In maintenance oral immunosuppressive treatment, 36 (56%) patients received an association of tacrolimus with mycophenolate mofetil (MMF) or mycophenolic acid (MPA), 18 (28.1%) tacrolimus with azathioprine and 35 (54.7%) with low doses steroids (median 5 mg) as a third immunosuppressive treatment. At first vaccine, 8 patients had lymphopenia <1,500/mm^3, 3 had hypogammaglobulinemia < 5 g/L and 26 (40%) had eGFR <60 mL/min.

All patients were vaccinated with the Pfizer SARS-CoV-2 mRNA BNT162b2 vaccine. Twenty patients received only 2 doses of vaccine including 11 with a known previous natural infection with SARS-CoV-2 (median time from infection to first vaccine injection 132 days IQR [81; 291], and 44 (62.5%) patients without known history of natural infection received three doses (**Figure 1B**).

Anti-Spike Antibody Levels in kTx Children After 2 or 3 BNT162b2 Vaccine Doses

Anti-spike IgG antibodies (anti-S Abs) were quantified in the plasma from 32 kTx children after two doses of vaccine (**Figure 2A**). 87.5% (28/32) had a positive response (defined as ≥33.8 BAU/mL according to the manufacturer) with a median antibody titer in responders at 1825 (IQR, 637–4,883) BAU/mL. The majority (81.3%, 26/32) had an antibody titer above 264 BAU/mL, Ab levels associated with 80% of vaccine efficacy [14]. KTx children after two vaccine doses were then classified according to their history of natural infection; based either on positive SARS-COV2 PCR or home-antigen test, or on positive anti-N humoral or cellular response. Fourteen of the 32 kTx children evaluated had a history of natural SARS CoV-2 infection before their 2nd vaccine dose and all of them (14/14) had a positive humoral response above the 264 BAU/mL cut-off after vaccination. In comparison, only 77.8% (14/18) of children without previous natural infection had a positive humoral response (p = 0.059) and 66.7% (12/18) reaching the 264 BAU/mL cut-off (p = 0.017). Among responders, anti-S antibody titers were significantly higher in children with natural infection [median: 3,265 (IQR, 1,492–8,178) BAU/mL]

TABLE 1 | Patients' and transplants' characteristic at the time of the first Pfizer SARS-CoV-2 mRNA BNT162b2 vaccine.

Patients' characteristics at first vaccine	Patients N = 64
Age (years), median (IQR)	16.9 (14.9; 17.6)
Male, *n* (%)	36 (56)
Primary renal diseases, *n* (%)	
CAKUT	20 (31.2)
Hereditary nephropathy	26 (37)
Glomerulonephritis and immunological diseases	12 (19)
Other	6 (9.3)
Donor type: Deceased donor, *n* (%)	49 (76.5)
Time from transplantation to vaccination (years), median (IQR)	3.8 (1.8; 8.3)
KRT before transplantation, *n* (%)	
Preemptive transplantation	15 (23.4)
Hemodialysis	37 (57.8)
Peritoneal dialysis	12 (18.7)
Induction treatment, *n* (%)	
Anti-thymocyte globulins	13 (20.3)
Anti-CD25	49 (76.5)
Maintenance immunosuppression, *n* (%)	
Tacrolimus	58 (90.5)
MMF/MPA	40 (62.5)
Azathioprine	19 (29.7)
Steroids	35 (54.7)
Steroid dose (Median)	5
mTOR inhibitors	3 (4.7)
Belatacept	1 (1.5)
Known history of natural infection SARS-CoV-2, *n* (%)	9 (14)
Biological data at first vaccine	
Lymphocytes (G/L), median (IQR)	2.3 (1.8; 2.9)
Lymphopenia <1,500/mm^3, *n* (%)	8 (12.5)
IgG (G/L), median (IQR)	9.4 (8.3; 12.3)
eGFR (mL/min/1.73 m^2, Schwartz 2009 equation), median (IQR)	66 (57; 81)
Tacrolimus trough levels (ng/mL), median (IQR)	5.5 (4.2; 6.8)

than in children without previous natural infection [median: 1,107 (IQR, 593–2,658) BAU/mL] (p = 0.007).

Anti-S Abs were quantified in the plasma for 40 kTx children after three vaccine doses (median time between 3rd dose and serology 39 days IQR (28; 72) (**Figure 1A**). Serological responses after the second dose of vaccine were determined only for 20 children (for 12 children in a laboratory that provides only qualitative results and for 8 in our central laboratory). Twenty children were only tested after their third dose (**Figure 1A**). Children were classified in three groups according to their humoral response to the 2nd vaccine dose: Responder for positive response, Non-responder for negative response and Unclassified (NC) for children with missing data. As expected, all (100%, 8/8) responders presented a positive humoral response after their 3rd vaccine dose, with seven (87.5%) achieving Ab levels above the 264 BAU/mL cut-off. Median antibody titer after the 3rd dose in these patients was 1805 (IQR, 783–2,485) BAU/mL. In comparison, the 3rd vaccine dose led to a positive humoral response in 75% (9/12) of non-responders with a lower median Ab titer in responders at 355 (IQR, 140–3,865 BAU/mL) (p = 0.028) and only 41.7% of them reaching the 264 BAU/mL cut-off (**Figure 2B**).

As mentioned above, eight kTx children were tested centrally after the second and the third doses of vaccine including 3 patients with no response after the 2nd dose (**Figure 2C**). The median antibody titers increased from 159 (IQR, 5–1,458) BAU/mL after 2 vaccine doses to 1,150 (IQR, 201–2,108) BAU/mL after 3 vaccine doses (p = 0.085). Ab level above the 264 BAU/mL cut-off were achieved respectively for 5/8 (62.5%) of children after the 2nd dose and 6/8 (75%) children after the 3rd dose. Interestingly, two of the 3 non responders after the 2nd dose developed a positive humoral response after their 3rd vaccine dose, while responders did not show a significant increase in their anti-S IgG titers after the 3rd injection.

Neutralizing Antibody Response Against Wild-Type, Delta and Omicron SARS-CoV-2 Strains in kTx Children After Two or Three BNT162b2 Vaccine Doses

Neutralizing antibodies against wild-type, Delta and Omicron strains were quantified using a SARS-CoV-2 pseudo-neutralization Antibody Detection kit (ELISA) [15] in the serum from 31 kTx children after two doses of vaccine (**Figure 3A**). The positive response (defined as ≥30% according to the manufacturer) against wild-type, Delta and Omicron strains was respectively achieved for 25/31 (80.6%), 24/31 (77.4%) and 18/31 (58.0%) of kTx children. The median neutralizing antibody activity decreased from 95.3% (IQR, 91–96.9) against the wild-type strain to 69.2% (IQR, 53.8–93.5) against Omicron (p = 0.003). Among children with a positive neutralizing antibody response, 72% of them had antibodies able to neutralize the three SARS-CoV-2 strains, 24% both the wild-type strain and Delta, and 4% the wild-type strain only (data not shown). All the children with a history of natural SARS-CoV-2 infection before their 2nd vaccine dose (14/14) had a positive neutralizing antibody response against the wild-type strain (**Figure 3A**, right panel). In comparison, only 64.7% (11/17) of children without previous natural infection had a positive neutralizing response (p = 0.008). In agreement, median of neutralizing antibody activity against wild-type strain was significantly stronger in children with natural infection [95.9% (IQR, 93.5–97.3)] than in children without previous natural infection [90.2% (IQR, −0.4–95.9)] (p = 0.008). Similar significant differences were observed for neutralizing antibody activity against Delta (p = 0.0026) and Omicron strains (p = 0.0002). It is however interesting to note that 12/14 (85.7%) of children with natural infection maintained a neutralizing response against Omicron instead of 6/17 (35.3%) of children without previous natural infection.

Neutralizing antibodies against wild-type, Delta and Omicron strains were quantified in the serum for 40 kTx children after three vaccine doses (**Figure 3B**). 75% (30/40) had a positive response against the wild-type strain. The percentage of children with positive response decreased with variants, reaching 40% (16/40) of children with Omicron (p = 0.0015). Similarly, the median neutralizing antibody activity decreased form 93.4% (60.9–96.7) against the wild-type strain to 69.6% (41.8–85.5) against Omicron (p = 0.0003). All (100%, 8/8) children with humoral response to the 2nd vaccine dose presented a positive neutralizing response

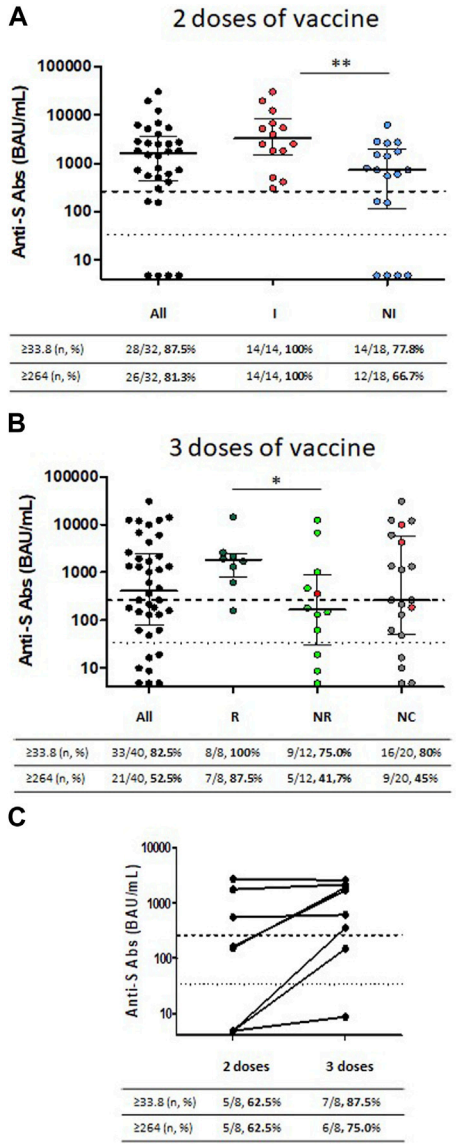


FIGURE 2 | Antibody response to SARS-CoV-2 following two or three injections of SARS-CoV-2 mRNA vaccine in KTx children. **(A)** Titers of anti-S IgG are shown in 32 KTx children following two injections of SARS-CoV-2 mRNA vaccine. Children are classified according to their history of previous natural SARS-CoV-2 infection before the second injection: with natural infection (I) or without natural infection (NI). **(B)** Antibody titers to SARS-CoV-2 following three injections of SARS-CoV-2 mRNA vaccine in 40 KTx children classified according to their humoral response following the second vaccine injection: Responder (R) for positive response, Non-responder (NR) for negative response and Unclassified (NC) for children with missing data. **(C)** Matched SARS-CoV-2 antibody titers for eight patients following the second and third vaccine doses. Dashed horizontal lines represent the threshold of positive humoral response (33.8 BAU/mL) and the ab level associated with 80% of vaccine efficacy against primary symptomatic COVID-19 (264 BAU/mL). Medians and interquartile ranges are shown.

after their 3rd vaccine dose against the wild-type strain with the median of neutralizing activity at 94.5% (IQR, 75.3–96.9) (**Figure 3A**, right panel). In comparison, the 3rd vaccine dose led to a neutralizing activity in 67.7% (8/12) of children classified non-responders after the 2nd dose, with a lower median at 73.7% (IQR, 42.7–95.4) ($p = 0.018$). Frequency of positive neutralizing response and median neutralizing activity levels decreased from the wild-type strain to Delta, then to Omicron. Only 4/8 (50%) of responders and 3/12 (25%) of non-responders presented a positive neutralizing response against Omicron after their third dose.

As mentioned above, only eight kTx children were centrally evaluated after both the second and the third doses. Among five patients with no neutralizing activity against the wild-type strain after the 2nd dose (**Figure 3C**), two developed a positive response after their 3rd vaccine dose. The median antibody titers increased from 3.2 (IQR, −5.8–85.8) % after 2 vaccine doses to 89.5 (IQR, 21.6–96.7) %L after 3 vaccine doses ($p = 0.085$). Interestingly, the neutralizing activity against Omicron was not improved after the 3rd injection.

Correlation Between Anti-Spike Antibody Levels and Neutralizing Antibody Responses Against Wild-Type, Delta and Omicron SARS-CoV-2 Strains in kTx Children After Two or Three BNT162b2 Vaccine Doses

Anti-S antibody levels strongly correlated with neutralizing antibody activity for the wild-type strain (**Figure 4A**), Delta (**Figure 4B**) and Omicron (**Figure 4C**). However, the percentage of children with a positive anti-S antibody response (≥33.8 BAU/mL) without a positive neutralizing activity (cut off 30%) increased from 6/71 for wild-type strain, to 11/71 for Delta, then 27/71 for Omicron. Interestingly, level of anti-S antibody in children without neutralizing activity against the wild type strain were all below the 264 BAU/mL cut-off associated with 80% of vaccine efficacy [14]. Conversely, some patients with high anti-S antibody titers showed no neutralizing activity against the VOCs despite antibody titers up to 736 and 1,690 BAU/mL for delta and omicron variants, respectively.

Specific Memory T Cells in kTx Children After Two or Three BNT162b2 Vaccine Doses

Spike-specific T cells were quantified in 28 kTx children after two vaccine doses and in 23 kTx children after three vaccine doses. Specific spike memory T cell were observed with a median of 6.5 (IQR, 2.0–32) SFC in children after two doses of vaccine. Medians of Spike-specific T cell response were, respectively, 13.5 (IQR, 2.3–51.8) SFC in the 12 children with previous natural infection, and 5 (IQR, 1–8.8) SFC in the 16 children without previous natural infection. Specific memory T cell were observed with a median of 7 (IQR, 1.0–23) SFC in children after three doses of vaccine. Median of Spike-specific T cell response tended to be higher in children with a humoral response after 2 doses (10 (IQR, 1–36) SFC) as compared to non-responders (3 (IQR, 1.5–145.5) SFC). Interestingly, children with the higher level of Spike-specific T cell response in the non-responder group had a

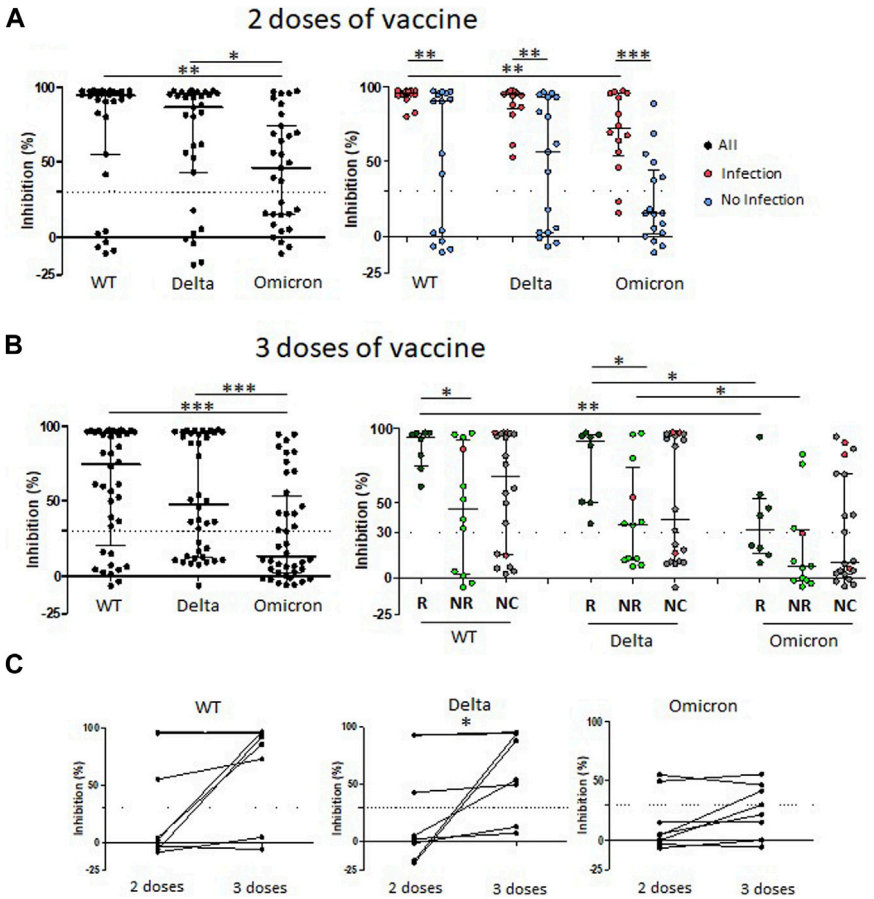


FIGURE 3 | Neutralizing antibody responses against wild-type, Delta and Omicron SARS-CoV-2 strains following two or three injections of SARS-CoV-2 mRNA vaccine in KTx children. **(A)** Neutralizing antibody activities against SARS-CoV-2 strains following two injections of SARS-CoV-2 mRNA vaccine classified according to their previous natural SARS-CoV-2 infection before the second injection: with natural infection (I) or without natural infection (NI). **(B)** Neutralizing antibody activities against SARS-CoV-2 strains following three injections of SARS-CoV-2 mRNA vaccine. Children are classified according to their humoral response following the second vaccine injection: Responder (R) for positive response, Non-responder (NR) for negative response and Unclassified (NC) for children with missing data. **(C)** Matched SARS-CoV-2 antibody neutralizing activities against SARS-CoV-2 strains for eight KTx children following the second and third vaccine doses. Dashed horizontal lines represent the threshold of positive neutralizing antibody response (30%). Medians and interquartile ranges are shown. Anti-SARS-CoV-2 neutralizing antibody activities were evaluated against the wild-type (WT) strain and the Delta and Omicron variants. Medians and interquartile ranges are shown.

natural infection between the 2nd and the third dose (**Figures 5A,B**).

Among 51 patients tested for both anti-Spike humoral and Spike-specific T cell responses, none had a spike-specific cellular response without a positive humoral response, whereas 9/51 had a positive humoral response without spike-specific cellular response detected (**Figure 5C**).

DISCUSSION

In this study, we confirm a higher humoral response rate after two injections of Pfizer SARS-CoV-2 mRNA BNT162b2 vaccine in pediatric kTx recipient (>80%) as compared to the rates reported in adult kTx recipients. We also demonstrate that a third dose of vaccine is able to induce a humoral response in 75% of the children that did not respond after two injections. Moreover, natural infection prior to vaccination significantly improves response rate since all patients with prior infection have stronger humoral responses and neutralizing antibody activities after two injections. Antibodies developed in kTx children with natural infection exhibit a lower loss of neutralizing activity against VOC than in kTx children without infection. Conversely, in responders after two doses, an additional dose of vaccine does not compensate for the sharp decrease in antibody neutralizing activities against VOC. Our data also highlight stronger discrepancies between anti-S IgG levels and neutralizing antibody activities for VOC. Finally, despite immunosuppressive therapy affecting the proliferative and/or effector functions of peripheral T cells, a significant number of kTx children developed anti-S specific T cell response after vaccination.

The high humoral response rate (85%) found in our cohort of pediatric kTx recipients is consistent with the publication of

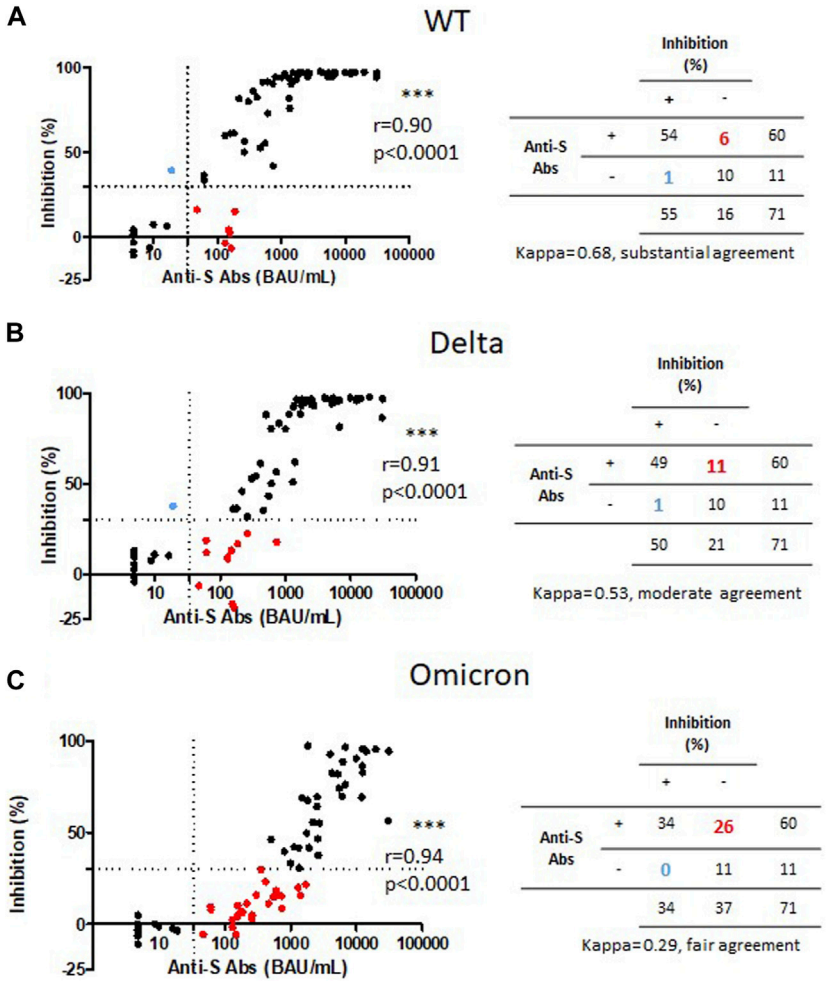


FIGURE 4 | Correlation of anti-S IgG titers and neutralizing antibody activities against the wild-type strain, Delta and Omicron variants of SARS-CoV-2 in KTx children. Correlation of anti-S IgG titers and neutralizing antibody activities against the wild-type strain (WT) **(A)**, Delta **(B)** and Omicron **(C)** strain in 71 KTx children (31 following two injections and 40 following three injections). Dashed lines represent the threshold of positive humoral response (33.8 BAU/mL) and the threshold of positive neutralizing antibody response (30%).

Sattler et al. which report a 90% rate of seroconversion after two doses of BNT162b2 mRNA COVID-19 vaccine among adolescent kTx recipients [16]. These rates are much higher than those reported among adult kTx recipients with around 40% of patients developing anti-spike IgG after two injections [17, 18]. Similarly, Crane et al. and Kermond et al. described lower seroconversion rates, respectively 56% and 50% in adolescent kTx recipients, with a response rate affected by the use of mycophenolate and prednisolone [13, 19]. Individual susceptibility to treatment or differences in treatment regimens may explain the observed discrepancies. As in Sattler et al. report, we observed that approximately 80% of kTx adolescents developed neutralizing antibody against the wild-type strain after two doses of vaccine. Interestingly, we also analyzed the protective responses against variants and showed that neutralizing activity of antibodies decreased with the increasing variability of VOC. Such observation is also described in adult kTx recipients and to a lesser extend in healthy adult donors [18].

The interpretation of low levels of anti-S IgG is already a challenge to predict a clinical protection against the wild-type strain. In our study, the six patients having a positive anti-S IgG response without neutralizing antibody activity, presented anti-S IgG levels below 264 BAU/mL. This cut-off, initially described with the alpha SARS-CoV-2 strain, was found associated with 80% of vaccine efficacy against primary symptomatic COVID-19 in previous studies [14]. Given the major discrepancies between anti-S IgG levels and neutralizing antibody activities against VOC, our results suggest that positivity threshold determined based on data with the alpha strain may not be applicable to other variants. Therefore, the development of assays and/or thresholds specifically designed for new VOC or the assessment of the neutralizing activity against VOC, which will become more accessible with the development of pseudoneutralization test,

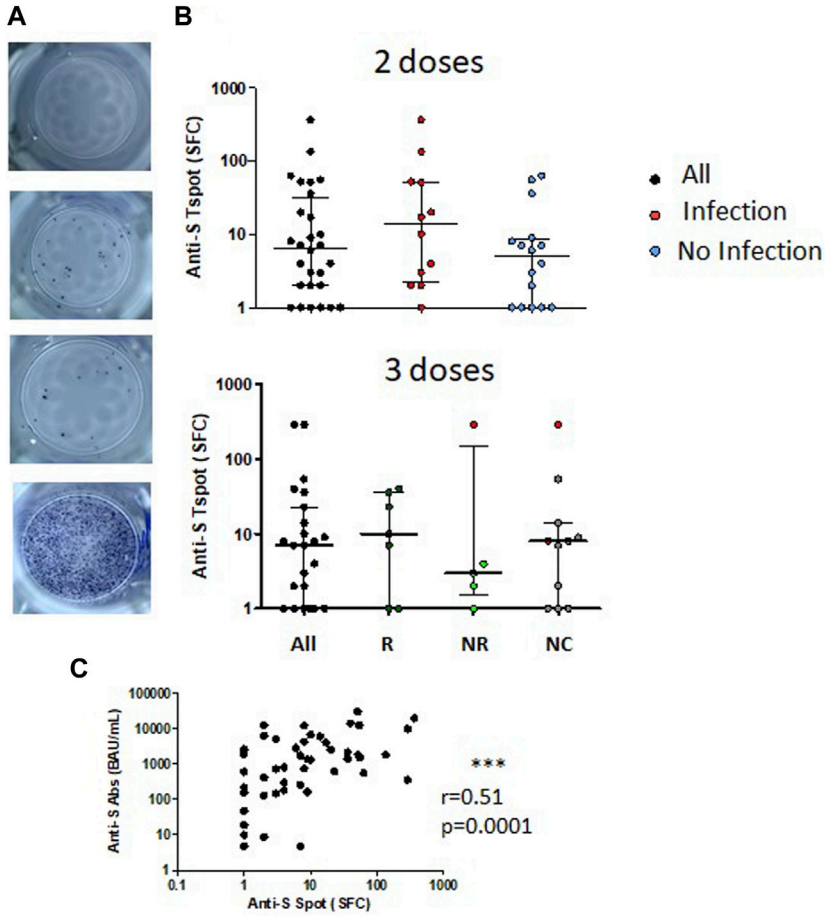


FIGURE 5 | Spike specific T cellular response following two or three injections of SARS-CoV-2 mRNA vaccine in KTx children. **(A)** SARS-CoV-2 specific T cell test results from top to bottom: negative control, anti-S T cell response, anti-N T cell response and positive control. **(B)** Numbers of anti-S T cells are shown in 28 KTx children following two injections of SARS-CoV-2 mRNA vaccine. Children are classified according to their previous natural SARS-CoV-2 infection before the second injection: with natural infection (I) or without natural infection (NI). Numbers of anti-S T cells following three injections of SARS-CoV-2 mRNA vaccine in 23 KTx children classified according to their humoral response following the second vaccine injection: Responder (R) for positive response, Non-responder (NR) for negative response and Unclassified (NC) for children with missing data. Medians and interquartile ranges are shown. **(C)** Correlation of anti-S humoral response and anti-S T cell response in 51 KTx children (28 KTx following two injections and 23 following three injections).

would be more reliable to evaluate clinical protection against VOC.

We also showed that all patients with a history of COVID-19 infection before vaccination had higher anti-spike antibody titers and neutralizing antibody activities after vaccination than patients without infection. Moreover, they also maintained a better ability to neutralize the Omicron variant compared to patients without infection. Among adult kTx recipients, Magicova et al. reported a major difference in the seroconversion rates between patients with (97%) and patients without (40%) previous infection [20]. Other reports support an improved response to vaccination in infected patients, both in terms of anti-SARS-CoV-2 Ig levels and antibody neutralizing activities, with a better and more sustained clinical protection against new variants [21–23]. Along with our results, this suggests that two injections may be sufficient for the initial vaccination of kTX recipients with a history of COVID-19 infection. However, longitudinal data on the sustainability of the humoral response after various vaccination protocols and after infection are needed to make a definitive conclusion on the best vaccination protocol in these patients.

The analysis of the group of patients receiving three doses demonstrated that a third dose induced a serological response in 75% of the non-responders after two doses. This again contrasts with previous studies in adults reporting much lower rates of seroconversion after a third dose (between 38% [7] and 44% [8]). More importantly, 42% of the non-responders after two doses presented after their third dose of vaccine antibody titers expected to provide an effective protection against severe COVID-19 infections (264 BAU/mL), in line with recent publications suggesting the benefit of a third SARS-CoV-2 vaccine for antibody response in adolescent with kTx [13, 19]. Whether this immunity will last over time and remain effective despite virus variability has to be demonstrated. Indeed, we showed that responders after two doses presented a high neutralizing antibody response against wild-type strain of SARS-CoV-2 but that half of them did not display neutralizing activity against the Omicron

stain. Unfortunately, an additional dose of Pfizer SARS-CoV-2 mRNA BNT162b2 vaccine did not overcome the loss of neutralizing activity due to higher virus variability and support the need for new vaccines specific for the variants.

We also assessed T cell specific immunity against SARS-CoV-2. Early in the pandemic, T cell response to mRNA COVID-19 vaccine received a lot of attention. However, only few data on T cell response are available in pediatric kTx recipients. Sattler et al. reported the same frequency of anti-S CD4 T cell in adolescents kTx recipients than in healthy adolescents after vaccination [12]. In our study, despite their immunosuppressive treatment, around 50% of kTx children developed anti-S specific T cell response, after two or three doses of vaccine. Among adult KTx recipients, cell response rates after three doses of vaccine greatly varied from 13% to 85% according to studies [7, 9, 24, 25]. Interestingly, half of kTx children with discrepancies between a positive anti-S humoral response and no neutralizing antibody activities against omicron, developed anti-S specific T cell response (data not shown). The discrepancies between neutralizing activity and T cell response has already been described by Fernandez-Ruiz et al. against the wild-type strain. In their study, the presence of T cell response without any neutralizing antibody activity is described in 13% of patients [26]. The importance of the specific T cell response after COVID-19 vaccination or infection is supported by the demonstration that T cell response is maintained even against variant of concerns (VOCs) [27]. This is indeed of great importance given the impaired neutralizing activity against emerging VOCs in seroconverted kidney transplant recipients after vaccination. This may provide some persistent protection against severe cases of COVID-19 despite substantial loss of neutralizing antibody activity [28].

Altogether, our results show that 1) Pediatric kidney transplant recipients have a high humoral response rate after two injections of Pfizer SARS-CoV-2 mRNA BNT162b2 vaccine, 2) the assessment of the humoral response after two injections is of interest to detect non-responders and perform a third injection, which will induce a response in the majority of the patients, 3) a supplementary vaccine dose did not counterbalance the strong decrease in neutralizing antibody activities against VOC highlighting the need for vaccines against new VOC. Further studies are however needed to assess the impact of the various vaccination strategies and the use of vaccine against new VOC on the maintenance of the immune response.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Robert Debré Hospital Ethics committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

Study design: CP, GC, and JH. Data collection and immunological testing: CP, IN, LD, CDu, CDe, MC, VB, PB, AC, EC, VG-E, and MN. Data analysis: CP, IN, GC, CDu, and JH. Writing of the manuscript: CP, IN, GC, and JH. All authors reviewed the manuscript and approved its final version. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

CONFLICT OF INTEREST

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

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ORIGINAL RESEARCH
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Normothermic *Ex Vivo* Machine Perfusion of Discarded Human Pancreas Allografts: A Feasibility Study

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Pancreas transplantation is the only curative treatment for patients with complicated diabetes, and organ shortage is a common and increasing problem. Strategies to expand the donor pool are needed, and normothermic *ex vivo* perfusion of the pancreas has the potential to test and repair grafts before implantation. Between January 2021 and April 2022, six human pancreases, declined for transplantation or islet isolation, were perfused using a previously established method by our group. All 6 cases were successfully perfused for 4 h, with minimal edema. The mean age of the donors was 44.16 ± 13.8 years. Five grafts were obtained from neurological death donors, and one was obtained from a donation after cardiac death. The mean glucose and lactate levels decreased throughout perfusion and insulin levels increased. All 6 grafts were metabolically active during perfusion and histopathology showed minimal tissue injury and no edema. Human normothermic *ex vivo* perfusion of the pancreas is feasible and safe and has the potential to expand the donor pool. Future studies will focus on tests and biomarkers for the assessment of grafts.

Keywords: pancreas transplantation, perfusion, normothermic machine perfusion, diabetes, human pancreas allografts

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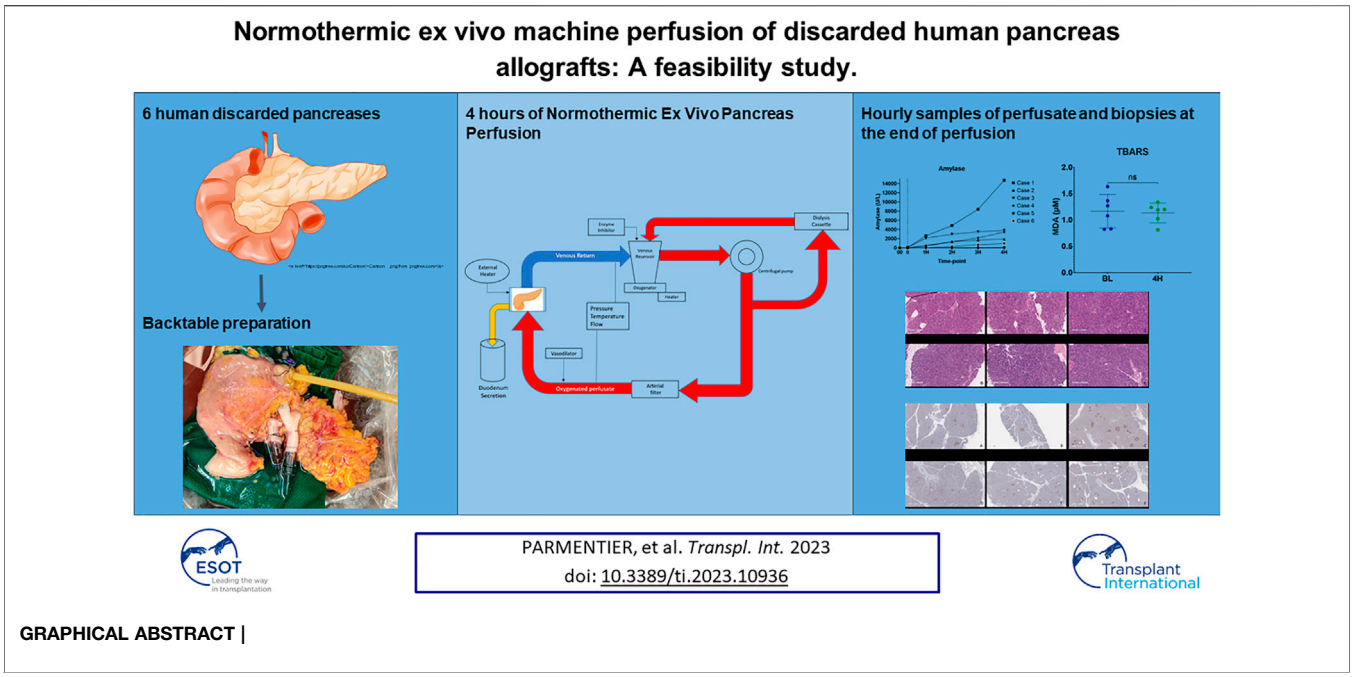
INTRODUCTION

Despite advances in insulin pump technologies and therapies, pancreas transplantation (PTx) is still the only curative treatment for patients with diabetes [1]. Historically, PTx either simultaneous (SPK) or pancreas after kidney (PAK) has been performed in patients with type 1 diabetes mellitus and concomitant kidney disease requiring a renal transplant. In select cases, pancreas transplant alone (PTA) has been performed in patients with life-threatening hypoglycemic unawareness. More recently the indications for PTx have been expanded and performed in patients with a diagnosis of type 2 diabetes mellitus with comparable results [2–4]. Broadening of the acceptance criteria for recipients has led to an increasing need for suitable pancreas grafts. However, the pool of suitable pancreas donors has remained largely stagnant [5].

There is a significant benefit to recipients of pancreas allografts demonstrating improvement in both quality of life and life expectancy [6, 7]. Although PTx does not reverse complications associated



GRAPHICAL ABSTRACT |

with diabetes, it has been shown to decrease the predicted cardiovascular risk by more than two-thirds at 5 and 10 years [1, 8].

Even with an increasing need for pancreas grafts, donor selection continues to be very restrictive, and the conversion rate from donation to transplant remains low [9]. In addition, the pancreas continues to be the most discarded organ. In Canada, in 2019, out of a total of 820 deceased donors, only 68 pancreases were transplanted [10]. In the UK, only 1/3 of accepted pancreases are transplanted [9, 11]. Not surprisingly, the number of PTx performed is considerably lower as compared to kidney, liver, heart, and lung [9, 12]. Strategies that will allow for the assessment and repair of pancreas allografts have the potential to reverse this trend in pancreas donation. Normothermic *ex vivo* machine perfusion (NEVPP) has been successfully used for the preservation of liver [13, 14], kidney [15, 16], heart [17], and lung allografts [18] but has only scarcely been studied for the pancreas.

Earlier studies of NEVPP have been limited as grafts develop severe edema and tissue injury [19, 20]. However, our previous work in a porcine model demonstrated that edema can be mitigated and that these grafts can be successfully transplanted after 3 h of perfusion [21]. The purpose of this study was to prove the feasibility and safety of this method in human allografts.

MATERIAL AND METHODS

Between January 2021 and April 2022, we received 7 human pancreas allografts recovered from multiorgan donors in Ontario, Canada. These grafts were declined for pancreas transplantation and islet cell isolation but donated for research purposes. The study was approved by the medical ethical committee of the Toronto General Hospital (Approval number: 20-5733). The allografts were retrieved by the multiorgan procurement team at Toronto General Hospital. All allografts were flushed and stored in University of Wisconsin (UW) preservation solution (Bridge to Life, London, United Kingdom). One of the grafts had to be discarded because of technical issues (heating pump failure) during the perfusion that did not allow for appropriate data collection.

Allograft Preparation

Recovered pancreas allografts were prepared for NEVPP utilizing a backtable preparation typical for human pancreas implantation. Briefly, the organ was inspected for any significant damage that would affect the perfusion. The spleen was removed by ligating the splenic artery and vein close to the hilum of the spleen. Iliac vessels were recovered from the donor and any small branches were suture ligated. Arterial reconstruction was performed using the donor iliac artery as a "Y graft." The external iliac artery and internal iliac artery were anastomosed to the splenic artery and the superior mesenteric artery with a 6-0 polypropylene suture, respectively. The common iliac artery was then used for cannulation. Similarly, an iliac vein was used as an extension graft by anastomosing the iliac vein to the graft portal vein in an end-to-end fashion using 6-0 polypropylene suture [22]. The artery and vein were cannulated with 1/4″ x 3/8″ reducers. The bowel was shortened if necessary and a Malecot catheter (Bard, 22 Fr, Covington, GA, USA) was inserted into the distal end to collect duodenal and pancreatic exocrine output during perfusion (**Figure 1**). The pancreas was weighed after completion of the back table and then flushed with 200 mL of 5% albumin before initiating NEVPP.

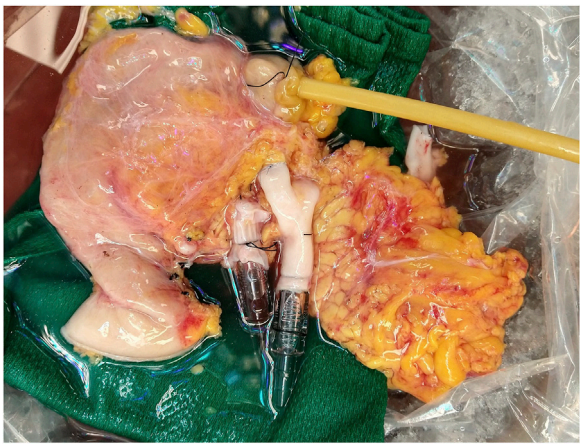


FIGURE 1 | Pancreas allograft after backtable.

Normothermic *Ex Vivo* Perfusion

Pancreas allografts were perfused for 4 h using the method described previously by our group [21, 23]. Briefly, a neonatal cardiopulmonary bypass system was used and fitted with a custom-made circuit (Sorin Group Canada Inc., Markham, Canada). In this system, the perfusate travels from the venous reservoir with the help of a centrifugal pump into an oxygenator. Following oxygenation, the circuit divides into two circuits with a part of the perfusate circulating through a dialysis filter and then back to the reservoir. The second circuit passes blood through an arterial bubble filter and then into the pancreas graft. The venous outflow goes back into the venous reservoir [21, 23] (**Figure 2**). The first 4 grafts were perfused with an O2/CO2 concentration of 95/5% and the last 2 grafts were perfused with a concentration of 91/9%. **Figure 3** shows a graft at the beginning and at the end of the perfusion. The perfusate's composition is shown in **Table 1**. Dialysate is infused at a rate of 1 L per hour and prepared before every experiment. The dialysate consisted of 22 mL of 45X concentrated hemodialysis solution (Baxter Corporation), 27 mL of 8.4% sodium bicarbonate, 3 mL of 8.4% potassium bicarbonate, 275 mg of sodium pyruvate, and 1.5 g of NaCl. The volume was then brought up to a liter with double reverse osmosis water.

A 4-hour perfusion time was decided upon since we believe that at least 2 h of perfusion are needed to perform any intervention on the graft. Doubling that period seemed to be a reasonable starting point for our first feasibility study with human grafts.

During the perfusion, arterial pressure and flow were measured and recorded every hour. Blood gas analysis from the perfusate was used to record acid-base and electrolyte balance and samples were taken every hour for storage. Duodenal output was measured every hour and recorded if present.

Histology

A core biopsy (Bard, Monopty disposable core biopsy instrument, 14g × 16 cm, Georgia, USA) was taken from the tail before the start of the perfusion, at 1-hour of perfusion. At the end of the perfusion 4 wedge biopsies were taken from the head, body, tail, and duodenum. These biopsies were fixed in formalin, snap frozen, and stored in RNA later (Stabilization Solution, Invitrogen, Thermo Fisher Scientific.

All the formalin samples (10% neutral buffered formalin) were stored for 48 h and then transferred to 70% alcohol. They were then all sent for paraffin block embedding and hematoxylin and eosin (H&E) staining. A semiquantitative scale, developed by our pathologist, was used to score fat and parenchyma necrosis (0 - no changes, 1 - mild changes, 2 - moderate changes, 3 - severe changes) [21].

For the assessment of islet cells, additional insulin staining was performed and reported as number if islet cells at a ×4 magnification. For the assessment of apoptosis, a TUNEL assay was performed on the end of perfusion samples and reported as negative, <30%, 30%–60% or >60%. For the assessment of vascularity of the grafts, a CD31 staining was performed. Interstitial edema was assessed on histopathology and classified as none, mild, moderate, or severe. All the histopathological analysis was performed by a GI/pancreas pathologist.

Oxidative Stress

Samples of the perfusate were stored at −80°C. These samples were thawed and used to measure thiobarbituric acid reactive

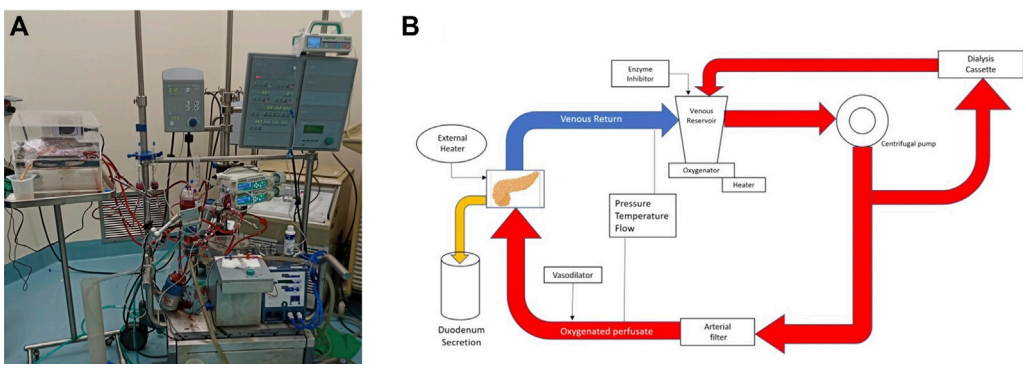


FIGURE 2 | Normothermic Machine. **(A)** Photography. **(B)** Schematic of the circuit.

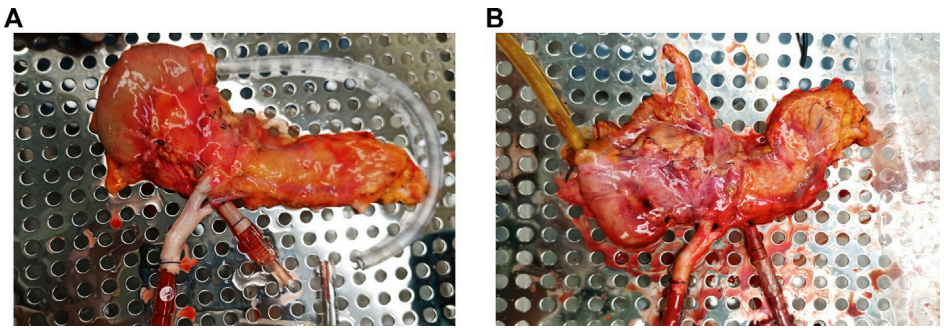


FIGURE 3 | Pancreas allograft. **(A)** at the beginning of perfusion. **(B)** At the end of perfusion.

TABLE 1 | Perfusate composition.

Ingredient	
Steen Solution	215 mL
Packed red blood cells	400 mL
Sodium bicarbonate (8.4%)	10 mL
Heparin (10000 IU/10 mL)	1.3 mL
Aprotinin	15 mg *
Continuous infusion: Epoprostenol – 0.5 mg dissolved in 250 mL of ringer's lactate and infused at 8 mL/h. *Aprotinin – 30 mg dissolved in 60 mL of ringer's lactate. 30 mL (15 mg) go are directly poured into the reservoir and the rest is infused at 10 mL/h.	

substances (TBARS) using a commercial assay kit (OxiSelect TBARS Assay Kit, Cell Biolabs, Inc.)

Data Analysis and Statistics

Continuous data are represented as mean and standard deviation and plotted versus time for each case. GraphPad Prism Software 9 was used for analysis and graphs.

For each case, the following variables were collected: age, cause of death (COD), type of donor (NDD or DCD), gender, height, BMI, cold ischemia time (CIT), blood type, and reason for discard.

RESULTS

Donor Characteristics

Characteristics of the donors and cold ischemia times are shown in **Table 2**. Half of the donors were male. Of the 6 included cases only one graft came from a DCD donor, with a warm ischemia of 17 min The mean age was 44.16 ± 13.79 years. The cause of death was anoxia in 2 donors, cardiac arrest in 2 donors, CVA/stroke in 1 donor and, head trauma in 1 donor. The mean cold ischemia time was 372.50 ± 137.69 min with a range of 173–547 min. The mean height was 172.50 ± 14.42 cm. The mean weight was 78.46 ± 25.70 kg and, and the mean BMI was 25.71 ± 5.81. The reason for discard was fatty infiltration in 2 grafts, older donor in 2 cases and high BMI in one case. Four out of the six donors presented a cardiac arrest event that required CPR and five out of six required vasopressors.

Graft characteristics

All the grafts (pancreas and duodenum) perfused evenly during the 4 h of perfusion, without any macroscopic evidence of poor circulation. The mean wet/dry weight ratio was 3.99 ± 0.39 before perfusion and 5.02 ± 0.63 after perfusion (p = 0.007) (**Figure 4A**) and a change in ratio that ranges from 6% to 42%. Individual values are shown in **Figure 4B**.

TABLE 2 | Donor characteristics.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Age (mean ± SD) (Range)			44.16 ± 13.79 (26–62)			
Height (cm) (mean ± SD) (Range)			172.50 ± 14.42 (151–183)			
Weight (kg) (mean ± SD) (Range)			78.46 ± 25.70 (38.8–114.8)			
BMI (kg/m^2) (mean ± SD) (Range)			25.71 ± 5.81 (17–34.3)			
CIT (minutes) (mean ± SD) (Range)			372.50 ± 137.69 (173–547)			
Gender	Male	Male	Male	Female	Female	Female
Type of donor	NDD	NDD	NDD	NDD	DCD	NDD
Cause of death	CVA/ Stroke	Anoxia	Anoxia	Cardiac arrest	Cardiac arrest	Head Trauma
Blood type	O positive	A positive	B positive	A positive	A positive	B positive
WIT (minutes)	N/A	N/A	N/A	N/A	17	N/A
Reason for Discard	BMI	Fatty infiltration of the graft	Fatty infiltration of the graft	Fatty infiltration of the graft	Age	Age

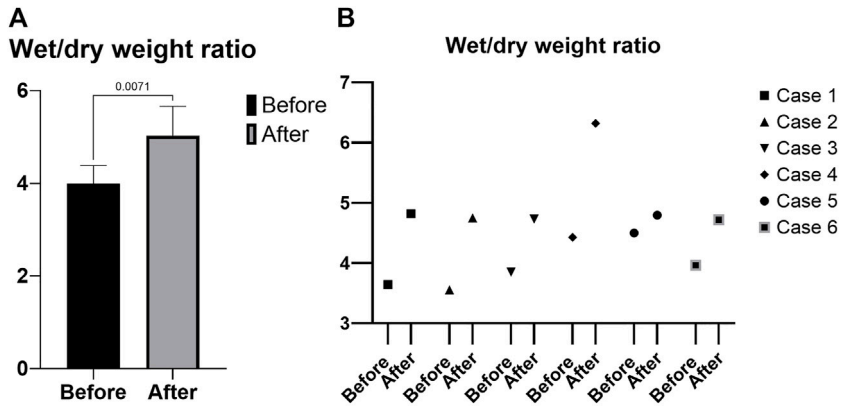


FIGURE 4 | Wet/dry weight ratio. **(A)** Mean. **(B)** Individual values.

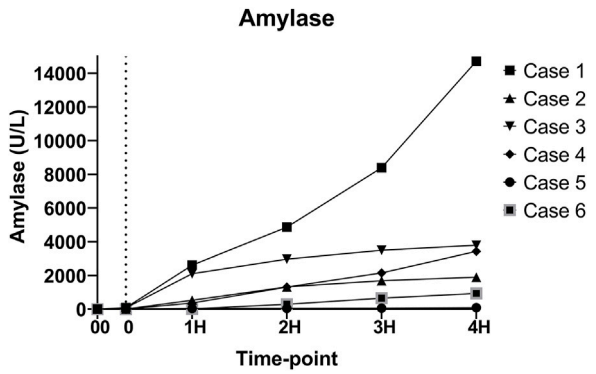


FIGURE 5 | Amylase levels during perfusion. (00 - before pancreas on pump, 0 – right after pancreas on pump, 1H - 1 h, 2H –2 h, 3H – 3 h, 4H – 4 h).

Laboratory Results

As expected, amylase levels increased during the 4 h of perfusion (median: 796.5U/L, IQR: 2430.75) (**Figure 5**). No significant difference was noted between amylase of the CO2 5% group vs. CO2 9% group. Glucose and lactate levels decreased during the 4 h of perfusion (median: 7.55 mmol/L, IQR: 4.025 mmol and median: 7.18 mmol/L, IQR: 4.59 mmol/L, respectively) (**Figure 6**). C-peptide levels (median: 1,084.5 pmol/L, IQR: 5559.75 pmol/L) during perfusion were more variable between cases (**Figure 7A**), and insulin levels increased in all the cases during the perfusion, except for case 5 (**Figure 7B**).

Levels of pH, HCO3 and, pCO2 were consistent throughout the perfusion (**Figures 8A–C**). However, pO2 levels were more variable during the perfusion but were always above 100 mmHg (**Figure 8D**).

Perfusion Characteristics

The arterial flow was stable throughout the 4 h of perfusion with a mean of 40.9 ± 16.19 mL/min/100 g (**Figure 9**).

Intravascular resistance was slightly higher for cases 2 (0.082 ± 0.013 mmHg/ml/min per 100 g), 4 (0.083 ± 0.028 mmHg/ml/min per 100 g), and 5 (0.084 ± 0.002 mmHg/ml/min per 100 g) as compared to cases 1 (0.042 ± 0.005 mmHg/ml/min per 100 g), 3 (0.038 ± 0.002 mmHg/ml/min per 100 g), and 6 (0.049 ± 0.002 mmHg/ml/min per 100 g) with a significant difference between means ($p < 0.001$) (**Figure 10**). CO2 5% group seemed to have a lower mean intravascular resistance than the CO2 9% group, but the difference was not statistically significant ($p = 0.31$).

Histopathology

Minimal tissue injury was noted in both the CO2 5% and CO2 9% groups and grafts from both groups were morphologically normal (**Figure 11**). Overall, the parenchyma was largely intact, with very mild focal necrosis, normal ducts, and mild hemorrhage/congestion. The duodenum showed mild to moderate erosive changes and mild autolysis. Islet cells were present in all the cases (**Figure 12**). No edema was observed in any of the grafts and TUNEL assay was negative for all the cases except for case 1 which presented less than 30% (approximately 5%) (**Figure 13**). All grafts were vascularized at the end of the perfusion as seen in the pancreatic tissue stained with CD31, with no evidence of thrombosis (**Figure 14**).

Oxidative Stress

TBARS were measured from the perfusate at baseline and at the end of the perfusion. No significant difference was noted between samples at baseline and at the end of the perfusion ($p = 0.84$) (**Figure 15**).

DISCUSSION

The pancreas is an organ vulnerable to edema and ischemic injury during retrieval and preservation leading to microcirculatory dysfunction [20]. This is likely one of the main reasons why perfusion of the pancreas did not gain as much interest as it has for other organs. In this study, all 6 cases were successfully

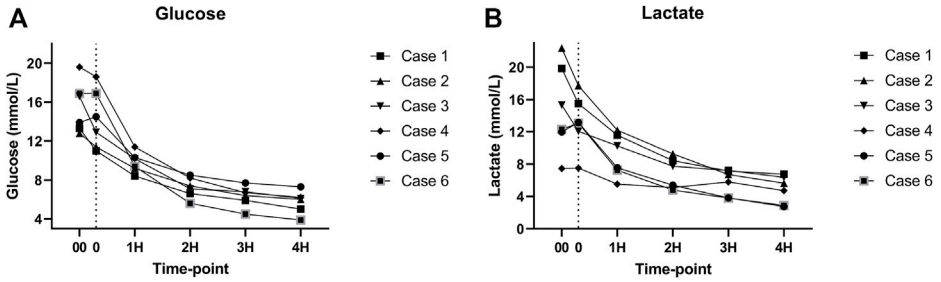


FIGURE 6 | (A) Glucose levels during perfusion. **(B)** Lactate levels during perfusion. (00 - before pancreas on pump, 0 – right after pancreas on pump, 1H - 1 h, 2H –2 h, 3H – 3 h, 4H – 4 h).

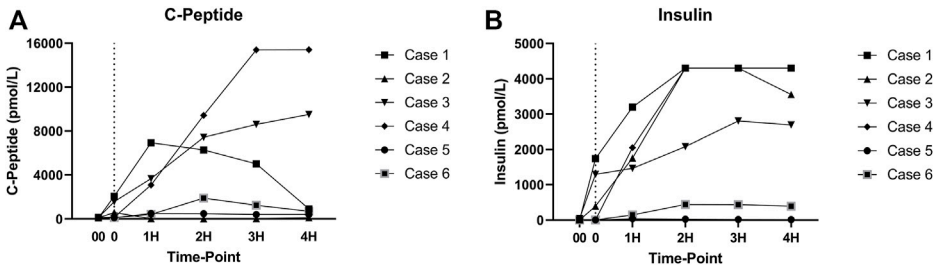


FIGURE 7 | (A) C-peptide levels during perfusion. **(B)** Insulin levels during perfusion. (00 - before pancreas on pump, 0 – right after pancreas on pump, 1H - 1 h, 2H –2 h, 3H – 3 h, 4H – 4 h).

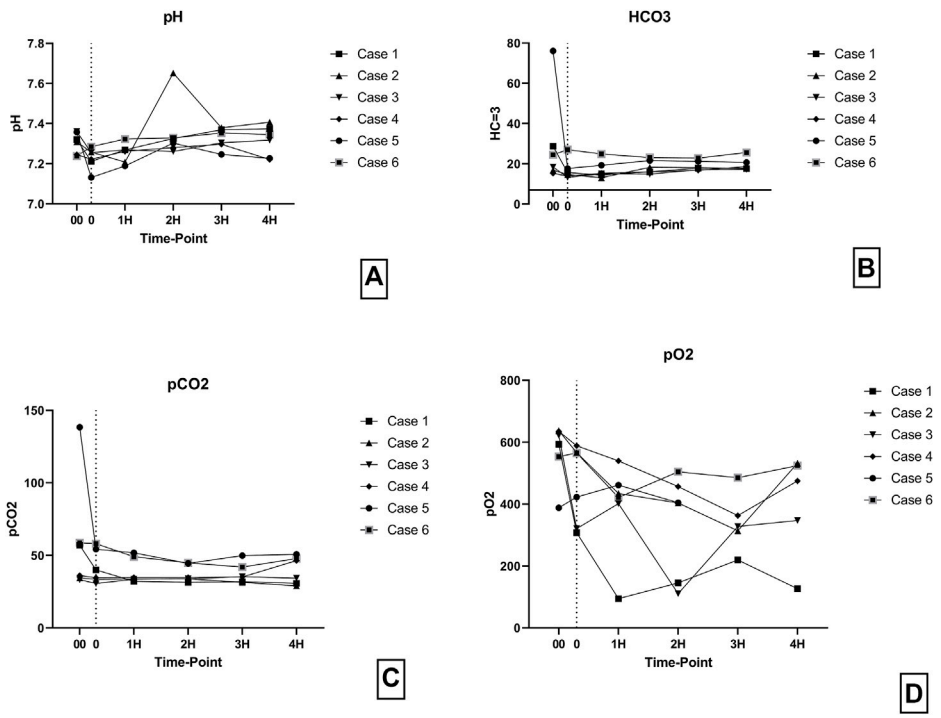


FIGURE 8 | (A) pH levels during perfusion. **(B)** HCO3 levels during perfusion. **(C)** pCO2 levels during perfusion. **(D)** pO2 levels during perfusion. (00 - before pancreas on pump, 0 – right after pancreas on pump, 1H - 1 h, 2H –2 h, 3H – 3 h, 4H – 4 h).

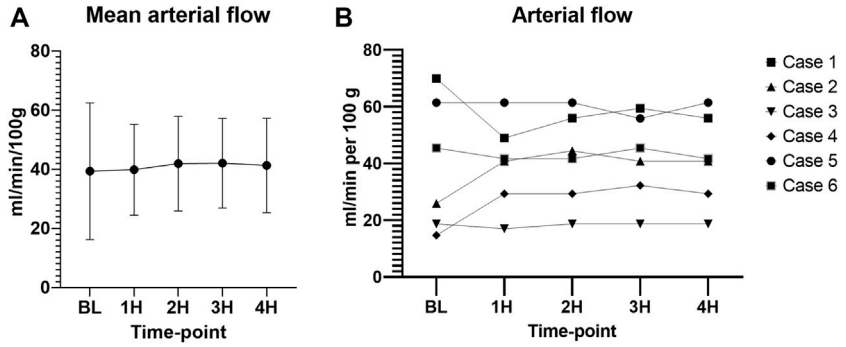


FIGURE 9 | Arterial flow during perfusion. **(A)** Mean values with standard deviation. **(B)** Individual values. (00 - before pancreas on pump, BL – right after pancreas on pump, 1H - 1 h, 2H –2 h, 3H – 3 h, 4H – 4 h).

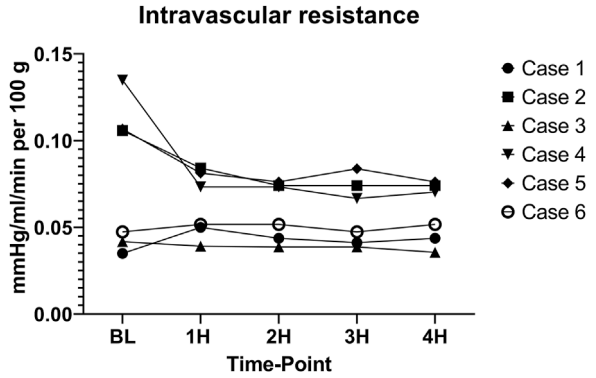


FIGURE 10 | Intravascular resistance. (BL – baseline, 1H - 1 h, 2H –2 h, 3H – 3 h, 4H – 4 h).

perfused for the established time with a stable arterial pressure, flow, temperature, and a good macroscopic and microscopic appearance. Insulin increase was noted during the perfusion and glucose and lactate levels were close to normal by 4 h. This was similar to the results in our porcine model and these grafts were successfully transplanted with minimal evidence of injury, normal glucose tolerance tests, and no signs of pancreatitis [21].

The first NEVPP of discarded human pancreases was reported by Barlow et al in 2015 [24]. They reported 4 cases with a 2 h perfusion, proving technical feasibility but with poor results on histopathology and no mention of graft weight gain. According to this paper, there were 5 cases but, the last one had to be discarded because of an ischemic appearance during perfusion thought to be due to 30 h of CIT. For the fourth case, perfusion had to be terminated after 60 min due to low perfusate volume. All the cases showed a significant degree of necrosis, and the authors deemed this method to be feasible but not suitable in its current state.

In our study, grafts were perfused for a longer period with excellent tissue viability and close to normal morphological histopathology appearance after 4 h. Unlike Barlow et.al., our arterial perfusion pressure was set to 15–25 mmHg, instead of 50–55 mmHg [24]. A lower perfusion pressure was found to be critical for successful perfusion in the porcine model and appears

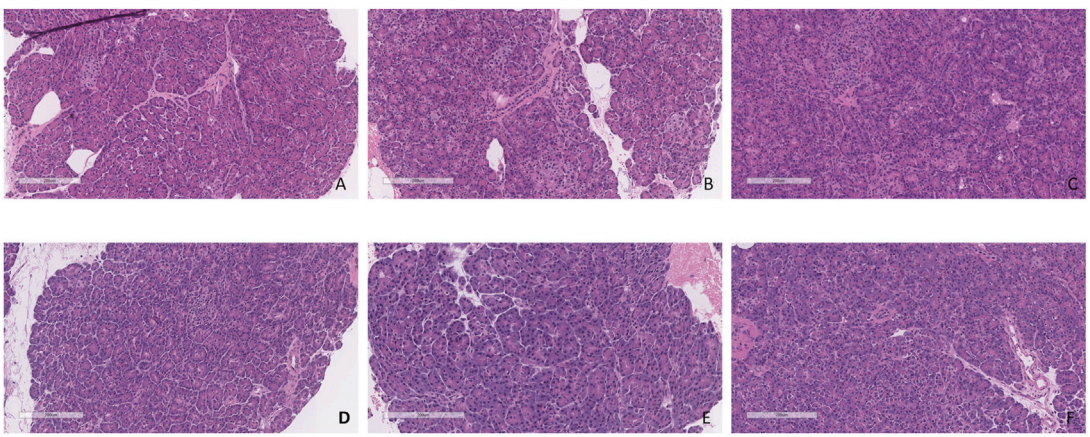


FIGURE 11 | H&E staining of pancreas tail biopsies. **(A)** CO2 5% before perfusion. **(B)** CO2 5% at 1 h of perfusion. **(C)** CO2 5% at the end of 4 h of perfusion. **(D)** CO2 9% before perfusion. **(E)** CO2 9% at 1 h of perfusion. **(F)** CO2 9% at the end of 4 h of perfusion. Bar = 200 μm.

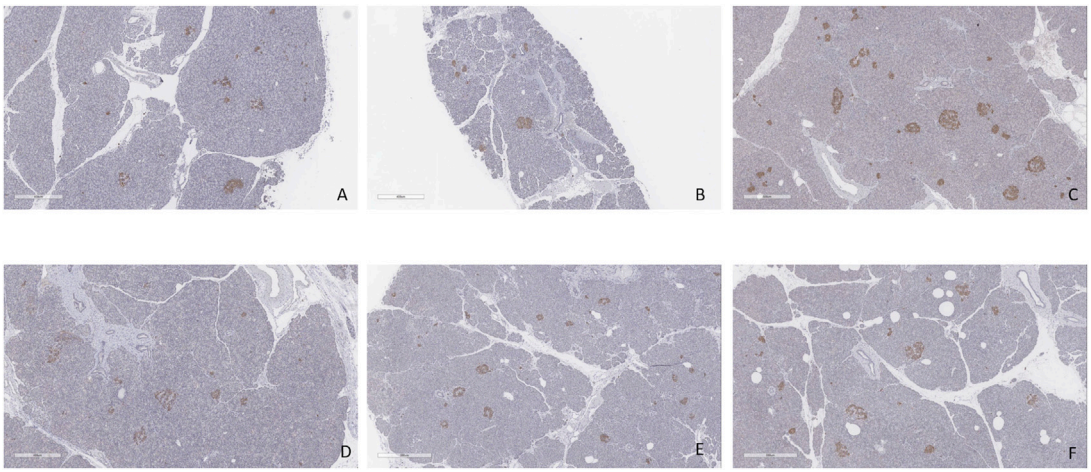


FIGURE 12 | Insulin staining. **(A)** Case 1. **(B)** Case 2. **(C)** Case 3. **(D)** Case 4. **(E)** Case 5. **(F)** Case 6. All biopsies taken at the end of perfusion (4 h). Bar = 500 µm.

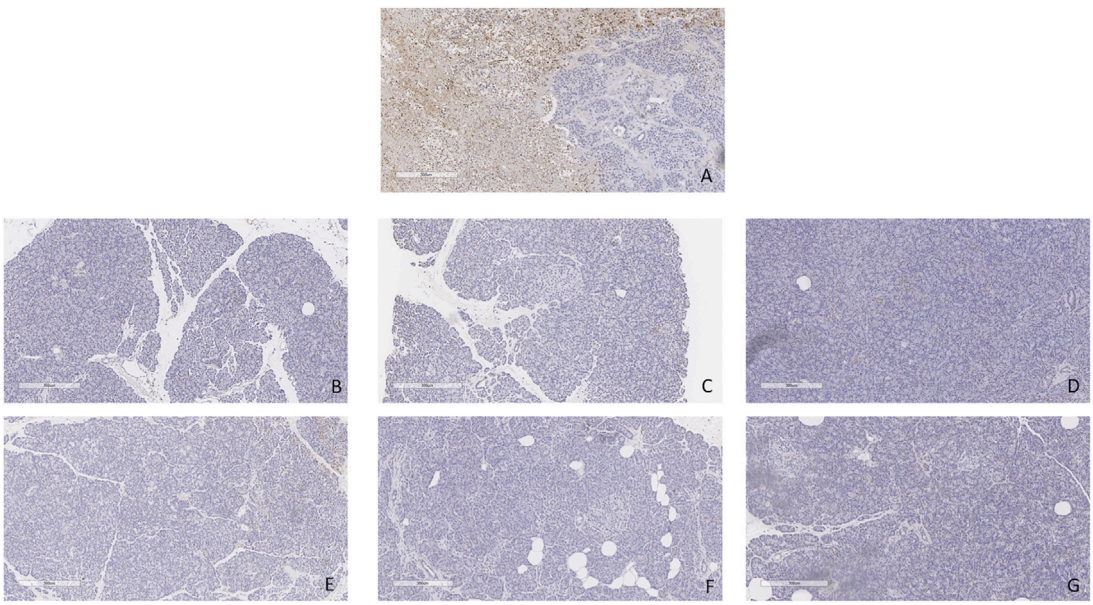


FIGURE 13 | TUNEL staining. **(A)** Positive and negative control. **(B)** Case 1. **(C)** Case 2. **(D)** Case 3. **(E)** Case 4. **(F)** Case 5. **(G)** Case 6. All biopsies taken at the end of perfusion (4 h). Bar = 300 µm.

to be similarly important in human grafts [21, 25]. Our system also incorporated a dialysis circuit which improved the degree of tissue edema that developed during perfusion. Finally, based on previous work, a protease inhibitor was added to the perfusate to mitigate any active enzyme that permeates into the system [21, 24].

According to studies in hypothermic machine perfusion of the pancreas, lower perfusion pressures obtain more stable perfusions and better results overall [19, 26]. Because of this, we decided to keep the pressure around 20–25 mmHg for the first 4 cases. In the latter part of the study, we noticed that when using a higher CO2 concentration, we could drop the pressure to 15 mmHg, without compromising the readings of pH, HCO3, pO2, or pCO2 concentrations in the perfusate. Cases 5 and 6 were perfused with a higher CO2 concentration (9% vs. 5%) which allowed a decrease in the overall arterial pressure.

The percentage change in wet/dry weight ratio before and after perfusion ranged from 6% to 42%. We noticed that the lowest change in ratio occurred in the grafts perfused with a higher CO2 concentration. Hypercapnia is a well-known vasodilator, but its use has been mainly described and studied for cerebral blood flow [27]. Studies in rats suggest that hypocapnia contributes to

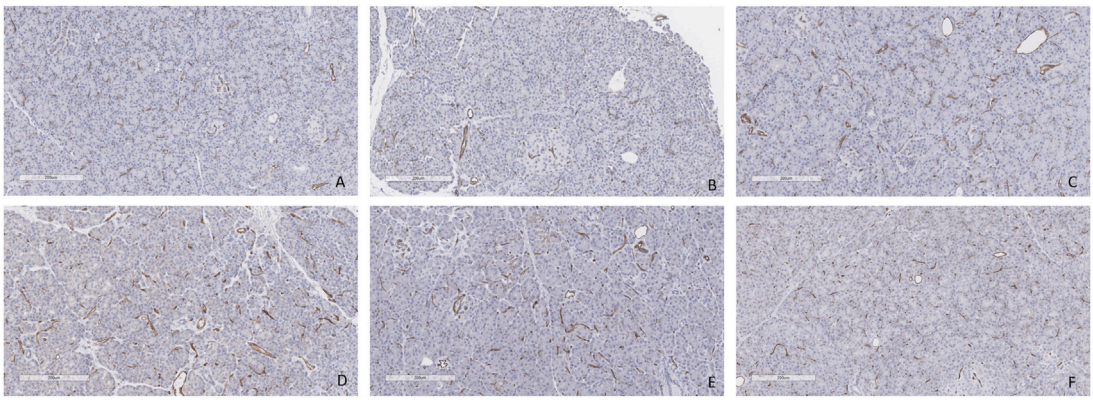


FIGURE 14 | CD31 staining at the end of the perfusion. **(A)** Case 1. **(B)** Case 2. **(C)** Case 3. **(D)** Case 4. **(E)** Case 5. **(F)** Case 6. All biopsies taken at the end of perfusion (4 h). Bar = 200 μm.

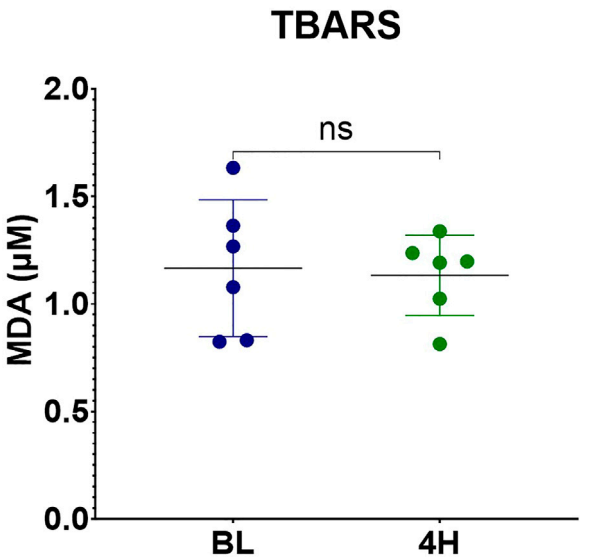


FIGURE 15 | MDA levels measured by TBARS assay.

hypoperfusion and edema [28]. From our experiments in swine, we noted that increasing the CO2 concentration in the perfusate decreased the intravascular resistance which allowed us to perfuse the grafts with a lower arterial pressure, as mentioned previously. In this study, intravascular resistance was slightly lower but not significant in the CO2 5% group. However, only 2 cases were perfused in the CO2 9% group and more experiments are needed in this arm to confirm this trend. Edema has been a recurrent problem during perfusion [20, 25]. We believe that both the dialysis filter and higher CO2 contributed to small increase in water content, but further studies are needed.

Since this is a closed system, amylase increased during the perfusion, as expected, but this does not seem to correlate with damage to the graft, according to previous reports [29] and our histopathology results, which showed intact parenchyma (**Figure 10**). Lipase was measured during the experiments, however, the maximum range (3,000 U/L) was reached very early in the experiments and did not prove to be a useful marker. The development of better biomarkers to measure graft injury is needed and will enhance the ability of NEVPP to be used for graft assessment.

The most common complication after pancreas transplantation is vascular thrombosis, followed by graft pancreatitis. According to Nadalin et.al., a physiological acute pancreatitis occurs in 100% of the patients undergoing PTx, due to ischemia-reperfusion injury and this is typically clinically silent [30]. In our previous studies [21, 23, 31], with porcine models and machine perfusion we had no cases of vascular thrombosis or signs that physiological graft pancreatitis was not successfully resolving by the day of the sacrifice. None of the grafts in this study were transplanted after perfusion, but we could hypothesize that human grafts would do as well as the porcine grafts after transplant. This hypothesis could be supported by the histopathological findings that demonstrated minimal signs of apoptosis by TUNEL assay, minimal endothelial damage seen with CD31 staining and no apparent evidence of thrombosis. We hope that NEVPP when used with marginal grafts will help mitigate severe ischemia-reperfusion injury (IRI) which occurs post transplantation and eliminate/reduce IRI-related complications and allow for successful transplantation.

It has been established that reactive oxygen species (ROS) are an important injurious factor for ischemia reperfusion injury [32, 33]. The studies regarding levels of malondialdehyde (MDA) and machine perfusion are scarce and mainly refer to Hypothermic Machine Perfusion (HMP). In 2017, Kosieradzki et al studied 50 kidney transplant recipients. Grafts were procured from 27 brain death donors and preserved in a pulsatile perfusion device for a total mean ischemia time of 36.7 ± 8 h. They concluded that the 18 recipients that presented delayed graft function presented higher levels of MDA in the preservation solution at the end of the perfusion [34].

In our study, no significant difference was noted in MDA concentration in the perfusate at baseline and at the end of the perfusion, these results are in accordance to what was previously reported by Brüggenwirth et.al. in 2020 in porcine livers submitted to hypothermic and normothermic machine perfusion [35]. Interpretation of the results might prove to be difficult, but we could hypothesize that normothermic machine perfusion is useful to slow down or mitigate the oxidative stress.

Our study has several limitations. First, the complicated setup of our machine is difficult to replicate, and the cost of every experiment is high (around 5000CAD). The grafts were only perfused and not transplanted afterward, so no follow-up is possible. All the grafts were discarded so none of these grafts were from ideal donors and could have already presented some degree of damage before perfusion. Currently, there is no suitable test to assess the quality of the graft during perfusion, and we still do not fully understand the physiology of the pancreas during normothermic machine perfusion. The addition of a dialysis filter helped with the control of edema but makes the interpretation of glucose and lactate levels challenging as they will normalize over time. In addition, the number of grafts is limited, as mentioned previously, however, we believe the data demonstrates that normothermic machine perfusion in human pancreas allografts is feasible. Future studies will be directed towards better understanding the physiology while undergoing NEVPP.

In conclusion, normothermic machine perfusion of the human pancreas is feasible, maintaining both the macroscopic and microscopic appearance of the pancreas at the end of the perfusion and could prove to be useful for the assessment of extended criteria donors pancreases both for whole organ transplantation and islet isolation. Future studies will focus on the development of tests and biomarkers for the assessment of grafts. Identifying optimal perfusion settings and modifying mechanisms of inflammation could allow us to bring this novel technology to the clinical setting. Normothermic pancreas perfusion holds the promise to increase the pancreas donor pool by improving graft preservation, assessment, and repair.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical ethical committee of the Toronto General Hospital. The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

CP participated in research design, writing of the paper, performance of the research and data analysis. SR, MK, YN, EN, SG, and BA participated in performance of the research and review. LM participated in research design, performance of the research and review. SM participated in research design, data analysis and review. MS and TR participated in research design, performance of the research, writing and review. 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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Metabolic Outcomes After Pancreas Transplant Alone From Donation After Circulatory Death Donors-The UK Transplant Registry Analysis

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Extrapolating data from early DCD (donation after circulatory death) kidney transplantation, pancreas transplants from DCD grafts were feared to have worse metabolic outcomes. Hence, we aimed to address the question of pancreas transplant alone (PTA) from DCD donors–are our concerns justified? A UK transplant registry analysis of 185 PTA performed between 2005 and 2018 was done. All early graft losses (<3 months) were excluded to allow focus on the metabolic outcomes (HbA_1c, weight gain and incidence of secondary diabetic macrovascular complications). The aim was to compare the metabolic outcomes, rejection rates (including the need for steroids), patient and graft survival between DBD (Donation after brainstem death) and DCD groups. After excluding early graft losses, data from 162 PTA (DBD = 114 and DCD = 48) were analyzed. Body mass index of the donor was less in DCD group (DBD = 23.40 vs. DCD = 22.25, p = 0.006) and the rest of the baseline transplant characteristics were comparable. There were no significant differences in the HbA_1c, weight gain, rejection rate, and incidence of secondary diabetic macrovascular complications post-transplant between DBD and DCD recipients. The 1-, 5-, and 10-year patient and graft survival were similar in both the groups. PTA from DCD donors have equivalent metabolic outcomes and survival (patient/graft) as that of DBD donors.

Keywords: pancreas transplantation, donation after circulatory death, metabolic outcomes, DCD donors, pancreas transplant alone

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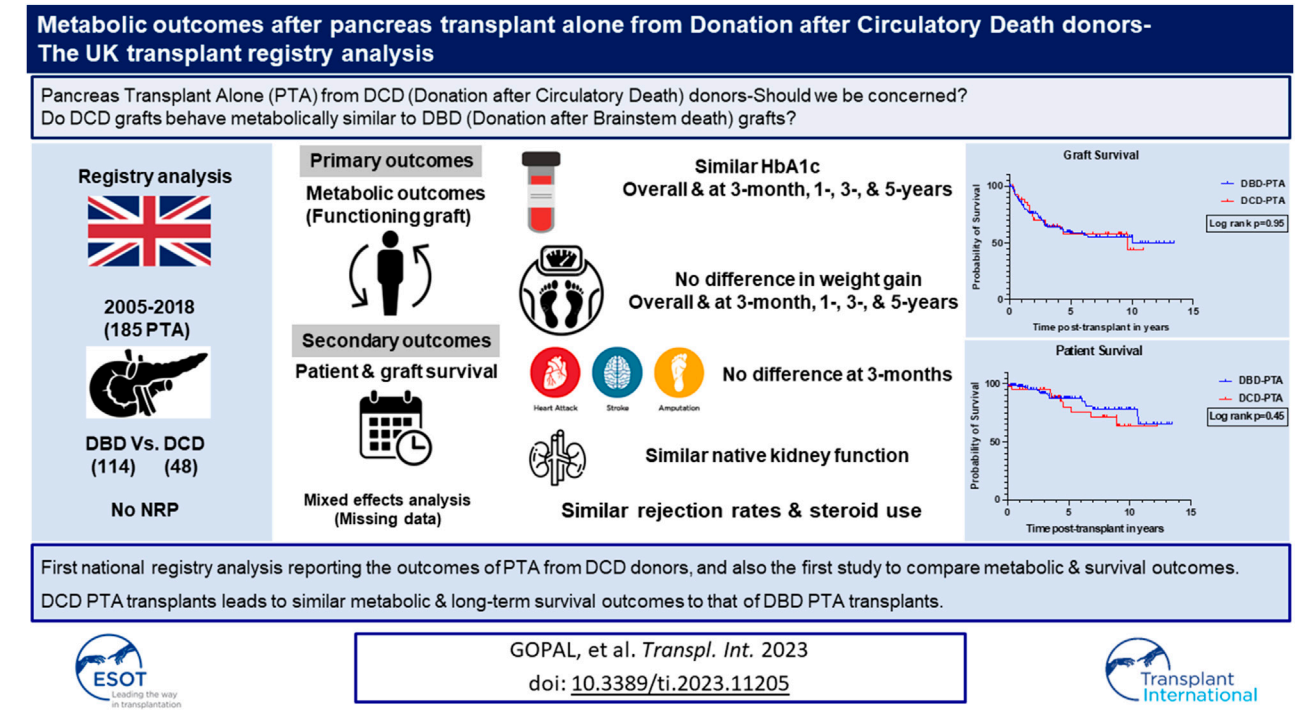
INTRODUCTION

Despite the increasing awareness regarding organ donation, scarcity of suitable donor organs is still a problem faced by the transplant community. The median months to transplantation for a pancreas transplant alone (PTA) in the US was 24.1 months in 2016–2017 [1]. In the Euro transplant region, 75% of the patients were still waiting for a pancreas transplant at 1 year from listing [2]. The waiting

Abbreviations: ACE, angiotensin converting enzyme; BMI, body mass index; cRF, Calculated reaction frequency; DCD, donation after circulatory death; DBD, donation after brainstem death; eGFR, estimated glomerular filtration rate; HbA_1c, hemoglobin A_1c; HLA, human leukocyte antigen; IQR, interquartile range; NHSBT, national health service blood and transplant; NORS, national organ retrieval service; NRP, normothermic regional perfusion; PTA; pancreas transplant alone; UK, United Kingdom; USA, United States of America; WLST, withdrawal of life sustaining treatment.



GRAPHICAL ABSTRACT |

list mortality is still significant. In the US, wait list mortality for PTA was 2.7% per 100 patient-years in 2019 and in the UK and Euro transplant region, the waitlist mortality was 3% within 1 year of listing [1–3].

Transplanting DCD organs has been a viable option to expand the donor pool. The UK has pioneered pancreas transplantation from DCD donors and in recent years about one-third of pancreas transplants performed are from controlled DCD donors, and the pancreas offer decline rate is better for DCD organs (45%) than DBD organs (54%) [3]. While the UK has been a global leader in DCD pancreas transplantation, DCD pancreas utilization rate in the US and Euro transplant region has remained low. In the US, less than 5% of the pancreas transplanted were from DCD donors and it has remained consistently low since 2008 [1]. A similar picture is noted in the Euro transplant region [2]. The main concerns for the differential usage are functional warm ischemia time and asystolic period prior to commencement of organ perfusion with the resultant ischemia reperfusion injury and consequent graft pancreatitis, sepsis and graft thrombosis.

Convincing evidence supporting DCD pancreas transplantation is being generated since 2000 [4–11]. None of the studies have looked into the metabolic outcomes and as such the metabolic outcomes after PTA from DCD grafts are unknown. A successful pancreas transplant, unlike intensive insulin regimen, restores euglycemia without the risk of hypoglycaemia and halts or reverses the progression of secondary complications of diabetes [12–14]. Hence, the real premise of pancreas transplantation especially in the setting of PTA is to achieve optimal metabolic control in addition to achieving insulin independence.

In patients with diabetes, chronic hyperglycaemia is known to be associated with an increased risk of cardiovascular disease, whereas, in patients without diabetes a higher HbA1c even within the normal range is associated with a significantly higher risk of coronary artery disease [15, 16]. This highlights the importance of stricter glycaemic control to achieve the maximum benefit. In addition, early post-transplant impaired glucose tolerance is associated with later pancreas graft failure [17]. Hence, it is vital to know the metabolic outcomes. Therefore, we aimed to study the metabolic outcomes after PTA and compare between DBD and DCD grafts.

MATERIALS AND METHODS

Data Collection

There are eight designated pancreas transplant centres in the UK and all of them report their follow up data to the UK Transplant Registry, which is a mandatory prospectively run database maintained by the National Health Service Blood and Transplant (NHSBT). The Pancreas Advisory Group, a subsection of NHSBT approved this study and provided access to the data. All patients who underwent PTA in the UK from 1 January 2005 to 31 December 2018 were identified, and pertinent data was obtained from the UK Transplant Registry.

HbA_1C was recorded as % prior to 2013 and as mmol/mol since then.

Indications and Restrictions for PTA

All patients waitlisted for PTA had insulin treated diabetes along with normal or near-normal renal function. They also had at least 2 severe hypoglycaemic episodes within the last 24 months and assessed by a diabetologist to have disabling hypoglycaemia. Body mass index (BMI) > 30 kg/sq.m for patients with type 2 diabetes was an absolute contraindication for PTA, whereas, insulin requirement >100 units/day, BMI > 30 kg/sq.m, and severe aorto-iliac or peripheral vascular disease were relative contraindications. The rest of the contraindications were similar to most of the solid organ transplants and are described elsewhere [18].

Donor Selection

The donor selection criteria were uniform across all pancreas transplant centres. The criteria were similar for DBD and DCD donors except for age (DCD ≤ 55 years of age; DBD ≤ 60 years of age). The following were contraindications to pancreas donation: history of diabetes in the donor (excludes insulin requirement in critical care), active or previous pancreatitis, previous pancreatic surgery, body mass index (BMI) > 40 kg/m^2, weight < 15 kg, as well as other contraindications for solid organ transplantation. The UK pancreas offering scheme has been national since 2010, with patient-specific offers and a combined list for solid organ pancreas and islet transplants. The national pancreas allocation scheme is described elsewhere [19]. All the waiting list patients were considered suitable for DCD organs without any distinction.

DCD Pancreas Procurement

In April 2010, the National Organ Retrieval Service (NORS) was established to carry out all the organ retrievals in the UK and before that, the corresponding implanting centres procured their own organs. At present, there are 10 abdominal NORS teams in the UK (six teams are associated with a pancreas transplant program) and eight teams are on-call on any given day. Depending on the location of the donor hospital and the availability of the nearest NORS teams, an appropriate NORS team will be mobilized for retrieval. Pre-mortem interventions such as heparinization or vascular cannulation are prohibited in the UK and organs were retrieved only from controlled DCD donors using a super-rapid technique. After obtaining informed consent from the next of kin, withdrawal of life-sustaining treatment (WLST) occurred either in the critical care unit or in the anaesthetic room of the operating theatre depending on the local hospital policy. After WLST, NORS team wait for 3 h for the onset of functional warm ischemia (defined as systolic blood pressure < 50 mmHg). Pancreases were procured if donor asystole occurred within 30 min following the onset of functional warm ischemia. NORS team will abandon pancreas procurement if asystole does not occur within the above time frame. There is a mandatory 5-min period following donor asystole before death can be declared and subsequently another 5-min "No touch" period following declaration of death and prior to commencement of organ procurement. Through a midline laparotomy, the donor distal aorta or common iliac vessels were cannulated and *in-situ* perfusion was commenced with University of Wisconsin solution (ViaspanTM, Bristol-Myers Squibb Pharmaceuticals, Uxbridge, United Kingdom). If the liver was procured, portal venous cannulation was performed with proximal venting of the portal vein. Normothermic regional perfusion (NRP) was not utilized in this study population.

Retrieval Training

Retrieval competency is governed by NHSBT. In order to gain competency in pancreas retrieval, trainee surgeons enter supervised training in any one of the commissioned NORS teams in the UK and will need to demonstrate appropriate knowledge, skills and attitudes which are compatible with unsupervised retrieval practice. The local NORS lead will be responsible to decide when the trainee surgeon is ready for unsupervised practice. Prior to unsupervised practice, all retrieval-related training and masterclass must be completed. A complete guidance for retrieval training is described elsewhere [20].

Pancreas Transplantation

Implantation techniques were as per the discretion of the implanting centre/recipient surgeon and both DBD and DCD organs were treated similarly. Immunosuppression protocol were according to the local practice in different centres.

Outcomes Studied

The primary aim was to compare the metabolic outcomes (HbA1c, weight gain, and secondary diabetic macrovascular complications) between the two groups and it was studied only in recipients with a functioning graft. The metabolic outcomes were compared alongside with the incidence of rejection episode and steroid usage. All the early graft losses (<3 months) were excluded when analysing the metabolic outcomes. The cut off for early graft loss was set at 3 months based on literature evidence [21–23]. When analysing metabolic outcomes failed grafts were excluded (censored at the point of graft failure). The secondary aim was to compare the survival outcomes (both graft and patient) between the two groups.

Definitions

Functioning graft was defined as being insulin independent post-transplantation. Secondary diabetic macrovascular complications were defined as any of the following events post-transplant: cerebrovascular accident, myocardial infarction, or limb amputation (minor or major). Recipients were classified based on the calculated reaction frequency (cRF) as either sensitised (cRF > 5%) or highly sensitised (cRF > 85%).

Statistical Analyses

Categorical variables are expressed as frequency (%) and continuous variables as median and interquartile range (IQR). Difference between the categorical variables were assessed by using Fisher's exact test and chi-squared test. Difference between the continuous variables were assessed by using Mann-Whitney test. The mixed-effects model approach was used in order to obtain unbiased results due to missing observation. The mixed-effects model for repeated observations was constructed without assuming sphericity of the data and performed without any interaction analysis or multiple comparisons. In the mixed-

effects model, recipients with a functioning graft at 3 months had longitudinal HbA1c and weight gain data inputted whereas longitudinal serum creatinine data was inputted irrespective of the graft function. Kaplan-Meir survival plots were used for survival analysis. For graft survival, censoring was done for grafts functioning at the time of analysis and death with a functioning graft. All the statistical analyses were performed using Graph Pad Prism software (Version 9.5.1).

RESULTS

In the study period 185 PTA's were performed. All early graft losses were excluded at all follow up time points (n = 23; DBD = 16/DCD = 7) to allow focus on metabolic outcomes. The early graft losses were included in survival analysis and there were no patient deaths in this group. Out of the 162 PTA's that were included, 114 were from DBD donors and 48 from DCD donors. The median follow-up period was 4.4 years (IQR: 2.1–8 years). The median asystolic period (downtime) for DCD donors was 11 min (Range: 5–30; n = 23). The median withdrawal time (time from withdrawal of life support to circulatory arrest) for DCD donors was 14 min (Range: 0–19; n = 11). The median functional warm ischemia time for DCD donors was 16 min (Range: 8–29; n = 29).

Baseline Characteristics

Donor, recipient and transplant characteristics as described in **Tables 1**–**3**. Apart from a lower BMI, the rest of the DCD donor characteristics (age, abdominal girth, sex, and ethnicity) were equivalent to DBD donors. Recipient characteristics (age, BMI, sex, ethnicity, duration of diabetes, HbA_1C at the time of registration, insulin use at the time of registration, and sensitization) and transplant characteristics (level of HLA mismatch, cold ischemia time, anastomosis time, exocrine drainage technique, induction immunosuppression, and proportion of re-transplants) were comparable between the two groups. 93% of the recipients were patients with type 1 diabetes mellitus (n = 150). Among the remaining 12 recipients, 7 had type 2 diabetes mellitus (DCD = 1; DBD = 6) and 5 had mixed (type 1 and 2) diabetes mellitus (DCD = 3; DBD = 2). A consistent proportion of PTA's were performed using DCD grafts across all eras.

Metabolic Outcomes

DBD and DCD recipients had similar median post-transplant HbA_1c millimole/mole at 3-month [35.5 (31.1–39.1) and 32.2 (27.3–37.1)], 1-year [34 (32–37) and 35.5 (32.6–39.4)], 3-year [35.3 (32–37) and 33.3 (32–36.5)], and 5-year post-transplant [36 (34–39) and 34.5 (33–37.7)] and the respective p values were 0.08, 0.25, 0.39, and 0.49 (**Figure 1**). The median HbA1c values in % for DBD and DCD recipients were also equivalent at 3-month [5.4 (5–5.7) and 5.1 (4.6–5.5)], 1-year [5.3 (5.1–5.5) and 5.4 (5.3–5.8)], 3-year [5.4 (5.1–5.5) and 5.2 (5.1–5.6)], and 5-year post-transplant [5.4 (5.2–5.7) and 5.3 (5.1–5.6)] and the respective p values were 0.09, 0.25, 0.69, and 0.50. In a mixed-effects model, there was no significant difference in the overall predicted mean HbA1c (millimole/mole) until 5 years post-transplant between the 2 groups (DBD = 39 and DCD = 37, p = 0.19).

HbA1c was also compared between the waitlisted candidates for PTA at the time of registration (n = 145) and those with a failed PTA graft with HbA1c recorded at the time of graft failure (n = 14). There was no significant difference in the median HbA1c (millimole/mole) between the groups [Waitlisted = 76 (63–91) Vs. Failed graft = 60.1 (48–114.3); p = 0.35].

Percentage weight gain post-transplant was calculated (weight post-transplant minus weight pre-transplant/100) and compared between the two groups. There was no significant difference in weight (median percentage weight gain) between the two groups at 3-month, 1-year, 3-year, and 5-year post-transplant and the respective p values were 0.20, 0.60, 0.41, and 0.95 (**Figure 2**). In a mixed-effects model, there was no significant difference in the overall predicted mean percentage weight gain until 5 years post-transplant between the 2 groups (DBD = 6.5 and DCD = −0.8, p = 0.86).

The incidence of secondary diabetic complications post-transplant was not significantly different between both the two groups at 3-month post-transplant (DBD = 1% vs. DCD = 2%, p = 0.51). There were no secondary diabetic complications in both the groups at 1-year, 3-year, and 5-year post-transplant.

There was no significant difference in the incidence of rejection between the two groups at 3-month (DBD = 10% vs. DCD = 13%, p = 0.63), 1-year (DBD = 19% vs. DCD = 10%, p = 0.15), 3-year (DBD = 12% vs. DCD = 10%, p = 0.71), and 5-year post-transplant (DBD = 10% vs. DCD = 10%, p = 1).

The overall steroid free maintenance rate was similar irrespective of the graft type (DBD = 75% vs. DCD = 73%, p = 0.79).

HbA_1C and weight were compared between DBD and DCD grafts in Era 1 (2005–2009) and Era 2 (2010–2014). In both the eras, there was no significant difference in HbA_1c or weight gain between the two groups at 3-month, 1-year, 3-year, and 5-year post-transplant (**Table 4**). In Era 3 (2015–2018) 18 PTA's were performed and out of which only 3 were performed utilizing DCD graft. Follow up data for analysing 3-year and 5-year outcomes was not available. Hence, it was not possible to compare Era 3 metabolic outcomes separately.

There was no significant difference in the median serum creatinine (micromole/L) between the DBD and DCD recipients at 3-month [104 (76–140) and 104 (82.7–140)], 1-year [107 (80–133) and 108 (80.2–153.5)], 3-year [115.5 (93.5–147) and 114 (96.2–140.8)], and 5-year [127 (96–162.3) and 110 (96–140.5)] post-transplant and their respective p values were 0.56, 0.57, 0.83, and 0.51. In a mixed-effects model, there was no significant difference in the overall predicted mean serum creatinine (micromole/L) until 5 years post-transplant between the two groups (DBD = 129.5 and DCD = 133.2, p = 0.74).

The evolution of the difference in HbA1c, weight gain, and serum creatinine between the two groups is shown in the scatter dot plot (**Figure 3**).

Survival Outcomes

On univariate analysis, there was no significant difference in the overall death -censored pancreas graft survival and overall patient

TABLE 1 | Comparison of donor characteristics.

Donor characteristics	Variable	DBD (N = 114)	DCD (N = 48)	*p*-value
Donor age, years		33 (21–48)	29 (18.5–43.8)	0.11
	Missing	0	0	
Donor BMI, kg/sq.m		23.4 (21.1–24.9)	22.2 (19.5–23.8)	0.006
	Missing	0	0	
Donor abdomen girth, cms		84 (76–92)	81.5 (74.2–85)	0.14
	Missing	23	16	
Donor sex	Male	56 (49%)	27 (56%)	0.49
	Female	58 (51%)	21 (44%)	
	Missing	0	0	
Donor ethnicity	Caucasian	104 (91%)	44 (92%)	0.99
	Non-Caucasian	10 (9%)	4 (8%)	
	Missing	0	0	

TABLE 2 | Comparison of recipient characteristics.

Recipient characteristics	Variable	DBD (N = 114)	DCD (N = 48)	*p*-value
Recipient age, years		41 (34.8–48)	43 (35.3–49.8)	0.63
	Missing	0	0	
Recipient BMI, kg/sq.m		24.7 (22.5–26.9)	24.4 (22.3–30.8)	0.62
	Missing	34	9	
Recipient sex	Male	41 (36%)	18 (38%)	0.85
	Female	73 (64%)	30 (62%)	
	Missing	0	0	
Recipient ethnicity	Caucasian	109 (96%)	47 (98%)	0.67
	Non-Caucasian	5 (4%)	1 (2%)	
	Missing	0	0	
Duration of diabetes (pre-transplant), years		26 (25.2–29.3)	28 (25.5–31.7)	0.54
	Missing	7	2	
Recipient HbA_1C at registration, mmol/mol		76 (62.9–92.1)	75 (61.6–91.8)	0.52
	Missing	20	2	
Recipient insulin use at registration, IU/day		40 (30–55)	40 (31.3–49.3)	0.80
	Missing	21	8	
Calculated Reaction Frequency, CRF	<85%	104 (91%)	43 (90%)	0.77
	>85%	10 (9%)	5 (10%)	
	Missing	0	0	

TABLE 3 | Comparison of transplant characteristics.

Transplant characteristics	Variable	DBD (N = 114)	DCD (N = 48)	*p*-value
Era of transplantation	2005–2009	36 (32%)	18 (37%)	0.76
	2010–2014	53 (46%)	20 (42%)	
	2015–2018	25 (22%)	10 (21%)	
Level of HLA mismatch	Level 1	4 (4%)	2 (4%)	0.25
	Level 2	13 (11%)	4 (8%)	
	Level 3	42 (37%)	11 (23%)	
	Level 4	55 (48%)	31 (65%)	
Cold ischemia time, mins		688 (548.5–781.5)	720 (578.0–832.0)	0.19
	Missing	14	9	
Anastomosis time, mins		33 (27–40.2)	37.5 (31.5–44)	0.05
	Missing	20	6	
Exocrine drainage technique	Enteric	64 (56%)	30 (63%)	0.19
	Bladder	38 (33%)	17 (35%)	
	Missing	12 (11%)	1 (2%)	
Induction immunosuppression	Depleting agent	93 (82%)	41 (85%)	0.60
	Non-depleting agent	20 (17%)	6 (13%)	
	Missing	1 (1%)	1 (2%)	
Re-transplants		10 (9%)	2 (4%)	0.30

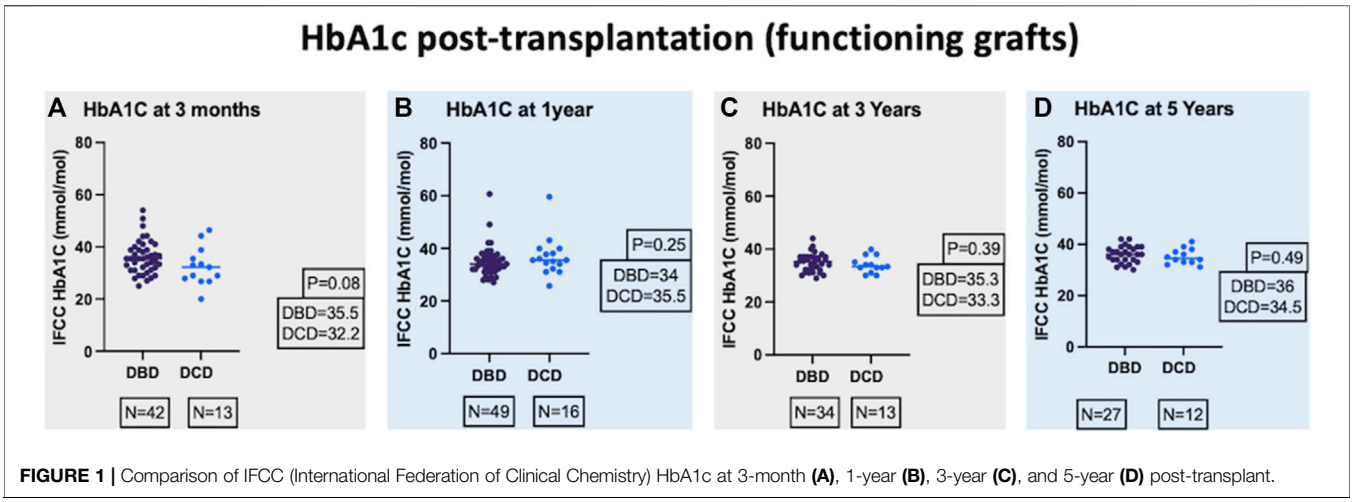


FIGURE 1 | Comparison of IFCC (International Federation of Clinical Chemistry) HbA1c at 3-month **(A)**, 1-year **(B)**, 3-year **(C)**, and 5-year **(D)** post-transplant.

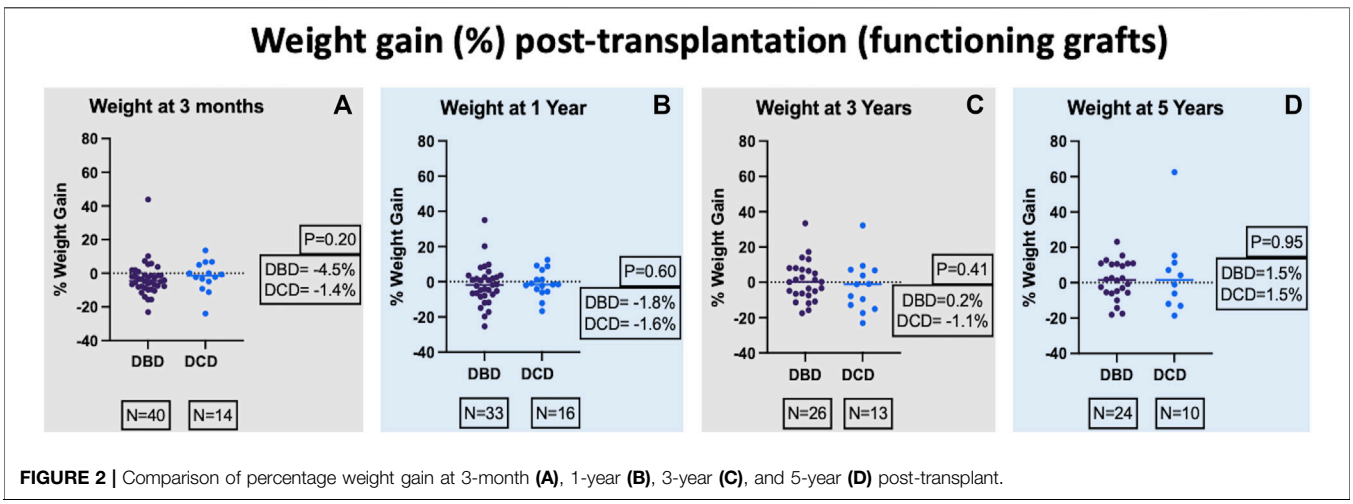


FIGURE 2 | Comparison of percentage weight gain at 3-month **(A)**, 1-year **(B)**, 3-year **(C)**, and 5-year **(D)** post-transplant.

survival between the DBD and DCD recipients (**Figure 4**; log rank $p = 0.95$ and $p = 0.45$, respectively). The 1-, 5-, and 10-year patient survival was 98%, 88%, 78% for DBD and 95%, 85%, 63% for DCD recipients. The 1-, 5-, and 10-year death-censored graft survival was 86%, 59%, 53% for DBD and 88%, 59%, 44% for DCD recipients. The proportion of early graft loss was also similar between the two groups. Data pertaining to early graft loss was not part of the study and hence a detailed analysis of the causes for early graft loss was not possible.

Missing Outcome Data

Graft function was not available for 30 patients (DBD = 20; DCD = 10) at 3-month and 38 patients at 1-year post-transplant (DBD = 23; DCD = 15). Among those with a functioning graft, HbA_1c data was not available for 73 patients at 3-month (DBD = 49; DCD = 24), 41 patients at 1-year (DBD = 26; DCD = 15), 26 patients at 3-year (DBD = 19; DCD = 7), and 18 patients at 5-year post-transplant (DBD = 13; DCD = 5). Pre-transplant weight was not available for 29 patients (DBD = 24; DCD = 5). Among those with a functioning graft, percentage weight gain data was not available for 74 patients at 3-month (DBD = 51; DCD = 23), 57 patients at 1-year (DBD = 42; DCD = 15), 34 patients at 3-year (DBD = 27; DCD = 7), and 23 patients at 5-year post-transplant (DBD = 16; DCD = 7).

DISCUSSION

This is the first study comparing metabolic outcomes alongside survival outcomes between DBD and DCD PTA recipients. Post-transplant HbA1c, in addition to being a marker of graft function, is also known to be an independent predictor of pancreas graft failure [24]. Hence it is vital to consider HbA1c alongside survival outcome. We noted comparable HbA1c for functioning DBD and DCD grafts at all time points. The University of Wisconsin have published similar results at 1-year post transplant but their DCD PTA group had only four patients [7]. In terms of weight gain post-transplant among functioning grafts, there was no significant difference between the two groups at all time points. Both the groups lost weight until 1 year and then

TABLE 4 | Comparison of metabolic outcomes based on era of transplantation.

Outcome/Era	Time post-transplant	DBD	DCD	*p*-Value
HbA_1C (mmol/mol)/Era-1	3-month	35.5 (31.1–36.6)	30.6 (27.1–38)	0.25
	1-year	34 (31.1–35.5)	35.5 (31.6–39.9)	0.44
	3-year	33.3 (30.6–36.1)	33.2 (30.8–38.2)	0.84
	5-year	36 (31.6–38)	36 (31.6–38.5)	0.89
Weight gain (%)/Era-1	3-month	−4.8 (−8.0 to −0.1)	−0.3 (−3.7–5.3)	0.05
	1-year	−6.6 (−11.0 to 2.4)	−1.6 (−4.2 to 1.2)	0.12
	3-year	−3.5 (−10.8 to 2.8)	1.3 (−10.9–13.4)	0.56
	5-year	−0.5 (−5.9–10.9)	−1.0 (−13.0 to 7.1)	0.57
HbA_1C (mmol/mol)/Era-2	3-month	37 (31.5–41.5)	33 (24.5–38.8)	0.32
	1-year	34 (32.2–36.7)	35.8 (33.2–39.2)	0.40
	3-year	36 (34–37)	34 (32–36.5)	0.16
	5-year	37 (34–39)	34 (33–37)	0.12
Weight gain (%)/Era-2	3-month	−6.6 (−8.7 to −2.0)	−6.7 (−20.8 to 4.4)	0.92
	1-year	1.0 (−4.8–5.7)	−0.9 (−14.4 to 9.6)	0.74
	3-year	5.7 (0.5–12.3)	−7.8 (−17.3 to 6.7)	0.07
	5-year	1.9 (−1.1–9.7)	11.3 (−11.9–15.3)	0.54

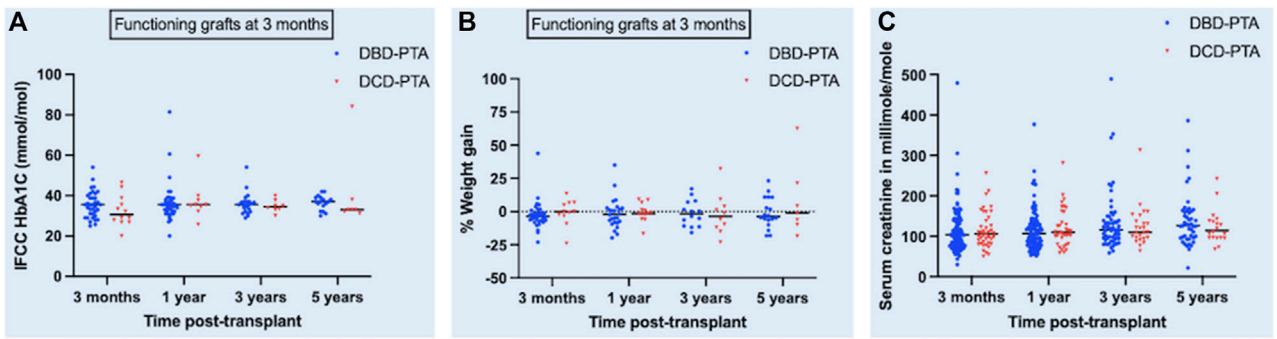


FIGURE 3 | Scatter dot plot showing the evolution of difference in HbA1c [panel **(A)**], percentage weight gain [panel **(B)**] and serum creatinine [panel **(C)**] post-transplant between DBD and DCD groups. Black horizonal line in the plot represents median of each dataset.

started to gain weight in spite of similar HbA1c, rejection rates and steroid usage. The weight gain outcome could have been biased by the missing data. Excessive weight gain post pancreas transplantation especially in the intermediate term have been reported in the setting of simultaneous pancreas kidney transplantation [25, 26]. Post-transplant weight gain has reported to be associated with the development of post pancreas transplant diabetes mellitus [27]. There is no literature evidence on weight gain after pancreas transplantation based on graft type. Weight gain has been included in the analysis of metabolic outcomes as treatment of rejection can lead to excessive weight gain and excessive weight gain can influence glycaemic control. Longer term follow-up could uncover the longitudinal trend of weight gain and its consequences such as post-transplant metabolic syndrome and cardiovascular complications.

When comparing the metabolic outcomes, rejection episodes were considered alongside, as treatment of rejection would influence the metabolic parameters. It is not uncommon to treat rejection episodes based on clinical suspicion and hence, it is difficult to distinguish whether these were actual rejection episodes or graft pancreatitis as both of them present with a similar clinical picture. The need for *de novo* steroids post-transplant were considered as a surrogate for rejection but whether steroids were introduced as part of modulation of the immunosuppression regime to counteract infections remain unknown. The incidence of rejection was similar in both the groups at all time points and this was in a setting where the HLA mismatch and the use of depleting agent for induction were similar as well. 73% of the DCD recipients were on steroid-free maintenance immunosuppression and was similar to DBD recipients. The rejection episodes reported to the UK Transplant registry were not classified as cellular or antibody mediated or mixed rejection and hence, an in-depth analysis was not possible.

It is well known that a successful pancreas transplant halts or reverses the progression of secondary diabetic complications [28, 29]. In addition, clinical trials have reported that maintaining normoglycemia with intensive insulin regimen reduces the cardiovascular complications in type 1 diabetes [30]. In this study, the secondary diabetic macrovascular complications such as non-fatal myocardial infarction, stroke/transient ischemic attack, and limb amputations were clustered together to form a composite endpoint and the incidence was similar in both the groups at 3-month post-transplant. There were no secondary events at the rest of the time points. This could be due to a stringent recipient selection or could be due to missing data. In a recent world

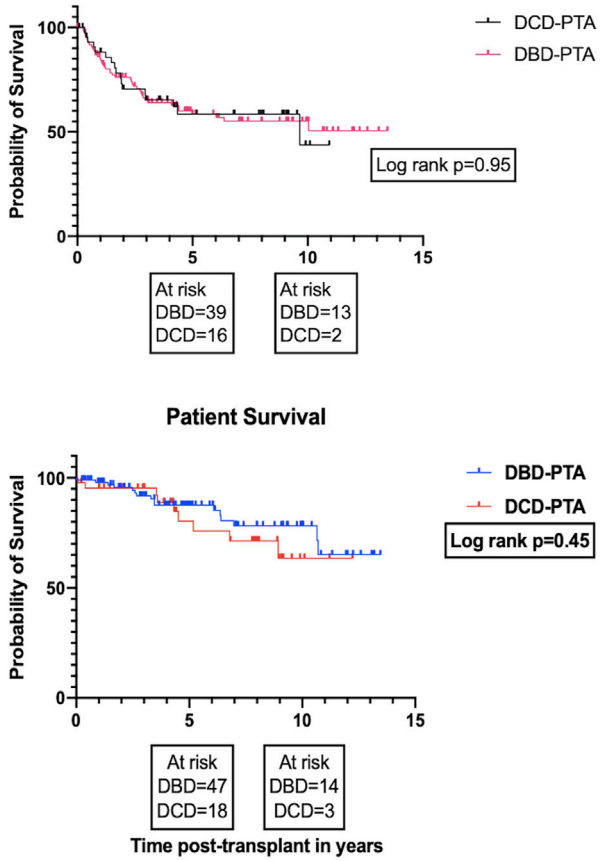


FIGURE 4 | Kaplan Meier plots for death-censored pancreas graft survival and patient survival, respectively.

consensus conference, experts could not draw a conclusion with regards to the effects of PTA on cardiovascular disease progression [31]. The registry does not collect data on modifiable risk factors for coronary artery disease/stroke such as tobacco use, physical activity, blood pressure, and dyslipidaemia. Some of the patients might not have adequate risk factor control. While comparing the incidence of peripheral vascular disease, only amputations were included. The other parameter indicating the progression of vascular disease such as the need for intervention (endovascular or open bypass) was not part of the standard dataset and so not included in our analysis. Hence, in the light of the above, no robust conclusions could be made.

The registry does not record data on diabetic microvascular complications such as retinopathy and neuropathy post-transplantation. Regarding nephropathy, we have compared creatinine post-transplantation between the DBD and DCD recipients and found no significant difference at all time points. There are concerns regarding the risk of accelerated decline in kidney function after a PTA [32, 33]. Even a moderate impairment of kidney function pre-transplant is associated with an increased risk for progression to end stage renal disease after a PTA [34]. Recipients who develop end stage renal failure after a PTA have a three-fold increased risk of mortality [35]. The use of calcineurin inhibitors may contribute to the decline in kidney function. However, improvement in glycaemic control post PTA could reverse the effects of diabetic nephropathy in the longer term [36]. In our study, correlation of creatinine along with proteinuria, concurrent use of ACE (Angiotensin converting enzyme) inhibitors and estimated glomerular filtration rate (eGFR) would have given a better overview about the native renal function. As the rest of the parameters were not part of the standard dataset, we could not correlate them. Future studies could include these parameters and focus on whether DCD grafts have a detrimental effect on the native kidney function after a PTA.

Evaluation of potential differences in HbA1c, weight gain and serum creatinine were performed at each time point separately due to its simple interpretation and its ability to use all the available data. However, per-time point analysis does not consider the overall difference, and inflates the type-1 error rate due to multiple testing. To counteract these deficiencies and the missing data, imputation techniques were necessary and refraining from their use might have led to biased results [37, 38]. Hence, we performed mixed-effects analysis of repeated measures to obtain unbiased results. There was no overall difference in HbA1c, weight gain and serum creatinine between DBD and DCD recipients in the mixed-effects analysis, which further strengthens our study results.

Among the studies reporting survival outcomes of PTA from DCD donors, this study has the highest number of PTA's performed from DCD donors. This study reports similar 1-, 5-, and 10-year graft and patient survival (unadjusted) for DBD and DCD recipients. In comparison with the previous UK transplant registry analysis by Muthusamy et al. [8], the 1-year graft survival in this study was slightly higher in both the groups, whereas the 1-year patient survival was similar. The slightly higher 1-year graft survival was probably due to a greater number of transplants over time in both the groups. The previously reported higher thrombotic graft loss (statistically insignificant) in DCD group was not observed in this study. Despite significantly improved outcomes and the ability to achieve long term normoglycemia without the risk of hypoglycaemia, PTA is still not widely recognized by healthcare professionals involved in diabetes care [28, 29]. There has been a conservative approach in offering PTA and even more so when it comes to acceptance of DCD grafts. This bias leading to selection of better-quality donors in both the groups could explain the similar survival outcomes observed. Pancreas Donor Risk Index (PDRI) could have been calculated (without using DBD/DCD) to compare the difference between the donors on the other variables but it was not performed as both the groups were comparable except for BMI and also recent literature evidence has questioned the inclusion of race as an indicator of pancreas donor quality [39, 40]. Future studies could shed more light on the outcomes of PTA from extended criteria DCD donors.

There was an observed male-to-female recipient ratio of 1: 2 in both DBD and DCD groups. This ratio stands at odds with the proportion of male-to-female incidence of type 1 diabetes mellitus based on the results from large population cohort

studies [41, 42]. One plausible explanation for this difference is that the number of female registrations were nearly equal to male registrations in the national pancreas transplant waiting list [43]. The national pancreas allocation policy does not provide any weightage for female patients. We are unable to comment whether this difference might have influenced our results.

Despite the strengths of the study, we acknowledge the following shortcomings. Firstly, this study suffers a bias due to the retrospective nature, that is inherent to all registry analysis; secondly, the sample size is small to perform a multivariate analysis but this would be an issue in most other studies due to the smaller proportion of the patients undergoing PTA especially from DCD donors. Future studies with multinational collaborative data may be able to generate sufficient numbers to allow a robust comparison. Finally, when comparing the metabolic outcomes, hypoglycaemic episodes, concurrent usage of oral hypoglycaemic agents, and other metabolic parameters such as C-peptide and glucose tolerance test were not compared as they were not part of the registry data. Incorporation of the above parameters along with the pancreas extraction times (cross clamp to organ out of the body) in addition to the standardized reporting would be helpful. Future studies could focus on reporting the functional outcomes utilizing the Igls criteria [44].

This is the first study reporting the outcomes of national data on PTA from DCD donors, and also the first study to compare metabolic outcomes alongside survival outcomes between DBD and DCD donors. PTA from DCD donors leads to similar metabolic and long-term survival outcomes to that of transplants from DBD donors. The findings of this study would alleviate the concerns surrounding the use of DCD graft for PTA and thereby supports their usage to expand the donor pool.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Research data is owned by NHS Blood and Transplant and cannot be provided by the authors. Data can be obtained upon request to NHS Blood and Transplant. Requests to access these datasets should be directed to statistical.enquiries@nhsbt.nhs.uk.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by NHS Blood and Transplant-Pancreas Advisory Group. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

AMu conceptualized the study, JG performed the analysis and wrote the manuscript, all the authors contributed to analysis and interpretation of data, reviewing and editing of the manuscript.

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AUTHOR DISCLAIMER

The data reported have been provided by NHS Blood and Transplant from UK Transplant Registry. The interpretation and reporting of the data are the responsibility of the authors and not by NHSBT.

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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Utilization of the Pancreas From Donors With an Extremely High Pancreas Donor Risk Index: Report of the National Registry of Pancreas Transplantation

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Pancreas transplants from expanded criteria donors are performed widely in Japan because there is a shortage of brain-dead donors. However, the effectiveness of this strategy is unknown. We retrospectively studied 371 pancreas transplants to evaluate the possibility of pancreas transplantation from expanded criteria donors by the Pancreas Donor Risk Index (PDRI). Patients were divided into five groups according to quintiles of PDRI values (Q1–Q5). The 1-year pancreas graft survival rates were 94.5% for Q1, 91.9% for Q2, 90.5% for Q3, 89.3% for Q4, and 79.6% for Q5, and were significantly lower with a lower PDRI ($p = 0.04$). A multivariate analysis showed that the PDRI, donor hemoglobin A1c values, and pancreas transplantation alone significantly predicted 1-year pancreas graft survival (all $p < 0.05$). Spline curve analysis showed that the PDRI was incrementally associated with an increased risk of 1-year graft failure. In the group with a PDRI ≥ 2.87, 8/56 patients had graft failures within 1 month, and all were due to graft thrombosis. The PDRI is a prognostic factor related to the 1-year graft survival rate. However, pancreas transplantation from high-PDRI donors shows acceptable results and could be an alternative when the donor pool is insufficient.

Keywords: pancreas transplantation, graft survival, type 1 diabetes mellitus, thrombosis, prognostic factor

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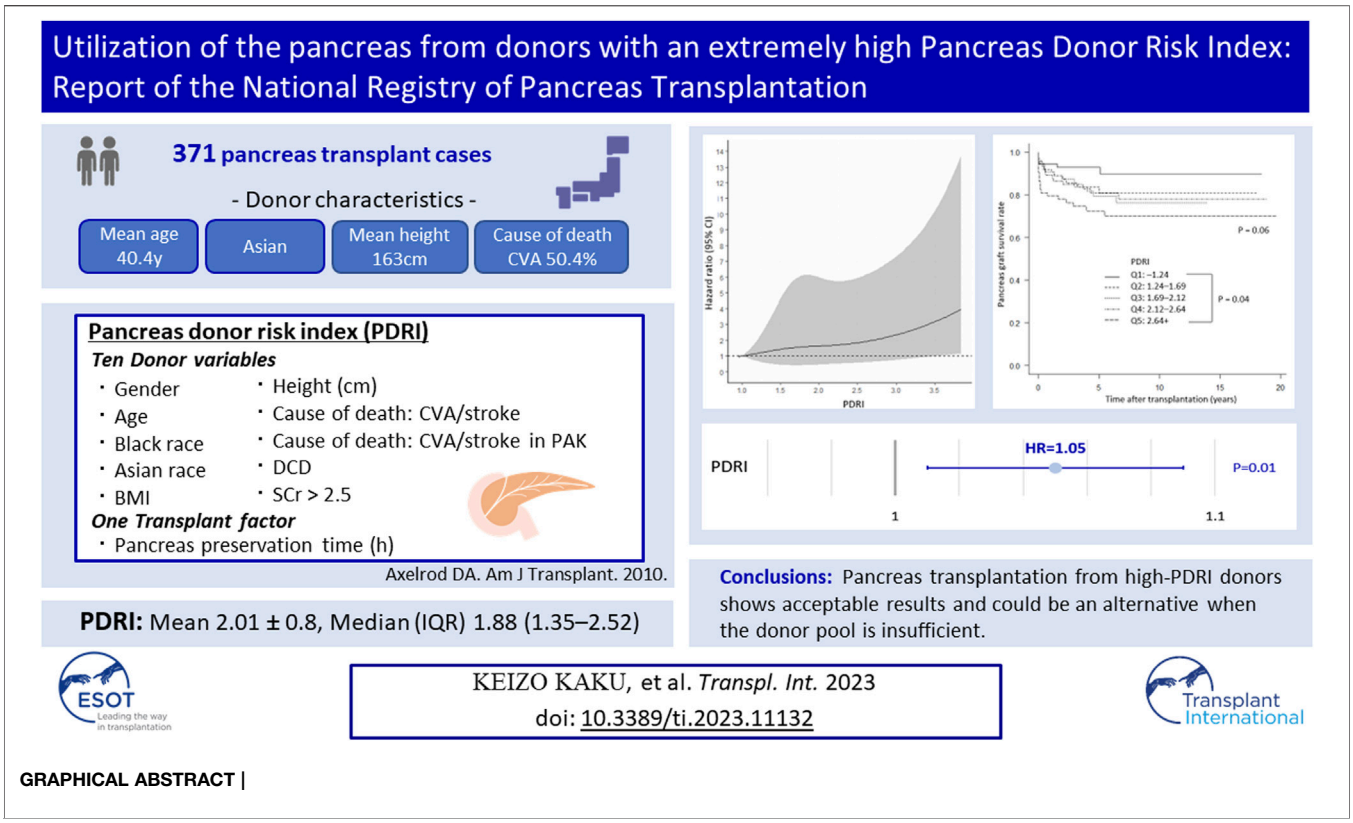
Abbreviations: BMI, body mass index; CI, confidence interval; CPR, cardiopulmonary resuscitation; CVA, cerebrovascular accident; GDA, gastroduodenal artery; HbA1c, hemoglobin A1c; HR, hazard ratio; PAK, pancreas transplantation after kidney transplantation; PDRI, pancreas donor risk index; SCr, serum creatinine; SPK, simultaneous pancreas and kidney transplantation; TIT, total ischemic time; PTA, pancreas transplantation alone.



GRAPHICAL ABSTRACT |

INTRODUCTION

Pancreas transplantation enables insulin withdrawal in patients with insulin-dependent diabetes and considerably improves patients' survival and quality of life [1–4]. However, in Japan, a shortage of brain-dead donors has resulted in a long waiting period. Pancreas transplantation from expanded criteria donors is widely performed because a prolonged waiting period worsens the prognosis of life [5, 6]. In Japan, the donor age is relatively high, with 43% of donors older than 45 years, and 51% of deaths are due to cerebrovascular accidents [7]. Although pancreas transplants are performed in such a special background with many expanded criteria donors, the results are relatively excellent [7]. However, a major drawback for expanded criteria donors is the lack of objective criteria. In practice, the donor's eligibility is determined by each facility's criteria on the basis of a comprehensive evaluation of factors, such as the donor's age, weight, body mass index (BMI), and hemoglobin A1c (HbA1c) values. Japanese national data analyses have reported that the donor's age is not associated with the prognosis [8] and that no single donor factor affects the prognosis [9], but which expanded criteria donors are acceptable remain unclear. There are a variety of factors that define an expanded criteria donor; therefore, it should be evaluated using a comprehensive and objective index.

In pancreas transplantation, the Pancreas Donor Risk Index (PDRI), which was reported by Axelrod et al. in 2010, is currently used to predict 1-year pancreas graft survival as a pre-procurement scoring system [10]. The PDRI was created using 10 donor factors and the pancreas preservation time for the US population. The donor factors consist of the following: sex, age, black race, Asian race, BMI, height, cerebrovascular accident (CVA)/stroke, CVA/stroke in pancreas transplantation after kidney transplantation (PAK), donation after circulatory death, and serum creatinine (SCr) concentrations. The PDRI is designed so that the median donor has a Donor Risk Index of 1.0. A higher Donor Risk Index indicates a higher risk of graft failure. An elevated PDRI is associated with an increased 1-year graft failure rate. A review of the reports that have evaluated the PDRI to date showed that the highest value of the PDRI was 3.40 [11]. Additionally, only a relatively narrow range of the PDRI has been used to evaluate the PDRI [12–18].

A high percentage of grafts are discarded because pancreas grafts are often evaluated under relatively strict criteria [19, 20]. In recent years, there has been a trend to make effective use of pancreatic grafts, which have been discarded in the past, for the purpose of effective use of organs. In the absence of other risk factors, deregulating the criteria for BMI and donor age is acceptable [19]. Furthermore, transplantation from a mildly obese donor can be safely performed [21]. In this trend of reregulating donor criteria and increasing transplantation opportunities, Japanese data, which have accumulated a large number of transplant results from expanded criteria donors, are considered to be effective for determining donor indications. This study aimed to evaluate pancreas transplant donors in Japan

using the PDRI and to examine the possibility of the effective use of expanded criteria donors.

PATIENTS AND METHODS

Study Population

A total of 400 pancreas transplants performed at 18 certified pancreas transplant centers in Japan between January 2001 and July 2019 were included in this study. Of these, 371 cases were included after excluding 27 cases of living pancreas transplantation and two cases of incomplete data. The primary disease was type 1 diabetes mellitus in all cases.

The following clinical data were retrospectively extracted from the national database administered by the Japan Society for Pancreas and Islet Transplantation: transplantation type, recipient age, recipient sex, recipient height, recipient BMI, duration of type 1 diabetes mellitus, episode of preoperative dialysis, duration of dialysis, donor age, donor sex, donor height, donor BMI, donor HbA1c concentrations, cause of death, episode of cardiopulmonary resuscitation, SCr concentrations, total ischemic time of the pancreas graft, pancreas graft position, ductal management, type of venous drainage, artery reconstruction, gastroduodenal artery reconstruction, and portal vein extension. Written informed consent was obtained for enrollment in the registry of the Japan Society for Pancreas and Islet Transplantation. The application and approval of the institutional review board were exempt because all data and information used in this study were de-identified. This study was conducted in accordance with the principles of the Declaration of Helsinki and Istanbul.

Study Design

The PDRI of Japanese patients with a pancreas transplant was calculated according to the formula reported by Axelrod et al. [10] Several cutoff values for the PDRI were set, and the short-term pancreas graft survival rate was verified. The short-term graft survival rate was defined as the 1-year graft survival rate. To analyze the long-term prognosis, the 5-year graft and patients' survival rates were verified. An analysis of prognostic factors related to 1-year pancreas graft survival was performed. The target population was narrowed down to patients with a high PDRI, and the 1-year graft survival rate was verified. Pancreas graft failure was defined as the time when the C-peptide value became <0.3 ng/mL or at the time of graft extraction.

Statistical Analysis

Categorical variables are presented as the count (percentage) and were compared using Fisher's exact test or the χ^2 test, as appropriate. Continuous variables are presented as the mean ± standard deviation and were analyzed using the Mann–Whitney U test. Kaplan–Meier curves with log rank tests were used to examine graft and patients' survival. Bonferroni correction was used to adjust for multiple comparisons. Potential risk factors for 1-year pancreas graft survival were assessed using univariate and multivariate Cox proportional hazards analyses. Restricted cubic spline curves were plotted to describe the multivariable-adjusted association between the PDRI and the hazard ratio (HR) with the 95% confidence interval (CI) for graft survival. The cutoff value of the PDRI calculated from receiver operating characteristic curve analysis was chosen as the reference for the spline plot. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using JMP 16.0.0 (SAS Institute, Cary, NC), EZR (Easy R) version 1.54 (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [22], and R version 4.1.2 (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Distribution of the PDRI

The distribution of the mean PDRI of the 371 patients in Japan is shown in **Figure 1A**. There were 308 simultaneous pancreas kidney transplantations, 49 PAKs, and 14 pancreas transplantations alone (PTAs), and all but three patients who underwent transplantation from cardiac death donors underwent transplantation from brain-dead donors. The mean PDRI was 2.01 ± 0.8 and the median PDRI (interquartile range; IQR) was 1.88 (1.35–2.52). The distribution of the PDRI according to the transplantation type is shown in **Figure 1B**.

Pancreas Graft Survival Rate and Patients' Survival Rate by the PDRI

Forty of the 371 patients had pancreas graft failure within 1 year of transplantation. The median (interquartile range) time to pancreas graft failure was 14 days (2.75–113.25 days). The causes of graft were thrombosis in 22 cases, rejection in seven cases, graft duodenal perforation in seven cases, non-adherence in two cases, recurrent type 1 diabetes in one case, and unknown in one case. Patients were divided into five groups (Q1–Q5) according to the quintile of the PDRI value (**Table 1**). Significant differences in donor age ($p < 0.001$), height ($p < 0.01$), BMI ($p < 0.001$), cause of death ($p < 0.001$), and TIT of the pancreas graft ($p = 0.01$), which are factors that constitute the PDRI, were found between the five groups. Other than the factors constituting the PDRI, significant differences were observed in HbA1c values ($p < 0.001$), cardiopulmonary resuscitation ($p < 0.01$), and graft position ($p = 0.01$) between the groups. The 1-year pancreas graft survival rates were 94.5% for Q1, 91.9% for Q2, 90.5% for Q3, 89.3% for Q4, and 79.6% for Q5, which were significantly lower with a lower PDRI ($p = 0.04$, **Figure 2A**). With regard to the long-term prognosis, the 5-year pancreas graft survival rates were 92.9% for Q1, 83.7% for Q2, 79.3% for Q3, 81.0% for Q4, and 72.5% Q5. The 5-year pancreas graft survival rate for Q5 was significantly lower than that for Q1 ($p = 0.04$, **Figure 2B**). The 5-year patients' survival rate was not significantly different between the groups (**Figure 2C**).

Comparison Between Japanese Donors and Reference Donors

Axelrod et al. defined the following as reference donors with a PDRI = 1: male sex, 28 years old, non-black, non-Asian, BMI of 24 kg/m^2, height of 173 cm, cause of death is not CVA, total ischemic time of 12 h for the pancreas graft, no donation after circulatory death, and creatinine concentrations < 2.5 mg/dL [10]. **Table 2**

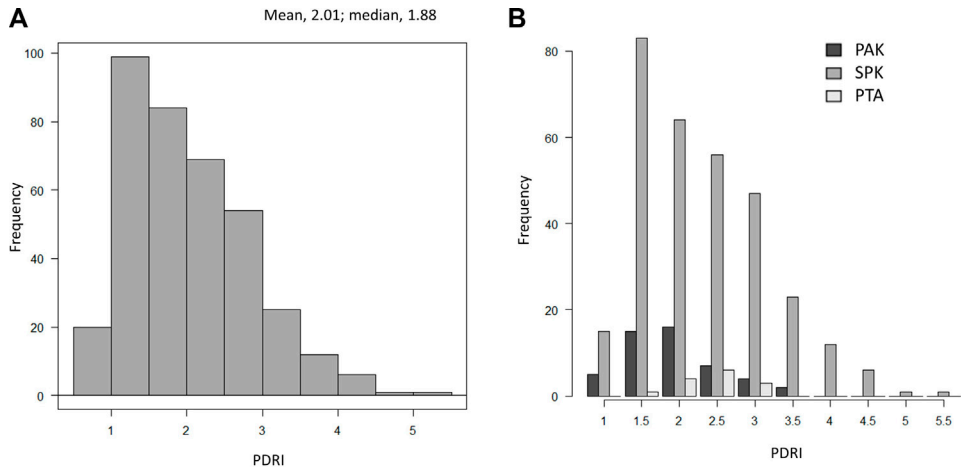


FIGURE 1 | Distribution of PDRI values. **(A)** Distribution of PDRI values in Japan. **(B)** Distribution of PDRI values in Japan by the transplant type. PDRI, pancreas donor risk index; PAK, pancreas transplantation after kidney transplantation; SPK, simultaneous pancreas and kidney transplantation; PTA, pancreas transplantation alone.

shows the features of the average donor in Japan. The median value of each variable was adopted for continuous variables, and factors that accounted for a larger proportion were adopted as categorical variables. As a result, the PDRI was 1.38 times higher for those aged 40.4 years, 1.17 times higher for Asians, 1.06 times higher for a height of 163 cm, and 1.23 times higher for death due to CVA. The incorporation of these factors increased the PDRI value of the average Japanese donor, with an average PDRI value as high as 2.01.

Univariate and Multivariate Analyses of Associations of Various Factors With 1-Year Pancreas Graft Failure

A Cox proportional hazards model was used to identify the factors associated with 1-year pancreas graft failure. The univariate analysis showed that the PDRI, donor HbA1c values, cause of death, PAK, and PTA were independent factors that significantly predicted 1-year pancreas graft survival (**Table 3**). The multivariate analysis excluding the cause of death and PAK included in the PDRI formula showed that the PDRI, donor HbA1c values, and PTA were significant independent factors that predicted 1-year pancreas graft survival (**Table 3**). A continuous multivariable-adjusted association between the PDRI and 1-year pancreas graft failure was also shown by a restricted cubic spline curve. A median PDRI value of 1.88 and a PDRI of 1.00 were chosen as the reference for each spline plot. The spline curve analysis showed that the PDRI was incrementally associated with an increased risk of 1-year graft failure (**Figures 3A,B**).

Transplant Outcomes From Donors With an Extremely High PDRI

The range of PDRI values evaluated by Axelrod et al. ranged from 0.64 to 2.86 [10], and results from donors with a PDRI > 2.86 have not been validated. Therefore, we focused our study on 56 patients with a PDRI ≥ 2.87. The patients were divided into two groups according to a PDRI of 2.87. There were significant differences in the PDRI ($p < 0.001$), age ($p < 0.001$), height ($p < 0.01$), BMI ($p < 0.001$), HbA1c level ($p < 0.01$), death at CVA ($p < 0.001$), and PAK ($p = 0.03$) between the two groups (**Supplementary Table S1**). When we compared the 1-year pancreas graft survival rate among the groups, the group with a PDRI ≥ 2.87 had a significantly lower survival rate than the group with a PDRI < 2.87 (78.4% vs. 91.0%) ($p < 0.01$, **Supplementary Figure S1**). In the group with a PDRI ≥ 2.87, 16 cases of graft failure were observed during the entire observation period (19.5 years). Additionally, 12 of the 16 cases showed graft failure within 1 year. The causes of the 12 graft failures were thrombosis in 8 patients, rejections in 2, duodenal perforation in 1, and unknown in 1. Furthermore, 8 of the 12 patients had graft failure within 1 month, and the reason for all of these graft failures was graft thrombosis.

DISCUSSION

In Japan, pancreas transplants from expanded criteria donors are frequently performed owing to the unique shortage of brain-dead donors. In this study, the mean and median PDRI values were 2.01 and 1.88, respectively. The reason for this finding is that the donors were older, and the cause of death was often cerebrovascular disease (**Table 2**). These data are clearly higher than those reported in Poland with a mean PDRI of 0.96 [12], in Netherlands with a median PDRI of 1.24 [14], in Spain with a mean PDRI of 1.08,[16] in the UK with a median PDRI of 1.30,[11] in Germany with a median PDRI of 1.30 [13], and in Norway with a median PDRI of 0.93 (**Table 4**) [17]. Despite the high number of expanded criteria donors in our study, the short- and long-term graft survival rates were acceptable (**Figures**

TABLE 1 | Cohort characteristics.

Characteristics	PDRI					*p*-value
	Q1–1.24 (*n* = 73)	Q2 1.24–1.69 (*n* = 75)	Q3 1.69–2.12 (*n* = 74)	Q4 2.12–2.64 (*n* = 75)	Q5 2.64+ (*n* = 74)	
Recipient factors						
Age (years)	43.8 ± 6.8	43.9 ± 9.2	44.3 ± 7.0	43.3 ± 7.8	45.8 ± 8.1	0.34
Sex (female), n (%)	46 (63.0)	43 (57.3)	49 (66.2)	51 (68.0)	42 (56.8)	0.51
Height (cm)	161.4 ± 9.5	161.3 ± 8.1	160.1 ± 7.4	160.5 ± 7.8	161.2 ± 7.8	0.85
BMI (kg/m^2)	20.7 ± 2.6	20.7 ± 2.7	21.2 ± 2.7	21.2 ± 2.8	20.5 ± 2.7	0.41
Duration of diabetes (years)	27.3 ± 7.7	29.1 ± 7.8	28.0 ± 8.8	28.0 ± 8.6	28.8 ± 7.4	0.69
Preoperation dialysis, n (%)	61 (83.6)	63 (84.0)	61 (82.4)	61 (81.3)	68 (91.9)	0.36
Duration of dialysis (years)	6.1 ± 5.3	6.6 ± 5.9	6.3 ± 5.8	6.0 ± 5.5	7.1 ± 5.6	0.79
Donor factors						
Age (years)	22.3 ± 5.4	30.9 ± 10.5	42.5 ± 6.5	48.5 ± 5.4	57.4 ± 5.8	<0.001
Sex (female), n (%)	26 (36.1)	29 (38.7)	32 (43.2)	35 (46.7)	39 (52.7)	0.27
Height (cm)	166.6 ± 11.0	161.7 ± 17.1	164.1 ± 8.7	165.4 ± 8.5	159.7 ± 7.9	<0.01
BMI (kg/m^2)	21.5 ± 3.4	20.5 ± 3.4	22.0 ± 3.4	22.9 ± 3.8	22.5 ± 3.1	<0.001
HbA1c (%)	5.4 ± 0.3	5.3 ± 0.3	5.4 ± 0.4	5.5 ± 0.3	5.6 ± 0.5	<0.001
Cause of death, n (%)						<0.001
CVA	6 (8.2)	20 (26.7)	39 (52.7)	57 (76.0)	65 (87.8)	
Anoxia	28 (38.4)	20 (26.7)	12 (16.2)	9 (12.0)	3 (4.1)	
Trauma	28 (38.4)	20 (26.7)	11 (14.9)	7 (9.3)	4 (5.4)	
Other	11 (15.1)	15 (20.0)	12 (16.2)	2 (2.7)	2 (2.7)	
CPR	41 (56.2)	39 (52.0)	44 (59.5)	26 (34.7)	27 (36.5)	<0.01
SCr (mg/dL)	0.78 ± 0.50	1.14 ± 1.75	1.04 ± 1.08	1.34 ± 1.56	1.23 ± 1.26	0.10
PDRI	1.06 ± 0.09	1.46 ± 0.13	1.89 ± 0.13	2.39 ± 0.15	3.25 ± 0.53	<0.001
Operative factors						
Era						0.28
2001–2010	11 (15.1)	18 (24.0)	17 (23.0)	23 (30.7)	17 (23.0)	
2011–2019	62 (84.9)	57 (76.0)	57 (77.0)	52 (69.3)	57 (77.0)	
Transplantation type						0.03
SPK	57 (78.1)	63 (84.0)	58 (78.4)	62 (82.9)	68 (91.9)	
PAK	16 (21.9)	9 (12.0)	13 (17.6)	7 (9.3)	4 (5.4)	
PTA	0 (0.0)	3 (4.0)	3 (4.1)	6 (8.0)	2 (2.7)	
TIT of the pancreas graft (h)	11.9 ± 2.3	11.9 ± 2.7	11.8 ± 3.0	12.6 ± 3.0	13.3 ± 2.8	<0.01
Graft position (Peritoneal/retroperitoneal)	42/31	51/24	45/28	60/15	58/16	0.01
Ductal management (ED/BD)	62/11	66/9	64/10	65/10	68/6	0.76
Systemic/portal drainage	72/1	74/1	72/2	72/3	74/0	0.44
Carrel patch/Y graft	64/9	63/12	68/6	59/16	66/8	0.16
GDA extension, n (%)	32 (43.8)	34 (45.3)	39 (52.7)	46 (61.3)	46 (62.2)	0.07
Portal vein extension, n (%)	14 (19.2)	18 (24.0)	16 (21.6)	20 (26.7)	16 (21.6)	0.85

Values represent n, n (%), or the mean ± standard deviation.

Abbreviations: PDRI, pancreas donor risk index; BMI, body mass index; HbA1c, hemoglobin A1c; CVA, cerebrovascular accident; CPR, cardiopulmonary resuscitation; SCr, serum creatinine; SPK, simultaneous pancreas and kidney transplantation; PAK, pancreas transplantation after kidney transplantation; PTA, pancreas transplantation alone; TIT, total ischemic time; ED, enteric drainage; BD, bladder drainage; GDA, gastroduodenal artery.

2A,B), and the patients' survival rates were also satisfactory (**Figure 2C**). These results are comparable to those in populations with a low PDRI [10–13, 16, 17] and in the United States [23]. This finding suggests that many donors with a high PDRI potentially have favorable outcomes.

In the multivariate analysis of factors involved in the 1-year graft prognosis, the PDRI, donor HbA1c levels, and PTA were prognostic factors. This analysis confirmed the validity of evaluating the PDRI using the Japanese data. There have been two types of reports on the effectiveness of PDRI as a prognostic factor. Some reports showed that the PDRI was effective [12–14], whereas others showed that the PDRI was not effective [15–17], which may be due to racial differences. Some studies reported that the PDRI was only effective in simultaneous pancreas and kidney transplantation only [11–18]. Our results suggest that although the PDRI is a prognostic factor, even donors with a high PDRI can have acceptable outcomes.

Increasing the donor pool is not a problem that can be accomplished in the short term, and donors with high PDRIs must also be used. However, the acceptable range of the PDRI must be discussed. In this study, as shown by the spline curves in **Figures 3A,B**, the HR increased as the PDRI increased. We found lower short- and long-term graft survival rates in Q5 with a PDRI of 2.64 or higher (**Figures 2A,B**). The increase in HR was steep from a PDRI of 2.64, and this value was proposed as the cutoff value (**Figures 3A,B**). The mean donor age for Q5 was 57.4 years (**Table 1**), which may be considered as a cutoff value for donor age.

In the study by Axelrod et al., PDRI values were validated only up to 2.87 [10]. In this study, 56 patients had a PDRI > 2.87. We found that the group with a PDRI < 2.87 had a

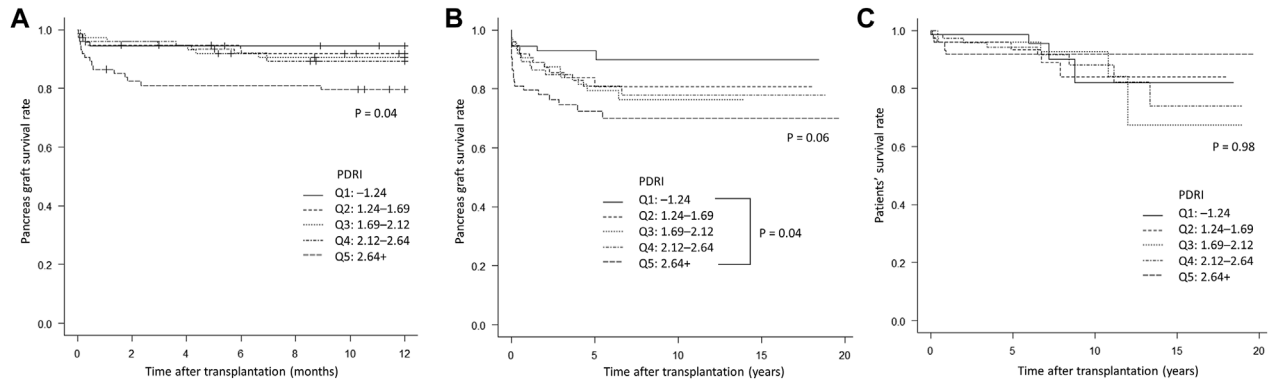


FIGURE 2 | Kaplan–Meier curves comparing the five groups (Q1–Q5) according to quintiles of PDRI values. **(A)** Short-term pancreas graft survival rate. **(B)** Long-term pancreas graft survival rate. **(C)** Patients' survival rate. PDRI, pancreas donor risk index.

TABLE 2 | Comparison between Japanese donors and reference donors.

Donor characteristics	Reference donor (PDRI = 1.00)	Japanese donor	Fluctuation in the PDRI
Sex	Male	Male	1.00
Age (years)	28	40.4	1.38
Black race	No	No	1.00
Asian race	No	Yes	1.17
BMI (kg/m^2)	24	21.9	1.00
Height (cm)	173	163	1.06
Cause of death: CVA/stroke	No	Yes	1.23
Cause of death: CVA/stroke in PAK	No	Yes	0.93
Pancreas preservation time (h)	12	12.3	1.00
DCD	No	No	1.00
SCr > 2.5 (mg/dL)	No	No	1.00

Abbreviations: PDRI, pancreas donor risk index; BMI, body mass index; CVA, cerebrovascular accident; PAK, pancreas transplantation after kidney transplantation; DCD, donation after circulatory death; SCr, serum creatinine.

better graft prognosis, but the group with a PDRI ≥ 2.87 also had an acceptable 1-year graft survival rate of 78.4%. However, the significantly low graft survival rate cannot be overlooked and should be limited to older recipients or patients who cannot wait any longer because of frequent hypoglycemic attacks.

A high rate of thrombosis occurs in transplants from donors with a high PDRI, which leads to graft failure. Donor risk factors for thrombosis are age [23–26], cerebrovascular cause of death, and a high BMI [26–28]. With regard to preservation factors, the total ischemic time has a considerable effect on graft failure due to thrombosis [29]. These factors are also components of the PDRI, and the results are congruent. Pancreas transplants from donors with an extremely high PDRI have a high incidence of thrombosis, resulting in early graft failure within 1 month. However, once this period is exceeded, stable results are obtained. Therefore, the use of anticoagulants, such as heparin, is left to the discretion of each institution in Japan, but the use of anticoagulants is strongly recommended in cases of an extremely high PDRI.

A strength of this study is that, to the best of our knowledge, this is the first report on the evaluation of the PDRI against a background of data from a large number of expanded criteria donors. We were able to show the results of patients with extremely high PDRI values, which have rarely been previously verified. Additionally, external evaluation of the PDRI has mainly been conducted in Western populations, and whether the PDRI is effective in Asian populations is unknown [30]. The present study shows an association between the PDRI and prognosis in the Japanese population. This finding suggests that the PDRI can be used as a tool for a pre-procurement scoring system even in the Asian population. However, notably, the range of the PDRI is different from that in the Western population. Other limitations of this study are that the number of cases was not large enough and it was a retrospective study. To validate the effectiveness of the PDRI, we evaluated the 1-year pancreas graft outcomes, which are affected not only by donor factors, but also by other factors (e.g., recipient factors, rejection, and recurrence of type 1 diabetes mellitus). However, the involvement of these factors cannot be ruled out completely. Regarding generalizability, all patients were from Japanese facilities and all patients were Japanese nationals. There is a lack of validation in other Asian

TABLE 3 | Univariate and multivariate analyses of associations of various factors with 1-year pancreas graft failure.

Coefficient variable	Univariate			Multivariate		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Recipient factors						
Age	1.01	0.97–1.05	0.80			
Sex (female)	1.45	0.74–2.84	0.29			
Height	0.97	0.93–1.01	0.08			
BMI	1.05	0.94–1.17	0.41			
Duration of diabetes	1.02	0.98–1.06	0.34			
Duration of dialysis	1.03	0.98–1.09	0.24			
Donor factors						
PDRI, per 0.1	1.05	1.02–1.09	<0.01	1.05	1.01–1.09	0.01
Age	1.02	1.00–1.05	0.05			
Sex (female)	0.96	0.51–1.79	0.89			
Height	1.00	0.97–1.02	0.76			
BMI	1.05	0.96–1.14	0.30			
HbA1c, per 0.1%	1.10	1.03–1.17	<0.01	1.08	1.01–1.15	0.03
Cause of death (CVA)	2.09	1.08–4.05	0.03			
CPR	1.09	0.59–2.03	0.78			
SCr	1.04	0.84–1.29	0.72			
Operative factors						
Era						
2001–2010	0.69	0.31–1.56	0.37			
2010–2019	1.45	0.64–3.27	0.37			
Transplantation type						
SPK	0.60	0.27–1.30	0.19			
PAK	2.39	1.23–4.63	<0.01			
PTA	3.63	1.42–9.26	<0.01	3.65	1.43–9.35	<0.01
TIT	1.07	0.96–1.19	0.20			
GDA reconstruction	0.79	0.42–1.47	0.45			
Portal vein extension	1.13	0.55–2.32	0.73			

Abbreviations: HR, hazard ratio; CI, confidence interval; BMI, body mass index; PDRI, pancreas donor risk index; HbA1c, hemoglobin A1c; CVA, cerebrovascular accident; CPR, cardiopulmonary resuscitation; SCr, serum creatinine; SPK, simultaneous pancreas and kidney transplantation; PAK, pancreas transplantation after kidney transplantation; PTA, pancreas transplantation alone; TIT, total ischemic time; GDA, gastroduodenal artery.

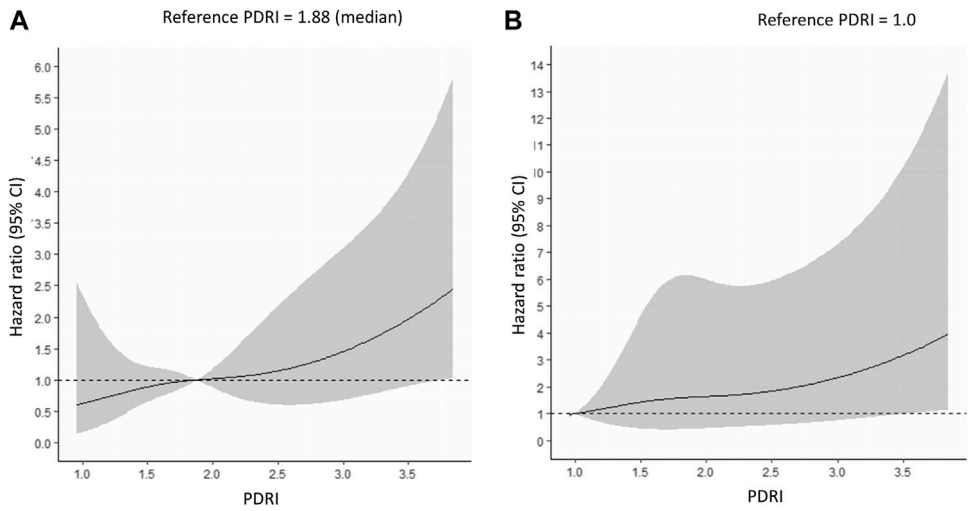


FIGURE 3 | Multivariable-adjusted restricted cubic spline plots of the hazard ratio for 1-year pancreas graft failure. The solid line represents the hazard ratio, and the gray area represents the 95% CI. **(A)** Reference PDRI = 1.88 (median). **(B)** Reference PDRI = 1.0. PDRI, pancreas donor risk index; CI, confidence interval.

countries. Although all transplants were performed at specialist-certified centers, surgeon-related factors may have contributed to the outcomes. Additionally, the study period was extended over almost two decades. The mean PDRI value in patients in 2001–2010 was 2.11 ± 0.76 and that in 2011–2019 was 1.98 ± 0.81 (data not shown in the

TABLE 4 | List of PDRI data from various national registries.

Study	Country	Total sample	Range of PDRIs	Mean PDRI	Median PDRI
Axelrod DA et al. [10]	United States	9,401	0.64–2.86	NA	1.00
Mittal S et al. [11]	United Kingdom	1,021	0.49–3.40	NA	1.30
Śmigielska K et al. [12]	Poland	407	0.59–1.33	0.96	NA
Ayami MS et al. [13]	Germany	327	0.54–2.40	NA	1.30
Blok JJ et al. [14]	Netherlands	349	0.68–2.31	NA	1.24
Franz C et al. [15]	Germany	108	0.96–1.38 (IQR)	NA	1.12
Salamanca-Bustos JJ et al. [16]	Spain	126	0.70–2.00	1.08	NA
Kjøsen G et al. [17]	Norway	344	0.58–2.41	NA	0.93
Mittal S et al. [18]	United Kingdom	90	0.69–2.74	NA	1.73
Present study	Japan	371	0.87–5.03	2.01	1.88

Abbreviations: PDRI, pancreas donor risk index; NA, not available; IQR, interquartile range.

text), which indicates that donor indications have become more rigorous over time. Advances in pharmacology, technology, and surgical methods during this period could have biased the results toward more recent cases, and older cases may no longer be representative of state-of-the-art situations.

In conclusion, the PDRI is an effective evaluation tool for pancreas transplantation in Japan. Pancreas transplantation from donors with a high PDRI can be performed with acceptable results as an alternative until the donor pool is increased. However, the early development of thrombosis should be noted in cases of an extremely high PDRI.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the Japan Society for Transplantation but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Requests to access the datasets should be directed to The Japan Society for Transplantation, http://www.asas.or.jp/jst/.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KK contributed to the design, data analysis, and implementation of the study, and to the writing of the manuscript. SK, YS, TM, HN, YO, YT, TI, and TK contributed to data collection and interpretation. MN conceived the original concept of the study and supervised the project. 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.2023.11132/full#supplementary-material

Supplementary Figure S1 | Kaplan–Meier curve for comparison of the group with a PDRI < 2.87 and the group with a PDRI ≥ 2.87, with a cutoff value of 2.87.

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ORIGINAL RESEARCH
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Incidence and Prognosis of Colorectal Cancer After Heart Transplantation: Data From the Spanish Post-Heart Transplant Tumor Registry

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In this observational and multicenter study, that included all patients who underwent a heart transplantation (HT) in Spain from 1984 to 2018, we analyzed the incidence, management, and prognosis of colorectal cancer (CRC) after HT. Of 6,244 patients with a HT and a median follow-up of 8.8 years since the procedure, 116 CRC cases (11.5% of noncutaneous solid cancers other than lymphoma registered) were diagnosed, mainly adenocarcinomas, after a mean of 9.3 years post-HT. The incidence of CRC increased with age at HT from 56.6 per 100,000 person-years among under 45 year olds to 436.4 per 100,000 person-years among over 64 year olds. The incidence rates for age-at-diagnosis groups were significantly greater than those estimated for the general Spanish population. Curative surgery, performed for 62 of 74 operable tumors, increased the probability of patient survival since a diagnosis of CRC, from 31.6% to 75.7% at 2 years, and from 15.8% to 48.6% at 5 years, compared to patients with inoperable tumors. Our results suggest that the incidence of CRC among HT patients is greater than in the general population, increasing with age at HT.

Keywords: heart transplantation, prognosis, incidence, colorectal cancer, management

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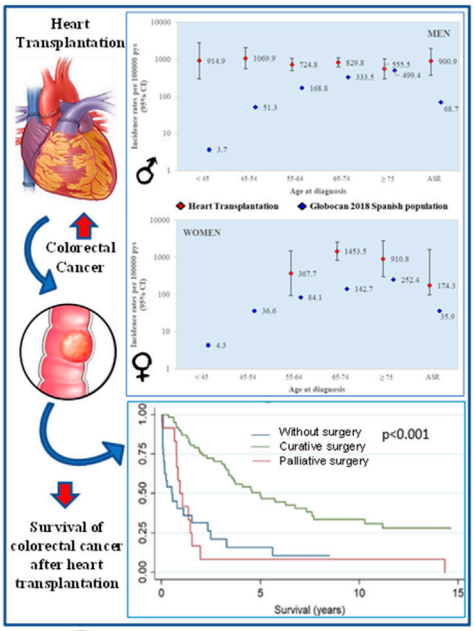
Abbreviations: CRC, colorectal cancer; CIs, confidence intervals; HT, heart transplantation; ISHLT, International society for heart and lung transplantation; Pys, person-years; SPHTTR, Spanish post-heart-transplant tumor registry.



Incidence and Prognosis of Colorectal Cancer after Heart Transplantation: Data from the Spanish Post-Heart Transplant Tumor Registry

Observational, multicenter study including 6,244 heart transplantation (HT) patients (all HT patients in Spain from 1984 to 2018).

- 116 colorectal cancer (CRC) cases were diagnosed, mainly adenocarcinomas, after a mean of 9.3 years post-HT.
- Incidence of CRC increased with age at HT:
  - < 45 years: 56.6 per 100,000 person-years
  - > 64 years: 436.4 per 100,000 person-years
- Incidence rates of CRC for age-at-diagnosis groups were significantly greater than estimated for the general Spanish population, both in women and men.
- Curative surgery, increased the probability of patient survival since a diagnosis of CRC, compared to patients with inoperable tumors:
  - At 5 years: from 15.8% to 48.6%

Our results suggest that the incidence of CRC among HT patients is greater than in the general population, increasing with age at HT.

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GRAPHICAL ABSTRACT |

INTRODUCTION

Throughout the last few decades, the life expectancy of patients with a heart transplant (HT) has increased mainly due to advances in immunosuppression, conferring more weight to long-term causes of morbidity–mortality [1], such as malignancies [2, 3]. The risk of *de novo* malignancy in HT recipients was reported to be 2—4 times higher than that in the general population [4–8]. According to the Spanish Post-Heart-Transplant Tumor Registry (SPHTTR), the most common cancer after HT is skin cancer, followed by noncutaneous solid cancers other than lymphoma. Within this latter group, gastrointestinal tumors are the second most frequent, behind lung cancer [9, 10]. Colorectal cancer (CRC) is the third most common malignancy in the general population worldwide, after lung and breast cancers, although it is the most common cancer in Spain [11]. Although CRC seems to increase slightly after transplantation, the representation of HT recipients in most such studies is low but results are controversial [12–14].

The aim of this study was to report on the incidence of CRC (overall and among different subgroups), its characteristics, the treatment received, and survival among HT recipients. This included analyzing SPHTTR data, which is updated yearly with information on tumors in all HT patients since the beginning of this therapy in Spain in 1984, and compare these results with a reference population.

MATERIALS AND METHODS

We conducted a retrospective, observational, multicenter study that included all patients who underwent a HT from the beginning of this therapy in Spain in 1984 to the 31st December 2018. As a source of information, we used the SPHTTR, which contains the records of HT patients of Spanish hospitals. From a total of 8,482 patients included in the SPHTTR, we excluded 490 pediatric transplants (<16 years), 1,520 patients who died in the first 3 months after HT, 112 combined transplants, and 116 due to retransplantation. The remaining 6,244 patients were followed up to December 2019.

In order to assess the incidence of CRC in different subgroups, the data considered were sex, age at HT, pre-HT smoking history, obesity, background of CRC pre-HT, immunosuppression treatment, anti-viral prophylaxis received, development of CRC, age at diagnosis of CRC, and duration of follow-up (terminated at the earlier of death or the 31st December 2019). To evaluate the effect of changes in immunosuppressive practice or HT protocols, the era in which the HT was performed was introduced as an independent variable. Two eras were considered: the period before (1984–2000) and after (2001–2018) the introduction of interleukin-2 receptor (IL-2R) blockers in Spain. For characterization of post-HT CRC, additional variables were taken into account such as time between HT and CRC diagnosis, localization of the tumor (colon or rectum), pathological features, extension at diagnosis (metastatic, including lymph nodes, or localized), treatment received

(surgery, chemotherapy, radiotherapy), surgical purpose (palliative, curative or none), and survival after CRC diagnosis.

Total incidence (age standardized with the direct method for the world standard population aged >15 years), and incidence in age-at-diagnosis groups (<45, 45–54, 55–64, 65–74, ≥75 years), were compared with GLOBOCAN 2018 estimates for the general Spanish population [15]. A discrepancy between the age of initiation of adulthood used in the SPHTTR (16 years) and the lower limit of the 15–44 years age group used for the world standard population was deemed of no consequence in this study.

This research protocol was approved by the institutional review board of each participating center.

Confidence intervals (CIs) for incidence rates in HT patient age groups were calculated using the quadratic approximation to the Poisson log likelihood for the log rate parameter [16]. Confidence intervals for GLOBOCAN 2018 age-group–specific incidence rate estimates were calculated using the exact method described by Armitage and Berry [17] with Epidat 4.2 [18]. Confidence intervals for age-standardized overall incidence rates were calculated as per Fay and Feuer [19, 20] using Epidat 4.2. Adjusted estimates of relative risk were obtained by means of a Poisson regression analysis. A world standard population was used to obtain adjusted rates in HT patients and the Spanish general population [20]. Postdiagnosis survival curves were constructed by a Kaplan-Meier method and compared using log rank tests to estimate the statistical significance of differences.

Except where otherwise stated, all statistical calculations were performed using Stata v12.0.

RESULTS

Study Population

This study included 6,244 patients (1,186 women [19.0%] and 5,058 men [81.0%]) with a total follow-up of 55396.5 person-years (pys), median follow-up of 8.8 years, and a mean age at HT of 52.1 years. A total of 976 (21.0%) patients were smokers pre-HT, 683 (11.3%) obese, and 16 (7.4%) had a history of CRC before HT surgery. A total of 2,553 patients (40.9%) underwent HT in the pre-IL2R–blocker era, and 3,691 (59.1%) in the most recent era. Of the total patients, 83.0% received induction therapy and 60.6% antivirals post-HT (**Table 1**). The used immunosuppressive agents are listed in (**Table 2**).

TABLE 1 | Patient characteristics.

Number of patients	6,244
Female	1,186 (19.0%)
Mean (SD) age at HT	52.1 (11.5)
Age at HT	
<45 years	1,340 (21.5%)
45–54 years	1,759 (28.2%)
55–64 years	2,516 (40.3%)
≥65 years	629 (10.0%)
Pre-HT smoking[a]	976 (21.0%)
Obesity[b]	683 (11.3%)
Pre-HT colon or rectum tumor[c]	16 (7.4%)
HT era	
1984–2000	2,553 (40.9%)
2001–2018	3,691 (59.1%)
Induction therapy	4,992 (83.0%)
Aciclovir or Ganciclovir after HT[d]	3,387 (60.6%)

[a]*Out of 4,653 patients for whom relevant data were available.*
[b]*Out of 6,048 patients for whom relevant data were available.*
[c]*Out of 215 patients for whom relevant data were available.*
[d]*Out of 5,586 patients for whom relevant data were available.*

Incidence of Colorectal Cancer After Heart Transplantation

With regard to tumors, 2,498 cases were registered, of which 116 were CRC (4.6% of all tumors and 11.5% of noncutaneous solid cancers other than lymphoma). Of these 116 cases, 99 were diagnosed in men and 17 in women. The incidence of CRC increased with age at HT from an average of 56.6 per 100,000 pys among under 45 year olds to 436.4 per 100,000 pys among over 64 year olds. No statistically significant differences were observed related to sex, pre-HT smoking history, obesity, HT era, immunosuppressive practice or antiviral prophylaxis (**Table 3**).

The mean age at diagnosis was 66.0 years (SD 8.8), with three patients under 45, nine aged 45–54 years, 29 aged 55–64 years, 62 aged 65–74 years, and 13 ≥ 75 years.

Incidence Comparison With the General Spanish Population

Contrary to what is observed in the general population, the incidence of CRC post-HT remained fairly constant with increasing age, particularly among males. However, both age- and sex-specific CRC incidences as well as age-standardized overall rates were several-fold higher in every group than GLOBOCAN 2018 estimates for the Spanish population, except for the subgroup of men over 75 for whom no statistically significant differences were found (**Figure 1**).

Colorectal Cancer Characteristics After Heart Transplantation

The mean time between HT and diagnosis of CRC was 9.3 years (SD 5.5 years). Information on tumor extension status at diagnosis was available in 107 cases, the cancer being metastatic in only 12 patients (11.3%). Regarding histopathological characteristics, 91 (85.1%) were adenocarcinomas (**Table 4**). We had no information on the treatment received by 18 patients. Curative surgery was performed on 62 patients (63.3%) and palliative surgery on 12 (12.2%), whereas 24 patients (24.5%) did not undergo any surgical treatment. Chemotherapy was indicated in 32 cases (33.7%) and radiotherapy in 13 (13.7%).

Prognostic Impact of Colorectal Cancer After Heart Transplantation

Within 2 and 5 years after diagnosis, overall Kaplan–Meier survival fell to 59.1% and 39.1%, respectively. No statistically

TABLE 2 | Patients receiving each kind of immunosuppressor (percentages), by period post-HT.

	<3 months	3–12 months	1–2 years	After 2 years	At any time
Cyclosporine	58.2	52.8	46.5	38.5	59.1
Azathioprine	36.2	32.4	27.7	19.4	37.2
Prednisone	85.6	76.6	56.4	44.4	86.0
Tacrolimus	30.1	26.5	19.5	18.3	39.3
MMF	49.7	42.3	34.0	35.4	62.6
Sirolimus	0.7	0.8	0.9	3.8	4.5
Everolimus	1.5	2.6	3.3	7.0	9.7
OKT3	23.4	0.2	0.1	0.1	23.6
Anti-thymocyte globulin	5.6	0.8	0.2	0.1	5.6
Basiliximab	27.7	0.1	0.0	0.0	27.7
Daclizumab	7.0	0.2	0.0	0.0	7.0
N = 6,242					

TABLE 3 | Incidence of colorectal tumors per 100,000 person-years among heart transplant patients and different subgroups. Follow-up, cases, incidence rates and relative risk.

Group	Follow-up (Pys)	Cases	Incidence rate	95% CI		RR	95% CI		p
Total	55396.5	116	209.4	174.6	251.2				
Sex									
Male	44763.6	99	221.2	181.6	269.3	1			
Female	10632.9	17	159.9	99.4	257.2	0.7	0.4	1.2	0.215
Age at HT (years)									
<45	14147.8	8	56.6	28.3	113.1	1			
45–54	16485.9	32	194.1	137.3	274.5	3.4	1.6	7.5	0.002
55–64	20638.3	58	281.0	217.3	363.5	5.0	2.3	10.4	<0.001
≥65	4124.5	18	436.4	275.0	692.7	7.7	3.4	17.8	<0.001
Pre-HT smoking	8721.0	13	149.1	8.6	256.7	0.6	0.4	1.1	0.124
Obesity	5520.2	9	163.0	84.8	313.3	0.8	0.4	1.5	0.471
Pre-HT colorectal tumor	115.9	2	1726.3	431.7	6902.5	8.3	2.1	33.5	<0.001
HT era									
1984–2000	28797.4	56	194.5	149.7	252.7	1			
2001–2018	26599.1	60	225.6	175.1	290.5	1.2	0.8	1.7	0.424
Immunosuppression									
OKT3 (Yes/No)	16864.4	44	260.9	194.2	350.6	1.4	0.9	2.0	0.104
ATG (Yes/no)	6530.4	14	214.4	127.0	362.0	1.0	0.6	1.8	0.982
Basiliximab (Yes/No)	14547.9	25	171.9	116.1	254.3	0.8	0.5	1.2	0.205
Daclizumab (Yes/no)	4096.0	11	268.6	148.7	484.9	1.3	0.7	2.4	0.424
Antiviral prophylaxis									
Aciclovir (Yes/No)	20118.5	42	208.8	154.3	282.5	0.9	0.7	1.4	0.788
Ganciclovir (Yes/No)	18822.8	39	207.2	151.4	283.6	0.9	0.6	1.4	0.768
Aciclovir or Ganciclovir	30373.0	67	220.6	173.6	280.2	1.1	0.73	1.56	0.734

significant differences were observed in the survival curves related to sex (women vs. men), nor to age at diagnosis (under vs. over 55). Curative surgery, performed in 62 of the 74 operable cases, increased the probability of survival since diagnosis from 31.6% to 75.7% at 2 years, and from 15.8% to 48.6% at 5 years, compared to inoperable patients (**Figure 2**).

DISCUSSION

Incidence of Colorectal Cancer After Heart Transplantation

In this study, we found the incidence of CRC considerably greater among Spanish HT patients than that which corresponded to the GLOBOCAN 2018 estimate for the general Spanish population.

Solid organ transplant recipients are at increased risk of cancer [3]. Regarding HT in particular, Youn et al. [4] found that more than 10% of adult HT recipients from the International Society for Heart and Lung Transplantation (ISHLT) registry developed *de novo* malignancy between years 1 and 5 after transplantation, and this outcome was associated with greater mortality. The largest increase was detected in skin cancer, followed by noncutaneous solid cancer, even though, more specifically, the proportion of CRC cases was lower than in our cohort. This result might be explained by the mean time between HT and the diagnosis of CRC in our study being over 9 years, whereas the ISHLT registry included only tumors within 5 years post-HT. The

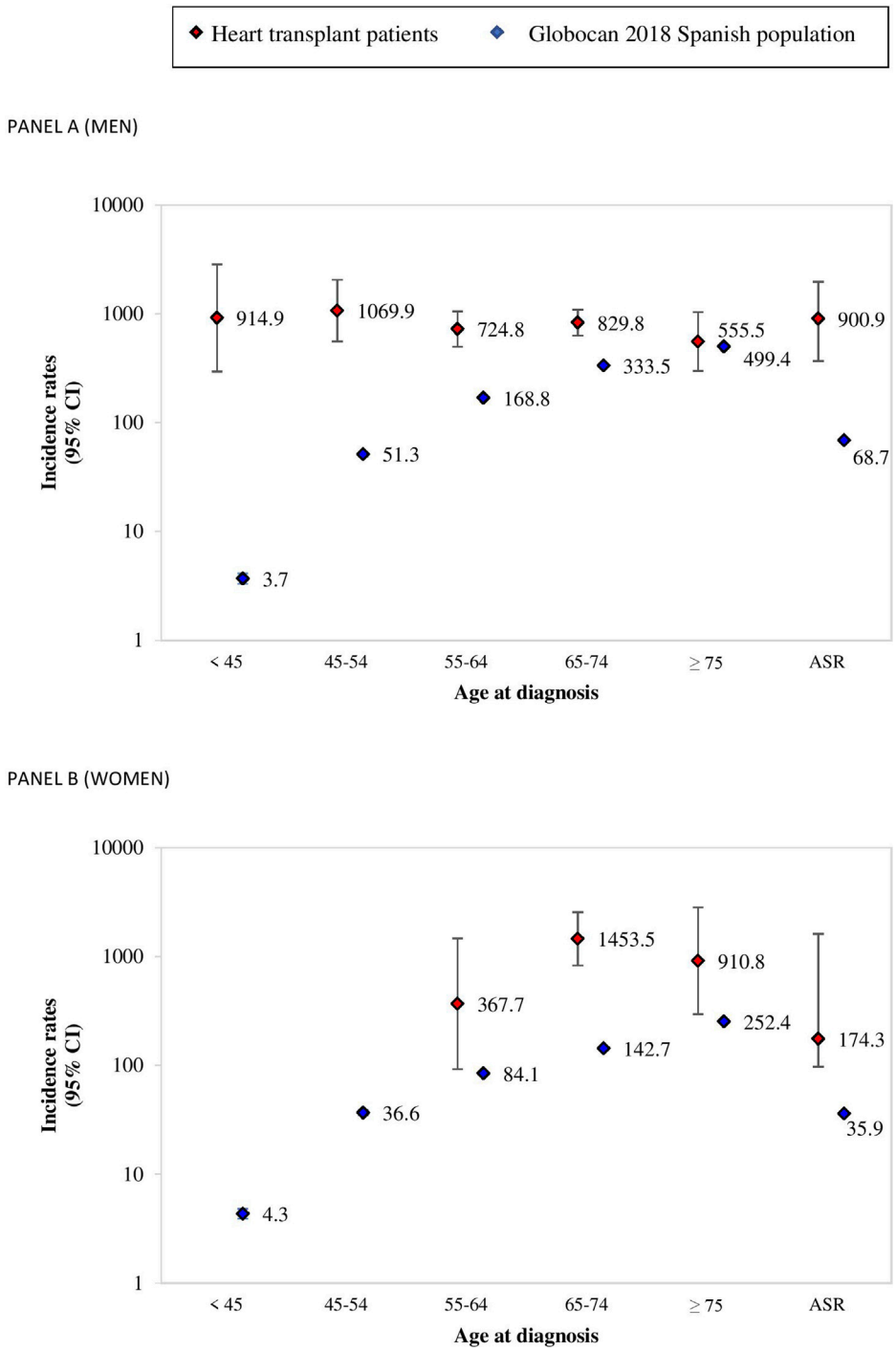

FIGURE 1 | Incidence rates per 100,000 person-years for age-at-diagnosis groups, and age-standardized overall rates for colorectal cancer among HT patients (SPHTTR data) and for the general Spanish population (GLOBOCAN 2018). **(A)** men; **(B)** women.

incidence rate of CRC after HT in our analysis was 209.4 per 100,000 pys (4.6% of the total malignancies); Van Keer et al. [21] reported similar numbers in Leuven.

The incidence of CRC increased with age at HT, which was expected, given the known relationship between age and cancer, but surprisingly this trend was not observed with age at diagnosis without a clear justification. Our group [22] had already noticed that the incidence rate ratio of lung cancer between HT and general populations fell with increasing age, suggesting, as a possible explanation, that with aging the effect of immunosuppression is relatively reduced due to a gradual decline of the immune system.

TABLE 4 | Characteristics of colorectal tumors at diagnosis (results expressed as n (%) unless otherwise stated).

Number of colorectal tumors	116
Mean (SD) age (years) at diagnosis	66.0 (8.8)
Mean (SD) time (years) since HT	9.3 (5.5)
Location	
Colon	92 (79.3)
Rectum	22 (19.0)
Anal	2 (1.7)
Histology[a]	107
Adenocarcinoma	81 (75.7)
Metastatic adenocarcinoma	10 (9.4)
Carcinoma	7 (6.5)
Metastatic carcinoma	2 (1.9)
Epidermoid carcinoma	3 (2.8)
Lynphoproliferative Syndromes	4 (3.7)
Surgery[b]	98
None	24 (24.5)
Paliative	12 (12.2)
Curative	62 (63.3)
Radiotherapy[c]	13 (13.7)
Chemotherapy[c]	32 (33.7)
Response to treatment[d]	87
Complete	50 (57.5)
Partial	17 (19.5)
None	20 (23.0)
Aciclovir or Ganciclovir	2 (1.7)

[a]*Out of 107 patients for whom relevant data were available.*
[b]*Out of 98 patients for whom relevant data were available.*
[c]*Out of 95 patients for whom relevant data were available.*
[d]*Out of 87 patients for whom relevant data were available.*

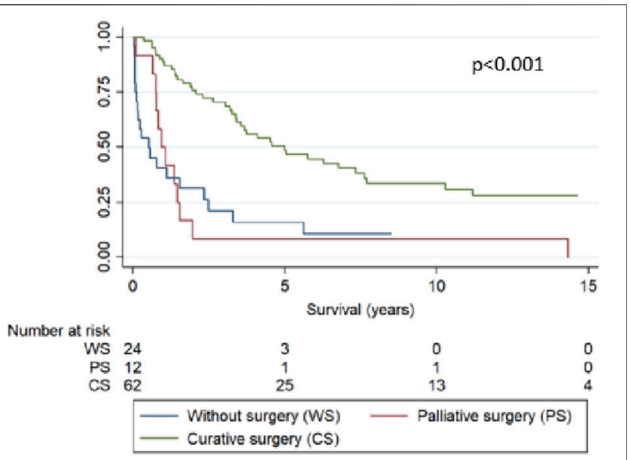

FIGURE 2 | Survival curves since diagnosis of colorectal cancer after HT by type of surgical treatment ($p < 0.001$).

Incidence Comparison With a Reference Population

Furthermore, the data reported in most studies are incidence rates that are not normalized to the general population. The SPHTTR data analyzed in our study showed an increased incidence of CRC in patients undergoing HT compared to GLOBOCAN 2018 estimates for the general population in Spain. This difference was maintained in successive age-at-diagnosis groups and age-standardized overall rates, except for the subgroup of men over 75 for whom although a rising trend was observed it was not statistically significant. In addition, the incidence of CRC has been increasing over the past few years, being at present the commonest tumor in Spain [11]. Therefore, to compare our cohort, which includes patients since 1984, to a general population obtained from GLOBOCAN 2018 might seem conservative but, nevertheless, the incidence post-HT was found to be higher than in the general population. Despite GLOBOCAN estimates that may possibly lead to an error owing to finite sampling, the aforementioned point, together with observed differences, suggest a greater risk of CRC after HT, which adds consistency to our results. Jäämaa-Holmberg et al. [23] recently assessed cancer incidence and mortality in Finnish HT recipients, both being markedly increased after HT, in comparison to the general Finnish population. Regarding CRC, they reached similar conclusions, observing that the incidence of colon cancer was 3.5–4 times higher than in the general Finnish population, although no rectal cancer was registered in their post-HT records. In contrast, Kellerman et al. [8] described no significant differences in relation to the incidence of CRC among the United States (US) HT and general populations. A possible explanation could be that the reference population considered in this US study might have been unsuitable for their HT cohort.

Potential Risk Factors Associated With Colorectal Cancer

Risk factors, such as obesity or smoking, have been associated with the development of CRC in the general population [24, 25], yet no statistically significant differences were observed in our analysis. Moreover, in spite of the fact that in our study the proportions of smokers and obese patients were lower than those of non-smokers and non-obese patients, respectively, the risk of CRC was still higher. These findings, together, suggest that the increased incidence is not due to these modifiable factors. Conversely, as was expected, even though the subgroup with a background of CRC pre-HT was quite small, the likelihood of this developing CRC after HT was eight times greater.

The association between immunosuppression and increased risk of neoplasia is well known. However, it is difficult to identify which immunosuppressive regimens are associated with an increased risk of cancers. No statistically significant differences were observed related to HT era or immunosuppressive agents in our study. Given a lack of information in the literature on the effect of immunosuppression specifically on CRC after HT, we can only compare such data with outcomes of malignancy in more general subgroups such as patients with noncutaneous solid tumors. In this subgroup, in terms of induction therapy, OKT3 and anti-thymocyte globulin have been associated with an increase in cancers [5], whereas in the Youn et al. analysis [4], mycophenolate mofetil, which is known to have anti-proliferative properties and prevent tumor dissemination by inhibiting endothelial cell proliferation and angiogenesis [25], showed a protective effect compared to azathioprine.

Colorectal Cancer Characteristics After Heart Transplantation

Regarding the characterization of post-HT colorectal cancer, consistent with CRC in general, the main histopathological type was adenocarcinoma. Furthermore, in our HT cohort most CRC cases were not metastatic at diagnosis, possibly due the close follow-up HT patients undergo, even though recommendations regarding CRC screening in HT recipients do not differ from those of the general population [26], which in Spain consists in fecal occult blood test beginning at age 50, followed by endoscopic study if positive. However, even though only 11.2% of cases were metastatic at diagnosis, surprisingly almost 25% were not considered operable. We do not know if that decision was due to high surgical risk or another reason. Curative surgery was performed in 63% of cases, increasing the probability of survival.

Limitations

The limitations of our study ought to be taken into account when interpreting the reported results. First, the study was retrospective. Second, because no Spanish national cancer registry existed, the incidence of CRC in the general population was obtained from GLOBOCAN 2018 estimates. However, as CRC incidence is increasing, comparing our data related to HT since 1984 to a 2018 reference population might underestimate the difference in incidences between both cohorts, strengthening our results. Third, the SPHTTR may have been missing some tumors because, while we do assure that every cancer included in our records is verified, some cases might not have been detected if diagnosed in a referral center where neither the patient nor their physician informed their corresponding transplant unit. Nevertheless, this would again underestimate the incidence of CRC in our HT population and, therefore, strengthen our results. Finally, although all Spanish hospitals performing HT in adults continually update data in the SPHTTR, it does not contain information that might have been of interest to analyze, such as CRC location (right or left sided), TNM staging, more specific details related to treatment, the implementation or not of CRC screening tests (fecal occult blood test or colonoscopy) in pre-HT protocols, and the use of statins [27] and aspirin [28] since some studies suggest these drugs might have a protective effect against CRC.

Conclusion

We conclude that the incidence of CRC among HT patients is greater than in the general population in our country, increasing with age at HT. Curative surgery increased the probability of survival compared to palliative surgery or that of inoperable patients. This suggests that a post-cardiac transplant follow-up might require specific screening for this cancer to achieve early diagnosis and treatment since this improves health outcomes, particularly after a recent recommendation to move the age at which to start screening for colorectal cancer in the general population forward from 50 to 45 years [29].

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de Ética de la investigación de A Coruña—Ferrol. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MS-F and MGC-L conceived of the presented idea. JM verified the analytical methods. All authors were involved in the data collection, discussed the results and contributed to the final manuscript.

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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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IN MEMORIAM
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In Memoriam: Georg K. Uhlschmid (1937–2023)

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Georg K. Uhlschmid was born in Graz (Austria) on 13 July 1937. He started his medical education at the Karl-Franzens-University Graz in 1956 after leaving the classical gymnasium with distinction and became Dr. med. univ. (*doctor medicinae universae)* in 1962. His early formation years as general surgeon were spent in Linz and Basel from 1963–1966. Prof. Rudolf Nissen attested him "an excellent clinical and operative talent combined with an agreeable, tactful way towards his patients, being greatly appreciated by his colleagues and nursing staff". Supported by a private fellowship he then worked at the Institute of Reconstructive Plastic Surgery, New York University Medical Center, in the transplantation laboratories under Profs. John Marquis Converse and Donald L. Ballantyne on experimental skin grafts and transplantation immunity (January 1967–April 1968), reporting on his work in two seminal papers published in 1969. On Converse's advice: "I need not emphasize to you the present trend in surgery toward a rapid evolution to transplantation research as an important part in the surgical field", Uhlschmid had the choice to join either Prof. Martin Allgöwer, then newly appointed to the chair of visceral surgery in Basel, the renowned plastic surgeon Prof. Bengt Johanson of Gothenburg (an uncle of Dr. Uhlschmid) or Prof. Åke Senning, head of Surgery in Zurich.

Uhlschmid chose Senning at the Surgical Clinic A at the University Hospital Zurich (USZ) where he remained until his retirement in 2001, first as surgical fellow with clinical responsibilities in visceral-, lung- and transplantation surgery. His brilliant expertise and creative ideas were soon appreciated by his superiors who quickly entrusted him with the leadership of the Surgical Research Unit in addition to his clinical activities. Uhlschmid's experimental investigations covered a wide range of problems in micro-, cryo-, laser-, and thoracic surgery with main emphasis in trachea- and kidney transplantation.

In 1973 Uhlschmid, also fluent in Swedish (his mother being a translator of Scandinavian literature), spent some time as a visitor at the Sahlgrenska University Hospital Gothenburg where he teamed up with Prof. Lars-Erik Gelin and his broad international collaborators in kidney and pancreas transplantation. Upon his return to Switzerland he joined the committee of organ conservation at Eurotransplant Leiden and established a data capturing system regarding procurement, preservation, allocation and transplantation for the USZ. After participating in the 1st Gelin-Memorial Symposium in Gothenburg in November 1981 Uhlschmid wrote in a document dated 11 April 1982 and sent to Dr. Guido Persijn: "it was felt that there was a need for a new society to be formed which would represent more accurately the aims and needs of transplantation surgery and surgeons in Europe." Subsequently, it was Uhlschmid who was chosen to organize the "Founding Assembly Meeting" in Zurich on 28 April 1982 with Prof. Roy Calne (Cambridge) as president, Prof. Maurice Slapak (Portsmouth) as vice-president, Dr. Georg Uhlschmid (Zurich) as secretary, Prof. Walter Land (Munich) as treasurer and Profs. Hans Brynger (Gothenburg), Max Dubernard (Lyon) and Dr. Raimund Margreiter (Innsbruck) as councillors. At this meeting Prof. Heinz Pichlmaier (Köln) supported Roy Calne's suggestion "to include *all* persons in organ transplantation, not just surgeons" and therefore, the society's name was changed from ESTS to ESOT (European Society for Organ Transplantation). In 1982 Uhlschmid also became Swiss Citizen and got his Venia Legendi of the Zurich University with a study on new experimental methods for elongation and replacement of the thoracic trachea.

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The first biannual ESOT Congress took place in Zurich in 23–25 November 1983 and was single-handedly organized by Uhlschmid. At this meeting a lot of attention was already paid to Sandimmune® (cyclosporine A) which had been approved by the FDA just a few weeks before! In the aftermath—to Uhlschmid's great disappointment—the transplant centers in Switzerland did not want to join ESOT, but instead created Swisstransplant in 4 March 1985. This prompted him to focus his research more towards general clinical and experimental visceral surgery. In 9–11 April 1984, he organized the 19th Congress of the European Society of Surgical Research (ESSR)—also in Zurich—chairing the first "Stapler Workshop" which brought him the accolade of the ESSR-presidency 1984/85.

His early investigations in reconstructive surgery in New York were now re-awakening his interest in angiogenesis and biomaterials research. His key-note contribution on "Angiogenesis—a new fascinating enigma for surgeons !?" at the first Swiss Conference on "Angiogenesis: Key Principles—Science—Technology—Medicine" in early March 1991 at St. Gallen, was highly appreciated by Judah Folkman (Harvard) and Robert Langer (MIT). A close collaboration with Prof. Ulrich Suter, then head of the Departement of Materials at the Swiss Federal Institute of Technology Zurich (ETHZ), soon resulted in two patented biopolymers for chemo-embolization, drug delivery and surgical applications (DegraPol®/DegraBloc®). Last but not least, Georg Uhlschmid encouraged many medical students to consider a career in surgery through his practical university course "Theory of surgical techniques" inaugurated in 1991 and conducted under his personal supervision. His last invited commentary at an interdisciplinary meeting at the Department of Visceral Surgery in Geneva in 26 June 2001 may be seen as his legacy with its challenging title "Surgery: evolution, revolution and entropy." Georg K. Uhlschmid will be remembered and sadly missed by his colleagues and friends.

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

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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