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Factors affecting graft survival after renal transplant: prevention of failure and follow up strategies

Edited by
Laila-Yasmin Mani and Sing-Chung Li

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Factors affecting graft survival after renal transplant: prevention of failure and follow up strategies

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Editorial: Factors affecting graft survival after renal transplant: prevention of failure and follow-up strategies

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KEYWORDS

kidney transplantation, IFTA, graft survival, viral infection, kidney donor, collapsing glomerulopathy, kidney MRI, arterial kinking

Editorial on the Research Topic
Factors affecting graft survival after renal transplant: prevention of failure and follow-up strategies

Since its first introduction in 1951 (1), kidney transplantation has become the best therapeutic option for patients affected by end-stage kidney disease. Indeed, kidney transplant recipients experience a clear survival benefit when compared to their matched counterparts on the waiting list (2). Thanks to the development of highly effective immunosuppressive regimens, the much-feared threat of acute organ rejection could be mastered. Surprisingly, however despite major advancements, a trend for decreased graft survival has been recorded over recent decades (3). Considered a final common pathway, the interstitial fibrosis and tubular atrophy (IFTA)-lesion of the kidney graft is thought to be multifactorial in origin secondary to immunological, cardiovascular, toxic and infectious causes (4). In this Research Topic, 15 articles of various formats from different geographic regions in the world invite us to shed light on diverse aspects of long-term kidney graft function.

Biopsy-proven causes for graft failure after a very long follow-up up to 26 years were examined by Betjes et al. in a prospective Dutch cohort of 737 kidney transplant recipients. The category of rejection accounted for the main part of death-censored graft failure while recipient's age, time after transplantation, and the presence of donor-specific antibodies before transplantation determined the relative contribution to overall graft loss and the type of rejection involved.

The influence of age and sex on graft survival was analyzed by Sancho et al. in a retrospective Spanish cohort of 1,101 kidney transplant recipients. The lower graft survival of female patients under 60 years of age was attributed to a more frequent use of expanded criteria donors and a higher prevalence of pre-transplant human leukocyte antigen sensitization.

Furthermore, the influence of donor race was examined in a retrospective clinico-pathological analysis from Columbia University NY on roughly 1,900 kidney transplant recipients. The authors confirmed a shorter allograft survival of kidney grafts from black donors and revealed a higher risk for the development of collapsing glomerulopathy in grafts from black donors (DiFranza et al.).

Infections as cause of late graft loss and complicated post-transplant course were the topic of several reports in this Research Topic. Brune et al. found no impact of 1st year urinary tract infection (UTI) episodes with extended-spectrum beta-lactamase (ESBL) *Escherichia coli* and *Klebsiella species* on graft survival in 389 kidney transplant recipients within the Swiss Transplant Cohort while hospitalization and UTI recurrence rates were higher compared to patients affected by UTI with non-ESBL-producing strains. In an Italian retrospective cohort of 939 kidney transplant recipients, MRI-confirmed acute graft pyelonephritis was associated with reduced death-censored graft survival influenced by donor age, multifocal presentation, and abcedation as well as anti-thymocyte globulin induction (Tarragoni et al.).

In addition to bacterial infections, viral infections are known to affect graft survival. Dai et al. report a case of graft loss after acute blood group antibody-dependent rejection in an ABO-incompatible living donor kidney graft recipient triggered by prolonged parvovirus B19 infection. In another case report by Hosek et al., postrenal acute kidney graft dysfunction was caused by cytomegalovirus (CMV)-positive nephrogenic adenoma of the transplant ureter and a potential link between the rare entities of CMV ureteritis and nephrogenic adenoma of the transplant ureter is discussed. BK virus infection is known to affect kidney graft outcomes. In their retrospective study in kidney transplant recipients from donation after circulatory death donors, Liu et al. use a machine-learning approach to identify risk factors for the progression of BK viruria to BK viremia.

Additionally, metabolic factors may affect graft survival. In their narrative review, Tang et al. describe the importance, risk factors, and current treatment options for post-transplant anemia. Zeng et al. conducted a prospective cohort study in 600 kidney transplant recipients and meta-analysis to evaluate the role of vitamin D-levels as predictor of graft loss.

Finally, functional kidney graft ischemia has been evoked as cause for the development of IFTA. In our center, we have examined the hypothesis that grafts are less oxygenated during the sitting position due to kinking or bending of the iliacal vessels analogous to iliacal claudication described in professional cyclists. Using a multiparametric functional kidney MRI protocol including blood oxygen level-dependent (BOLD)-MRI, diffusion-MRI and arterial spin labeling-MRI during neutral and flexed hip position, the Bent Knee Study showed an acute impact of hip flexion on graft perfusion and oxygenation (Mani et al.).

Immune-dependent factors play a well-known role for kidney graft survival with rejection episodes contributing to early and late graft loss. Therefore monitoring of immunosuppression has a major role in preventing rejection and avoiding infectious and toxic complications. Reineke et al. correlated Torque teno virus load in 106 kidney transplant recipients undergoing indication biopsies to histological findings and conclude that Torque teno virus load may reflect changes in immunosuppressive therapy even after the 1st year post-transplant. In a proof-of-principle study by Born et al. in 39 kidney transplant recipients, the feasibility of tacrolimus monitoring in hair samples has been studied in order to allow self-collection by patients and reduce the frequency of medical visits. Füessl et al. report on the potential benefit of the twice-daily use of extended-release tacrolimus in a kidney transplant recipient identified as fast metabolizer for tacrolimus leading to normalized trough levels and area under the concentration-time curve and improved graft function.

Lastly, the prediction of graft survival was studied by Hiramitsu et al. using a prediction model for the ideal perioperative estimated glomerular filtration rate (eGFR) in a cohort of 1,174 living-donor kidney transplant recipients. In this study, the predicted ideal eGFR/actual eGFR at 1, 2, and 3 weeks after transplantation was predictive for graft loss.

Taken together, ongoing research efforts continue defining and refining optimal post-transplant care for kidney transplant recipients with the ultimate goal and challenge to achieve improved long-term kidney graft survival.

Author contributions

L-YM: Conceptualization, Writing – original draft.

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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ORIGINAL RESEARCH
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Hip Position Acutely Affects Oxygenation and Perfusion of Kidney Grafts as Measured by Functional Magnetic Resonance Imaging Methods—The Bent Knee Study

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Background: Kidney perfusion and oxygenation are two important determinants of kidney graft function. In kidney transplantation, repeated graft hypoperfusion may occur during hip flexion, for example in the sitting position, due to the progressive development of fibrotic tissue around iliac arteries. The aim of this study was to assess the changes in oxygenation and perfusion of kidney grafts during hip flexion and extension using a new functional magnetic resonance imaging (fMRI) protocol.

Methods: Nineteen kidney graft recipients prospectively underwent MRI on a 3T scanner including diffusion-weighted, blood oxygenation level dependent (BOLD), and arterial spin labeling sequences in hip positions 0° and >90° before and after intravenous administration of 20 mg furosemide.

Results: Unexpectedly, graft perfusion values were significantly higher in flexed compared to neutral hip position. Main diffusion-derived parameters were not affected by hip position. BOLD-derived cortico-medullary R2* ratio was significantly modified during hip flexion suggesting an intrarenal redistribution of the oxygenation in favor of the medulla and to the detriment of the cortex. Furthermore, the increase in medullary oxygenation induced by furosemide was significantly blunted during hip flexion ($p < 0.001$).

Conclusion: Hip flexion has an acute impact on perfusion and tissue oxygenation in kidney grafts. Whether these position-dependent changes affect the long-term function and outcome of kidney transplants needs further investigation.

Keywords: hip flexion, kidney transplantation, perfusion, oxygenation, functional MRI, BOLD, arterial spin labeling, multiparametric magnetic resonance imaging

INTRODUCTION

Kidney transplantation is the therapy of choice for patients with end-stage kidney disease conferring survival benefit regardless of graft source (1). However, long-term graft survival has failed to improve in recent decades despite the great reduction in acute rejection episodes achieved by current immunosuppressive regimens (2). A major cause is the progressive interstitial fibrosis and tubular atrophy (IFTA) of the organ considered multifactorial in origin (3, 4).

In spite of advanced operative techniques, vascular complications after kidney transplantation remain of concern (5, 6). Surgical re-interventions are generally complicated by the marked fibrotic peri-graft reaction developing in the early post-transplant period (7).

Intriguingly, lower extremity claudication has been reported in professional cyclists due to kinking of iliac arteries during hip flexion visualized by magnetic resonance (MR) angiography (8, 9). Kinking was caused by tethering of iliac arteries by psoas or other arterial side branches, by fibrous fixation of the iliac bifurcation, or by chronic arterial stretching during repeated hip hyperflexion (10, 11). The claudication responded well to surgical release of the artery (9). In healthy subjects, maximal hip flexion has been shown to induce shortening, bending, and twisting of iliac arteries (12).

Little is known about dynamic perfusion changes in kidney grafts depending on body posture. Kidney grafts typically placed into iliac fossa might be exposed to similar position-dependent perfusion problems. Specifically, kinking or narrowing of the transplant renal or iliac artery may occur during hip flexion due to tethering by adjacent fibrotic tissue leading to iterative hypoperfusion episodes as well as chronic ischemic graft damage and IFTA in the long-term. Considering common sedentary lifestyle majorly in the sitting position, this influence could be significant. Furthermore, pre-existing endovascular lesions in this high-cardiovascular-risk population or subclinical vascular anastomotic problems may have an additional effect. To the best of our knowledge, this question has not been addressed so far.

Today, novel functional MR imaging (fMRI) techniques allow for the non-invasive investigation of renal tissue oxygenation, perfusion, and diffusion with the use of blood oxygenation level dependent (BOLD)-MRI, arterial spin labeling (ASL)-MRI, and diffusion-weighted imaging (DWI) (13).

The aim of this prospective interventional study was therefore to assess the influence of hip flexion on kidney graft oxygenation and perfusion using fMRI techniques in kidney transplant recipients. Our hypothesis was that hip flexion >90° (as achieved in the usual static sitting position) would lead to an instant and temporary reduction of renal tissue oxygenation and perfusion as measured by BOLD-MRI, ASL-MRI, and DWI compared to neutral hip position. To correlate results with vascular anatomy (presence of functional kinking and/or pre-existing endovascular lesions), non-contrast-enhanced time-of-flight (TOF) angiography during hip flexion and duplex-ultrasound scans (DUS) were performed. Additionally, we analyzed the correlation of oxygenation and perfusion changes with clinical parameters.

Abbreviations: ADC_D, diffusion coefficient; ASL, arterial spin labeling; BOLD, blood oxygenation level dependent; CNI, calcineurin inhibitors; CKD-EPI, chronic kidney disease epidemiology collaboration; DUS, duplex ultrasound scan; DWI, diffusion-weighted imaging; eGFR, estimated glomerular filtration rate; fMRI, functional magnetic resonance imaging; FOV, field of view; F_P, perfusion fraction; IFTA, interstitial fibrosis and tubular atrophy; MCR R2*, medullary to cortical R2* ratio; MR, magnetic resonance; MRI, magnetic resonance imaging; RAASI, renin-angiotensin-aldosterone system inhibitors; R2*, transverse relaxation rate; ROI, region of interest; SD, standard deviation; TE, echo time; TOF, time of flight; TR, repetition time; wo/w, without/with.

MATERIALS AND METHODS

The protocol of this prospective single-center interventional study was approved by the local ethics committee (Canton of Bern, Switzerland, protocol number 2181, approval number 042/12) and conducted in accordance with the Declarations of Helsinki and Istanbul (14, 15).

Study Population

Patients ≥18 years having received a kidney graft ≥6 months ago into the iliac fossa with a stable graft function (≤30% deviation of last three serum creatinine values) and an estimated glomerular filtration rate (eGFR) ≥30 ml/min/1.73 m^2 according to Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)-equation and preserved faculties of judgment were eligible.

Exclusion criteria were pregnancy; New York Heart Association stage IV dyspnea or orthopnea; acute infection; active neoplasia; surgical intervention, severe trauma, or acute ischemic or thromboembolic event within the preceding 2 months; inability of ipsilateral hip flexion; classical contraindications to MRI; body weight >200 kg; as well as any implanted metallic material without prior 3T-MRI after implantation.

Study Design

From June 2015 through January 2016, 97 consecutive patients were screened at the outpatient University Clinic for Nephrology and Hypertension in Bern, 19 of whom fulfilled eligibility criteria and accepted to participate. Written informed consent was obtained from each participant prior to inclusion. A standardized hydration protocol was followed (2 L water intake over the preceding day; on the study day, 5 ml/kg upon awakening, followed by 3 ml/kg/h) and diuretics were held on the study day and the preceding day if considered safe by the treating nephrologist (16, 17). A light meal was allowed on the study day. Baseline clinical characteristics were obtained based on medical record review and blood was drawn for serum creatinine analysis.

There were four study phases during one patient's session: anatomical MRI, DWI, BOLD-MRI, and ASL-MRI were first performed during neutral hip position and then repeated during maximally achievable hip flexion (≥90° from bed level); to account for position-induced confounding effects on the BOLD signal as well as to include a functional test, the same measurements were repeated 10 min after the intravenous administration of 20 mg furosemide (LASIX®; Sanofi-Aventis, Vernier, Switzerland) (17–21). At the end of the last study phase,

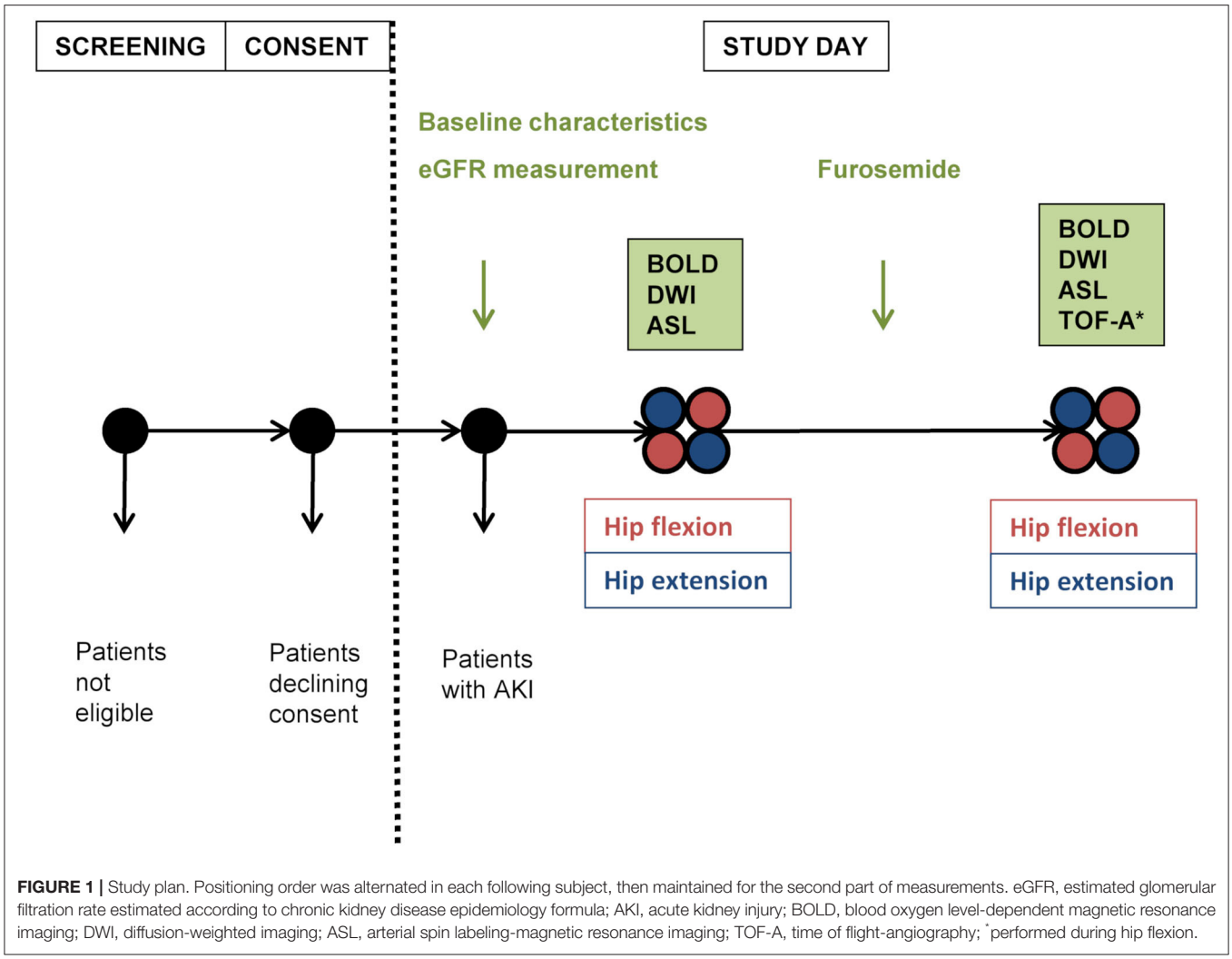

FIGURE 1 | Study plan. Positioning order was alternated in each following subject, then maintained for the second part of measurements. eGFR, estimated glomerular filtration rate estimated according to chronic kidney disease epidemiology formula; AKI, acute kidney injury; BOLD, blood oxygen level-dependent magnetic resonance imaging; DWI, diffusion-weighted imaging; ASL, arterial spin labeling-magnetic resonance imaging; TOF-A, time of flight-angiography; *performed during hip flexion.

TOF angiography was performed during hip flexion. In order to minimize bias, the sequence of positions was alternated in each consecutive subject but maintained unchanged after furosemide administration in each subject (**Figure 1**).

MRI Protocol

MRI data were acquired on a 3.0 T whole body MR Scanner (Magnetom Verio®; Siemens Healthcare, Erlangen, Germany). To facilitate hip flexion >90°, measurements were performed in the lateral decubitus position requiring subject positioning far off-center. Maintenance of hip flexion was assured by an MR-safe belt.

BOLD-MRI is a non-invasive method using deoxygenated hemoglobin as an endogenous contrast agent which influences the relaxation time T2*; outcome measure is the transverse relaxation rate R2* (equal to 1/T2*) which correlates to tissue oxygen content when confounding factors such as blood volume or hydration state are excluded. Rather than providing absolute values of oxygenation, this technique is especially useful to demonstrate relative changes in response to various interventions (13, 22). In order to standardize BOLD measurements and add a functional test, an additional maneuver had to be included; in this case, measurements were repeated after the administration of furosemide. BOLD-MRI was performed in the coronal plane using a multiple-gradient-echo sequence in a single end-expiratory breath-hold of 17 s per slice with 12 echoes equally spaced (6–52.3 ms) and the following parameters: repetition time (TR) = 65 ms, field-of-view (FOV) = 400 × 400 mm^2, matrix = 256 × 256, slice thickness = 5 mm. All 12 images acquired from BOLD-MRI were used to estimate the R2* parameters in a linear fitting model.

ASL-MRI allows quantitative perfusion measurements using magnetically labeled water as an endogenous diffusible tracer (13, 23, 24). ASL-MRI was performed using a flow-sensitive alternating inversion recovery perfusion preparation combined with a true-fast imaging with steady-state precession data acquisition according to an established protocol with pixel-based calculation of perfusion values (23, 25). Parameters were as follows: TR/echo time (TE) = 4.0/2.0 ms, slice thickness = 7 mm, matrix = 128 × 128, FOV = 360 × 360 mm^2, inversion time = 1200 ms, averages = 30 (15 scans with inversion pulse and 15 without).

DWI allows the quantification of diffusion parameters and microperfusion yielding ADC_D and the perfusion fraction F_P (13, 22). DWI was performed with eight different b-values (0–600 s/mm^2), two repetitions, TR of 3300 ms, TE of 56 ms, slice thickness of 5 mm, matrix of 128 × 128 and FOV of 300 × 300 mm^2.

TOF angiography is an MR technique permitting the visualization of vascular flow without the need for contrast agents. Based on the phenomenon of flow-related enhancement of spins entering into an imaging slice, vascular anatomy can be reconstructed in a three-dimensional view.

A maximum of six regions of interest (ROIs) were analyzed in every slice (BOLD, DWI: 2–4 slices, ASL: one slice) for each of the four measurements (neutral and flexed position, before and after furosemide). ROIs were manually defined by the same blinded investigator on images handed out in a random fashion (mixed between patients and study phases). ROIs containing approximately 10 voxels were traced in the medulla and cortex. All images were co-registered facilitating comparable ROI position for all methods. Data were analyzed using in-house custom-scripts written in IDL® and MATLAB®.

Laboratory Analyses

Serum creatinine was measured by an enzymatic creatinine assay (Roche Creatinine Plus®, Roche Diagnostics, Basel, Switzerland).

Doppler Studies

Doppler studies were performed according to clinical routine in the outpatient clinic of the University clinic for Nephrology and Hypertension in Bern on an Acuson® 2000S device (Siemens Healthcare, Erlangen, Germany) including graft morphology, urinary outflow, and perfusion (resistance indices, flow velocities).

Outcome Measures

The primary outcome was the change in mean medullary and cortical R2* values, in medullary to cortical R2* ratio (MCR R2*) and in R2* ratio without/with (wo/w) furosemide during hip flexion compared to neutral hip position. Secondary outcomes were the changes in mean ASL perfusion values and mean F_P by DWI during hip flexion compared to neutral hip position, the presence of renal transplant/iliac artery kinking visualized

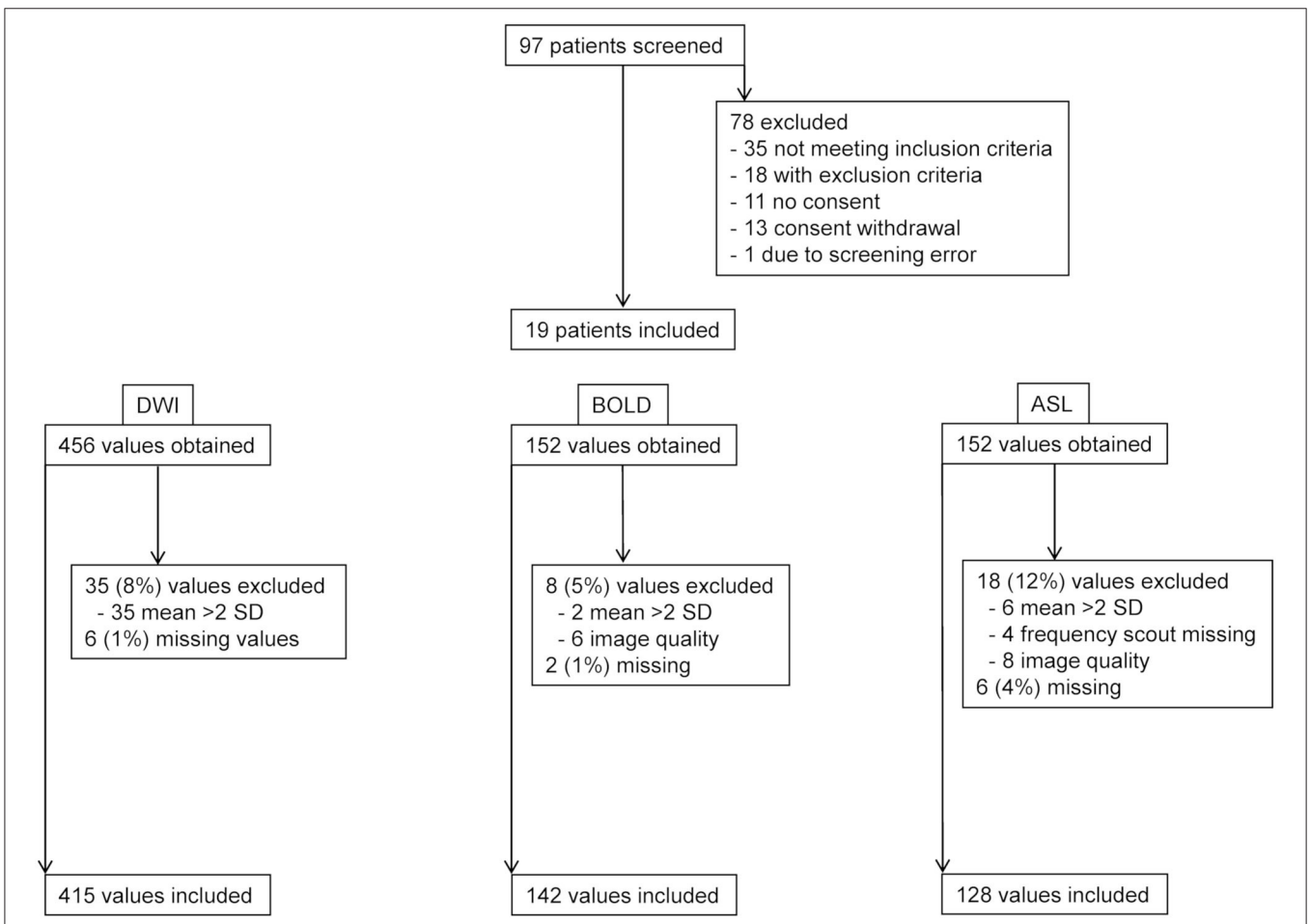

FIGURE 2 | Flow chart. Patient screening and data exclusion. DWI, diffusion-weighted imaging; BOLD, blood oxygen level dependent-magnetic resonance imaging; ASL, arterial spin labeling-magnetic resonance imaging.

by TOF angiography during hip flexion, the correlation of oxygenation (R2*, MCR R2*, R2* ratio wo/w furosemide) and perfusion changes with the presence of functional kinking, endovascular lesions of transplant renal/iliac artery (evidenced by DUS) and with clinical parameters [age, eGFR, renin-angiotensin-aldosterone system inhibitor (RAASI) medication, calcineurin inhibitor (CNI) medication, implantation site, and donor source], as well as correlation of these clinical parameters with functional kinking.

Statistical Analysis

Based on previous studies, we estimated that a change in R2*; the diffusion coefficient ADC_D; and perfusion value of <7, 4, and 15%, respectively, could be detected in 19 patients on a significance level of 0.05 and 80% statistical power (22, 25–29), each subject serving as its own control. Quantitative variables were expressed as means with standard deviation (SD) or medians with range between minimal and maximal value. Normality testing was performed using the Kolmogorov–Smirnov test. Paired Student's *t*-test was used to compare study phases. Correlations between position-induced changes in fMRI parameters with clinical parameters were determined by Pearson and point-biserial correlation coefficient analysis as appropriate. Data were analyzed using IBM SPSS Statistics 25® and MS Office®.

RESULTS

Subjects

Nineteen kidney transplant recipients completed the study protocol (**Figure 2**). Baseline characteristics are shown in **Table 1** and **Supplementary Table 1**. Subjects were mainly male Caucasian (median age 49 years) first transplant recipients (42% from living donors) with a median eGFR of 53 ml/min/1.73 m^2. All but one subject were on antihypertensive medications including RAASI. In half the patients, vascular anastomoses involved >1 vessel and/or angioplasty. Nearly 75% of patients were on a CNI-based immunosuppressive regimen.

TABLE 1 | Baseline characteristics of the subjects ($n = 19$).

Age (years)	48 ± 13 (49; 20–69)
Male gender (%)	74
Race (%)	
- Asian	16
- Caucasian	84
Living donor (%)	42
Dialysis vintage (years)	2 ± 2
Transplant episode	
- first	89
- second	11
Transplant vintage (years)	8 ± 7 (6; 0.58–20)
eGFR (ml/min/1.73 m^2)	57 ± 19 (53; 32–102.1)
AHT medication (*n*)	2 ± 1 (0–4)
- RAASI (%)	100
CNI-based regimen (%)	74
Steroid medication (%)	63
Ipsilateral implantation (%)	11
Arteries (*n*)	1 ± 1
- with vascular intervention (%)	47
Veins (*n*)	1 ± 0
- with vascular intervention (%)	63

Values are given as mean ± standard deviation (median; range) or as percentage of patients, as appropriate. n, number; eGFR, estimated glomerular filtration rate estimated according to chronic kidney disease epidemiology collaboration formula; AHT, antihypertensive; RAASI, renin angiotensin aldosterone system inhibitor; CNI, calcineurin inhibitor.

Measurement Quality

The MRI protocol including morphological sequences, BOLD, ASL, and DWI was successfully performed in all 19 patients in both hip positions before and in 18, 16, and 17 patients after furosemide administration, respectively. TOF angiography was performed in 16 patients during flexion after furosemide administration. Three subjects (patients 2, 4, and 12) received an incomplete dose of furosemide due to venous access problems. A mean scanning time per study phase of 15–20 min was met, resulting in an overall measurement time for all four scans <90 min including a break leaving the magnet and the time for furosemide injection. Overall visual image quality was judged good despite unusual lateral decubitus in both positions and intermediate for TOF angiography, however, without performing a formal image quality analysis (**Figure 3**). BOLD, ASL, and DWI-derived mean values and SD ranges were in line with previously reported values (22, 26, 30). Low SD and significant correlations between MRI parameters in hip flexion and extension as well as before and after furosemide confirmed measurement stability (**Supplementary Figures 1, 2**). Overall, 7–16% of values in each modality were not available for analysis due to missing data or were excluded due to poor image quality or outlier values (deviation >mean ± 2 SD) (**Figure 2**). Normality testing using the Kolmogorov–Smirnov test yielded *p*-values of >0.05, that is, the hypothesis of a normal distribution cannot be rejected, in 71 out of 72 tests. Solely for medullary ADC_D with furosemide in flexed position, the test yielded $p = 0.04$.

BOLD-MRI

Mean medullary and cortical R2* values as a marker of tissue oxygenation level (higher values meaning decreased tissue pO2) are shown in **Table 2** and **Figure 4**. As expected, R2* values were significantly higher in the medulla. Medullary R2* decreased significantly after furosemide administration, as reported for native kidneys (16, 31, 32). During hip flexion, no significant changes in absolute R2* values were noted. However, there was a significant decrease of the MCR R2* during hip flexion suggesting a medullary oxygen redistribution, which was not observed after furosemide. The medullary R2* ratio wo/w furosemide corresponding to the response to furosemide decreased highly significantly during hip flexion. In the cortex, no significant difference was observed.

ASL-MRI

Mean medullary and cortical perfusion values obtained by ASL-MRI indicating macro-perfusion are shown in **Table 2** and

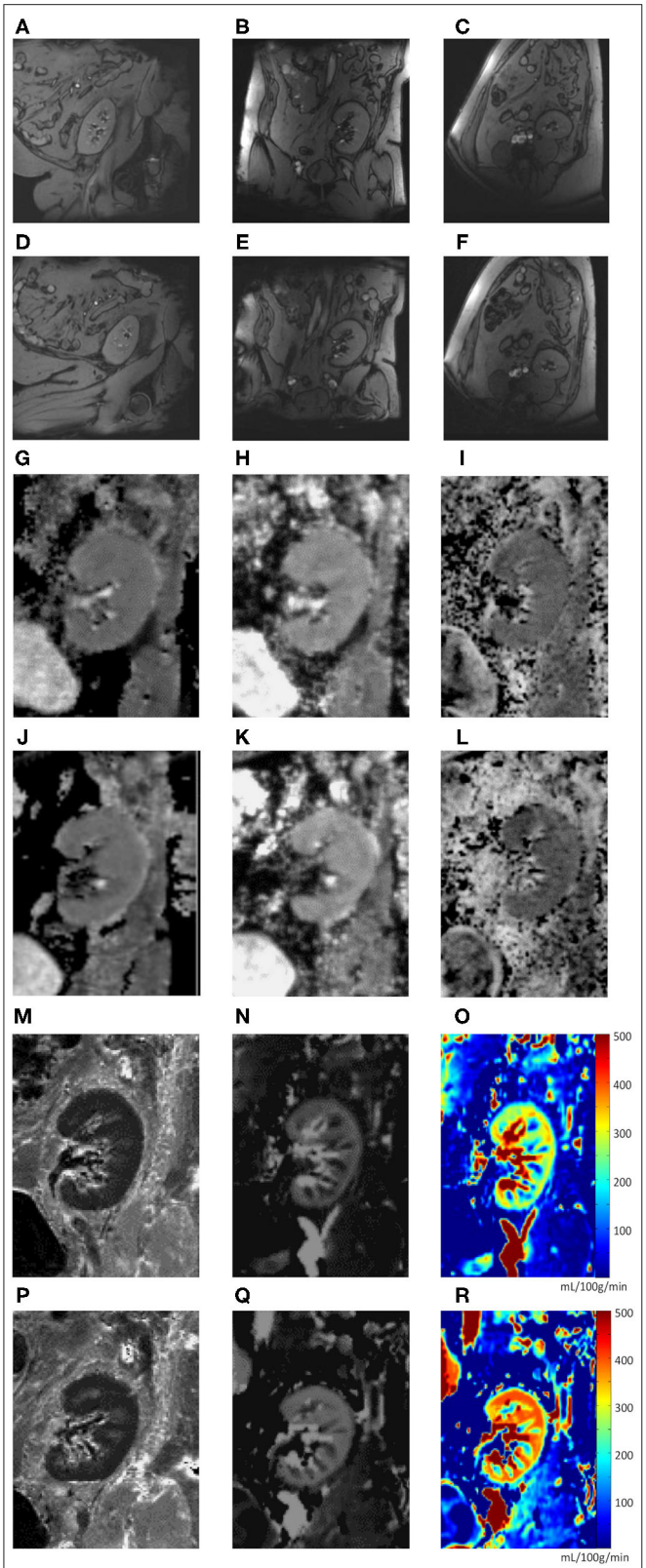

FIGURE 3 | (A–R). Image examples (one subject). Anatomical sequence (T1 map) during neutral hip position **(A–C)** and hip flexion **(D–F)** in sagittal **(A,D)**, *(Continued)*

FIGURE 3 | coronal **(B,E),** and transverse **(C,F)** planes. Functional MRI sequences during neutral hip position **(G–I, M–O)** and hip flexion **(J–L, P–R)**: total apparent diffusion coefficient (ADC_T) map **(G,J)**; pure diffusion coefficient (ADC_D) map **(H,K)**; fraction of perfusion (F_P) map **(I,L)**; resonance transverse relaxation rate (R2*) map **(M,P)**; arterial spin labeling (ASL) map **(N,O,Q,R)**.

Figure 5. There was a consistent increase in mean perfusion values during hip flexion reaching statistical significance in medullary measurements as well as in the cortex after furosemide injection. The perfusion increase was stronger in the medulla than in the cortex resulting in a significantly higher medullary to cortical-ratio during hip flexion.

DWI

Mean medullary and cortical values for the diffusion coefficient ADC_D as a marker of pure diffusion are shown in **Table 2**. As expected, no change induced by hip flexion was observed in the cortex. In medulla, ADC_D values were significantly higher during hip flexion after administration of furosemide. Perfusion fraction F_P, a parameter of microperfusion, showed no significant changes dependent on hip position (**Table 2**).

TOF Angiography

TOF angiography did not identify kinking phenomena during hip flexion (data not shown).

Correlation to Clinical Parameters

To investigate determinants of position-induced oxygenation and perfusion changes, correlation testing was performed between fMRI and selected clinical parameters (age, transplant vintage, eGFR, donor source, and CNI use). To reduce the number of comparisons, only significant changes between positions were tested. Two significant correlations were found:

First, in BOLD, the change in MCR R2* from neutral to flexed position without furosemide correlated positively with eGFR ($R = 0.52$, $p = 0.039$). This might indicate a stronger cortical R2* increase (oxygenation decrease) during flexion in patients with reduced graft function.

Second, in ASL, the increase in cortical perfusion from neutral to flexed position without furosemide correlated negatively and significantly with age ($R = -0.52$, $p = 0.041$) and transplant vintage ($R = -0.745$, $p = 0.001$) as well as with CNI use after furosemide ($r_{pb} = -0.604$, $p = 0.049$).

Duplex Studies

DUS performed in all subjects within 6 months of the study day ruled out renal and iliac arterial or venous flow restrictions; in one patient, the arterial anastomosis could not be visualized.

DISCUSSION

The main findings of this study are the following: (1) hip flexion induces a consistent increase in renal perfusion as measured by ASL; (2) hip flexion causes a redistribution of renal tissue oxygenation with a significant decrease of the medulla/cortex ratio R2*. This effect on renal oxygenation was not observed

TABLE 2 | Functional magnetic resonance results and derived parameters.

	wo F		with F		wo/with F	
	Neutral	**Flexion**	**Neutral**	**Flexion**	**Neutral**	**Flexion**
			R2* [1/s]			
Cortex	17.7 ± 1.8	18.5 ± 1.4	17.3 ± 2.3	17.5 ± 2.2	1.03 ± 0.11	1.04 ± 0.10
p	0.14		0.77		0.53	
Medulla	28.3 ± 2.9	27.6 ± 3.6	24.0 ± 3.3	25.1 ± 4.1	1.22 ± 0.17	1.11 ± 0.22
p	0.22		0.23		0.00077	
MCR R2*	1.60 ± 0.27	1.48 ± 0.15	1.40 ± 0.20	1.44 ± 0.22	1.17 ± 0.16	1.03 ± 0.14
p	0.015		0.39		0.0036	
			ASL [mL/100g/min]			
Cortex	299.5 ± 60.1	332.0 ± 66.4	277.6 ± 80.6	336.7 ± 69.8	1.10 ± 0.22	0.98 ± 0.16
p	0.051		0.027		0.16	
Medulla	99.2 ± 35.5	139.9 ± 59.8	81.7 ± 22.1	147.6 ± 68.5	1.23 ± 0.59	1.14 ± 0.54
p	0.011		0.0052		0.41	
MCR ASL	0.34 ± 0.09	0.44 ± 0.15	0.32 ± 0.11	0.48 ± 0.21	1.10 ± 0.39	1.04 ± 0.42
p	0.026		0.033		0.37	
			ADC_D [$*10^{-5}$ mm^2/s]			
Cortex	208 ± 13	210 ± 13	206 ± 12	211 ± 14	1.01 ± 0.07	0.99 ± 0.05
p	0.5		0.163		0.42	
Medulla	208 ± 15	206 ± 15	202 ± 13	208 ± 15	1.03 ± 0.07	0.99 ± 0.07
p	0.95		0.022		0.39	
MCR ADC_D	1.01 ± 0.06	0.99 ± 0.05	0.98 ± 0.05	0.99 ± 0.07	1.03 ± 0.07	1.01 ± 0.09
p	0.62		0.77		0.8	
			F_P [%]			
Cortex	11.7 ± 3.3	12.9 ± 3.6	11.3 ± 4.2	11.1 ± 4.9	1.06 ± 0.42	1.34 ± 0.50
p	0.5		0.34		0.076	
Medulla	5.9 ± 4.6	7.4 ± 4.5	10.1 ± 3.6	10.7 ± 4.0	0.64 ± 0.48	0.80 ± 0.64
p	0.51		0.78		0.66	
MCR F_P	0.60 ± 0.51	0.54 ± 0.27	0.93 ± 0.35	1.11 ± 0.62	0.72 ± 0.52	0.67 ± 0.53
p	0.63		0.16		0.6	

Values are given as mean ± standard deviation. p-values are calculated according to Student's t-test. Wo, without; F, furosemide; neutral/flexion denotes hip position; R2, transverse relaxation rate; MCR, medullary to cortical ratio; ASL, arterial spin labeling; ADC_D, pure diffusion coefficient; F_P, fraction of perfusion.*

after furosemide. (3) Hip flexion diminishes the increase in oxygenation after furosemide. At last, our study demonstrates for the first time the feasibility and reliability of a multiparametric functional MRI protocol with examination of kidney graft oxygenation and perfusion during two different body positions in a single session.

Our initial hypothesis was that hip flexion would lead to an instant and temporary reduction of graft perfusion associated with a decrease in graft oxygenation. To our surprise, perfusion values measured by ASL-MRI consistently increased during hip flexion. ASL technique involves the magnetic labeling of inflowing blood with the creation of a subtraction image of the kidney with and without labeling (25). In models of renal ischemia due to renal artery stenosis, decreased perfusion values have been described with this technique (33). There are several possible explanations for the unexpected increase in graft perfusion upon hip flexion.

The first is an increase in arterial inflow due to the partial obstruction distally to the graft leading to increased flow through the external and internal iliac artery. In line with this hypothesis, maximal hip flexion has been shown to induce a shortening, bending, and twisting of iliac arteries in healthy subjects (12, 34). In patients with leg amputation, significant changes in arterial hemodynamics proximal to the amputation have been observed, characterized by early return of reflected waves and an increase in shear stress (34). In patients with traumatic lower limb amputation, these changes have been associated with an increased risk for cardiovascular diseases including aortic aneurysms.

A second hypothesis is that hip flexion causes an increase in arterial blood pressure secondary to a global increase in systemic vascular resistance. Analogously, the squatting position classically adopted by patients with cyanotic heart disease leads to blood pressure elevations by increasing venous return and systemic arterial resistance. Yet, this effect might be restricted to the standing position (35, 36). However, in our experiments, the effect was even greater after furosemide administration, which should lead to a decrease rather than increase in systemic blood pressure.

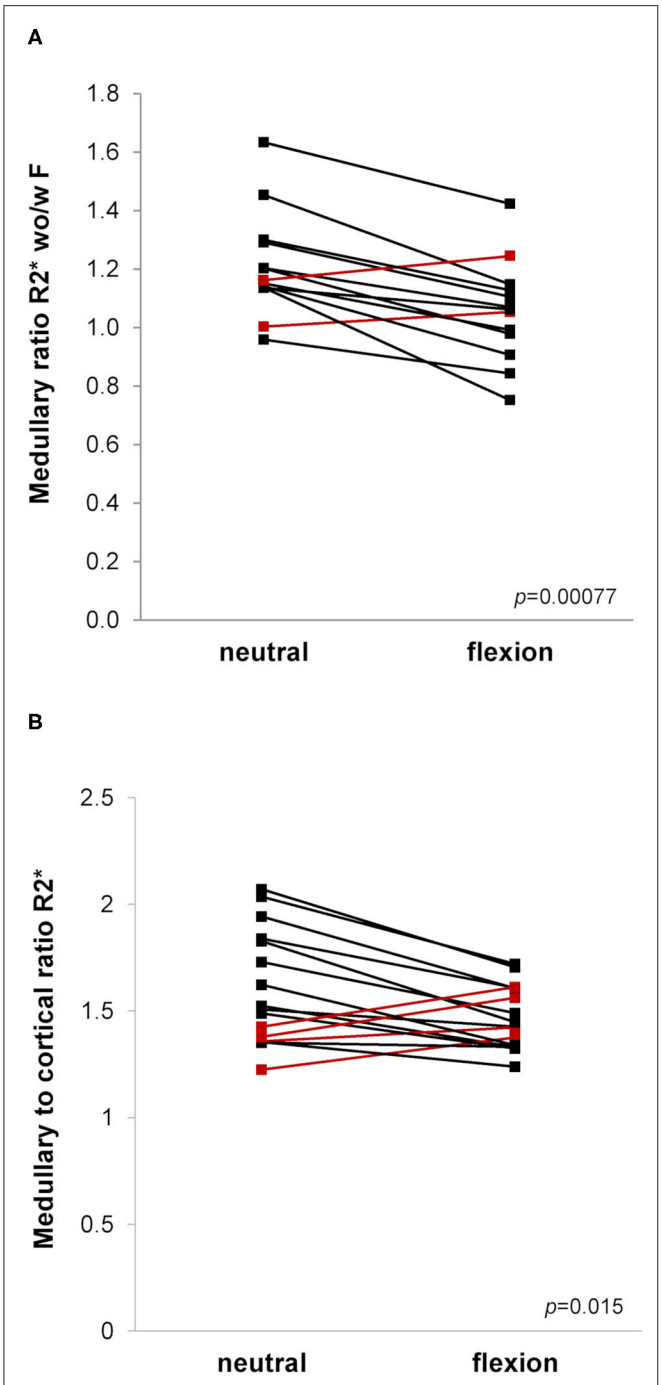

FIGURE 4 | (A,B). Blood oxygen level dependent (BOLD)-MRI during neutral and flexed hip position. Ladder plots showing resonance transverse relaxation rate (R2*)-derived parameters during neutral and flexed hip position: **(A)** Medullary ratio R2* without/with (wo/w) furosemide (F), $n = 13$; **(B)** Medullary to cortical ratio R2*, $n = 16$. Paired Student's *t*-test-derived p-value comparing neutral and flexed hip position (*p*). Colors denote opposite trends.

The increase in renal perfusion could also represent a counter-regulatory mechanism secondary to the changes in tissue oxygenation. Indeed, the scan order putting BOLD before ASL sequences might explain inverse trends in BOLD and ASL values. Thus, in a recent study, sympathetic stimulation by handgrip exercise has been reported to decrease renal artery flow and increase medullary oxygenation measured by BOLD-MRI. A decreased reabsorptive workload due to reduced distal sodium delivery was hypothesized by the authors (37).

Venous outflow obstruction with backflow to the graft during hip flexion is still another mechanism whereby hip flexion could increase graft perfusion. Venous obstruction might actually explain both the increased renal perfusion and the reduced oxygenation response to furosemide. In this situation, the marked prolonged position change might have overcome the hurdle of venous valves. The hydration protocol inducing a larger plasma volume may have increased this effect. Similarly, the two subjects with ipsilateral graft implantation (with a risk of arterio-venous crossing) showed a particularly reduced perfusion ratio neutral/flexed hip position possibly pointing to outflow obstruction. Importantly, potential changes in arterial inflow may have been masked in this case. Thus, in this view, the positive correlation between age, transplant vintage and CNI use on one hand and the cortical perfusion ratio neutral/flexed hip position on the other may be explained by a reduced arterial component of overall flow. However, this hypothesis remains speculative.

The perfusion increase in the cortex and medulla during hip flexion after furosemide is augmented by the observed (albeit non-significantly) lower baseline perfusion values during neutral hip position after furosemide compared to the values before the administration of furosemide. This is in line with previous microelectrode measurements in rats and ASL-MRI measurements in humans (38, 39).

In accordance with our initial hypothesis, hip flexion resulted in changes of renal tissue oxygenation characterized by a significant decrease in the medullary-to-cortical R2* ratio, suggesting a redistribution of intrarenal tissue oxygenation toward the medulla. The decrease of MCR R2* during hip flexion correlated with lower eGFR values. MCR R2* has previously been defined as the normalized intrarenal oxygen bioavailability (40). The significant decrease in MCR R2* during hip flexion points to a decreased cortical oxygen availability or medullary redistribution similar to that described in CKD and vascular allograft rejection (40–43). However, after furosemide administration this effect was not reproduced. This may be due to the strong medullary R2* decrease induced by furosemide which might have outweighed the difference induced by the positional change. When considering absolute R2* values showing no significant differences between both body positions in this study, the confounding factor of the regional blood volume has also to be discussed. Indeed, vasoconstriction might lead to only minor R2* increases and even R2* decreases despite reduced oxygen delivery due to a decreased blood volume fraction (44).

Another finding of our study supporting the initial hypothesis is that the improvement in medullary oxygenation observed upon administration of furosemide under normal conditions was attenuated during hip flexion. As shown previously in native kidneys, furosemide decreases R2*, reflecting an increased tissue oxygenation. This effect is thought to result from decreased oxygen consumption through Na-K-2Cl-cotransporter

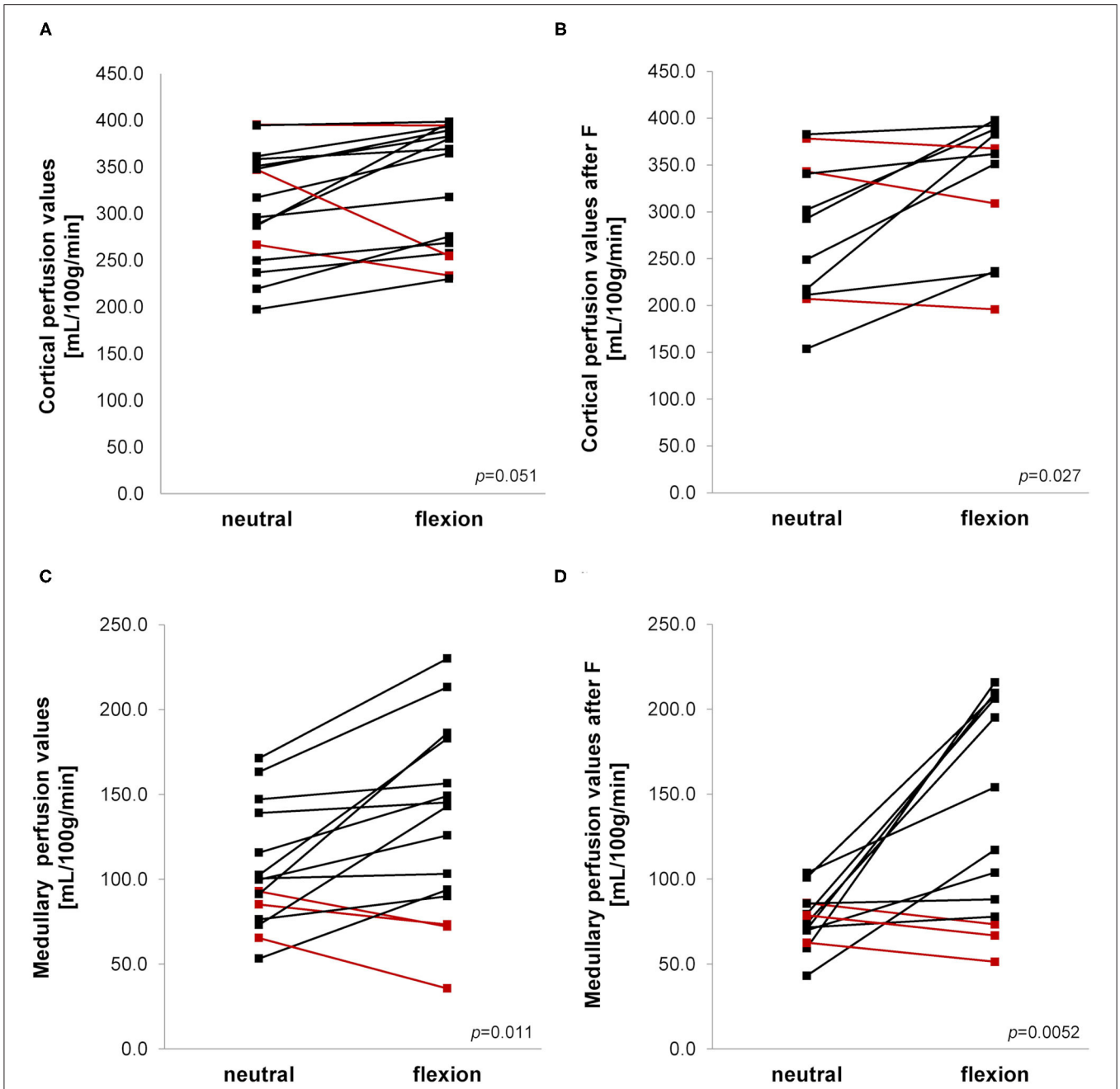

FIGURE 5 | (A–D) Arterial spin labeling-MRI during neutral and flexed hip position. Ladder plots showing perfusion values measured in cortex **(A,B)** and medulla **(C,D)** without **(A,C)** and after **(B,D)** furosemide (F) administration during neutral and flexed hip position. **(A)** $n = 16$; **(B)** $n = 11$; **(C)** $n = 15$; **(D)** $n = 13$. Paired Student's *t*-test-derived *p*-value comparing neutral and flexed hip position (*p*). Colors denote opposite trends.

inhibition (16, 31, 32, 38, 45). The blunted effect of furosemide during hip flexion could be due either to a resistance to furosemide caused by an acute tubular dysfunction or to increased sodium reabsorption secondary to an activation of the renin-angiotensin-aldosterone system, although most of the patients were on a blocker of the renin-angiotensin system. It may also be the consequence of a relative reduction of oxygen supply during hip flexion. Our findings are in accordance with reports on reduced BOLD response to furosemide without effect on absolute R2* values in acute and chronic kidney disease states, in chronic arterial hypertension and during aging (17, 42, 45–47). Furosemide administration has indeed been used as a test of renal functional reserve enhancing the sensitivity of BOLD-MRI similarly to furosemide stress test in acute kidney injury (48).

Our study has several limitations: high-field-strength MRI does not allow imaging in an upright position; therefore, the

sitting position had to be simulated in lateral decubitus excluding the additional influence of gravity potentially operating during the usual sitting position. Blood pressure was not measured simultaneously to evaluate the influence on perfusion values. For ethical reasons, contrast-enhanced angiography was not performed to evaluate for the presence of arterial kinking of iliac arteries and preexisting vascular lesions. Instead, TOF angiography and DUS were used enabling the exclusion of renal artery stenosis or arterial abnormalities in these patients. Another potential limitation is the absence of a measure of graft function during hip flexion. The MRI protocol was too complex to perform simultaneous functional measurements, but this aspect would deserve additional studies. For security reasons, patients with foreign material such as vascular stents and no previous 3T-MRI were excluded. Thus, we may have selected a less atherosclerotic population. The number of subjects in this proof of principle-study was limited. Due to the number of cases, only univariate analysis of clinical associations was performed. Lastly, correction for multiple comparisons was not carried out in this exploratory analysis of clinical correlations.

In conclusion, this physiological proof of principle-study suggests for the first time that redistribution of oxygenation and functional hypo-oxygenation of renal transplants may occur depending on hip position. Whether this phenomenon contributes to the development of chronic fibrosis and ultimately to graft dysfunction is not known. However, one could hypothesize that besides immunological and known non-immunological factors, recurrent graft hypo-oxygenation might also play a role in the long-term loss of kidney grafts. The potential implication of our observations could be a recommendation to avoid a prolonged sitting position in kidney graft recipients and to favor exercises without major sustained hip flexion, such as rowing. Further research is required to assess the functional and/or histological impact of our observation in patients showing a pathologic response during this maneuver.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics commitee of the Canton of Bern, Switzerland (protocol number 2181, approval number 042/12). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LYM participated in research design, performance of the research, data analysis, and writing of the manuscript. MS participated in research design, performance of the research, data collection, and revision of the manuscript. FN participated in data analysis and the revision of the manuscript. DT participated in data analysis and the revision of the manuscript. GD participated in data analysis. PM contributed the arterial spin labeling–magnetic resonance imaging sequence and participated in the revision of the manuscript. MB participated in the writing and revision of the manuscript. BV participated in the research design, performance of the research, and revision of the manuscript. PV participated in the research design, performance of the research, data collection, data analysis, and revision of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.697055/full#supplementary-material

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Causes of Kidney Graft Failure in a Cohort of Recipients With a Very Long-Time Follow-Up After Transplantation

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Background: Biopsy-proven causes of graft loss many years after kidney transplantation are scarcely documented.

Methods: Patients transplanted between 1995 and 2005 ($n = 737$) in a single center were followed on a regular basis until 2021. The recipients were divided according to age at transplantation into 3 groups; 18–39 years (young), 40–55 years (middle age), and older than 55 years (elderly). For cause biopsies of renal transplants were clustered into the categories, rejection, IFTA, return original disease, and diagnosis of *de novo* kidney disease.

Results: Rejection was the main cause of graft failure censored for death at every time period after transplantation. The incidence of T cell-mediated rejection (TCMR) became rare 6 years after transplantation while the cumulative incidence of antibody-mediated rejection (ABMR) increased over time (1.1% per year). ABMR was not diagnosed anymore beyond 15 years of follow-up in recipients without pre-transplant donor-specific antibodies (DSA). An episode of TCMR was associated with an increased incidence of ABMR diagnosis in the short-term but did not increase the overall incidence of AMBR not in the long-term. Death as a cause of graft failure was an important competitive risk factor long after transplantation and resulted in a significantly lower frequency of rejection-related graft loss in the elderly group (11 vs. 23% in the young group at 15 year follow-up).

Conclusion: Rejection is a major cause of graft loss but recipient's age, time after transplantation, and the presence of DSA before transplantation determine the relative contribution to overall graft loss and the type of rejection involved.

Keywords: kidney transplantation, ABMR, antibody-mediated rejection, TCMR, graft failure risk, long term

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

INTRODUCTION

Graft survival of the transplanted kidney is documented in detail for the first years after transplantation in many publications. The causes for graft loss are predominantly acute T cell-mediated rejection (TCMR), primary non-function in case of deceased donor donation, surgical complications, and increased risk of death because of cardiovascular events or infection. Data of long-term graft survival are usually derived from large registries and, in general, provide an analysis of graft loss because of death with functioning graft or graft loss censored for death. However, there is a growing interest in the causes of kidney graft loss in the (very) long term but the number of publications is still limited. A major paradigm shift has occurred by leaving the ill-defined concept of chronic allograft nephropathy (1, 2) and redefining graft loss by regularly updated pathology criteria (Banff criteria) which include the categories (chronic-active) antibody-mediated rejection (ABMR) and interstitial fibrosis with tubular atrophy (IFTA) among others (3). In particular ABMR was recognized as a major cause of kidney graft loss in the long-term (4–6). However, a close follow-up of recipients with a high degree of diagnostic biopsies was usually lacking. In addition, the role of recipients age and time after transplantation is generally not taken into account. For instance, the incidence of TCMR is recipients age-dependent and the incidence is highest within the first months after transplantation (7–10). For ABMR the relation with recipients age is not documented and whether the incidence changes in the years after transplantation is also not known.

In addition, death with functioning graft is a major cause of graft loss and is a competitive risk factor for all other causes of graft failure, particularly in the elderly. This issue is recognized but poorly addressed, although a recent publication drew attention for this cause of graft loss (11). Another recent publication, by Mayrdorfer et al., showed that the cause of graft loss changes over time after transplantation and revealed that usually a number of clinical adverse events contribute to the final progression to graft loss (12). The general lack of data of the (very) long follow-up of kidney graft recipients is likely explained by the fact that many transplantation centers do not prospectively collect their data in a dedicated database.

In this study, a cohort of kidney transplant recipients with prospective collection of relevant data and a high level of kidney biopsies-proven diagnoses was analyzed to describe the changes in cause of graft loss in different age groups over a very long time after transplantation, taking death with a functioning graft into account.

MATERIALS AND METHODS

This study included all 737 kidney transplantations performed between January 1995 and December 2005 at the Erasmus Medical Center in the Netherlands. The last follow-up date before data analysis was 1 March 2021. Recipients were seen at least once a year at our out-patient clinic for follow-up and data were registered in a national database (see below) which was locally supplemented with additional clinical parameters. If the regular visits were discontinued recipients were considered lost to follow-up from their last visit. **Figure 1** shows the flow chart of patients at 1 year and at the end of follow-up.

All transplantations were performed with a negative complement-dependent cytotoxicity cross-match with both current and historic sera and ABO blood group-compatible. The standard immune suppressive medication protocol with a calcineurin inhibitor was either tacrolimus (aiming for predose concentrations of 10–15 ng/ml in weeks 1–2, 8–12 ng/ml in weeks 3–4, and 5–10 ng/ml, thereafter) or ciclosporin (aiming for predose concentrations of 150–200 μg/L tapered to 100–150 μg/L at 6 months), combined with mycophenolate mofetil (starting dose of 1 g b.i.d., aiming for predose concentrations of 1.5–3.0 mg/L) and glucocorticoids. All patients received 50 mg prednisolone b.i.d. intravenously on days 0–3. Thereafter, 20 mg oral prednisolone was started and subsequently tapered to 5 mg at month 3.

For analysis, the baseline and clinical follow-up transplantation data were retrieved from the Netherlands Organ Transplant Registry (NOTR), which was over 99% complete for our center at time of this study. The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism" and in accordance with the declaration of Helsinki. The use of clinical data and assessment of donor-specific antibodies in stored serum samples was approved by the Research Ethics Committee for Biobanks and the Medical Ethics Committee of the University Medical Center Utrecht.

All renal biopsies were performed because of progressive loss of graft function and no *per* protocol biopsies were performed. All kidney biopsies were evaluated by experienced renal pathologists and commented on in detail. The original descriptions of the glomerular and tubular-interstitial compartment were used to reclassify the biopsies with rejection according to the Banff 2018 reference guide (3). Rejection episodes were classified as cellular (TCMR), humoral (ABMR), or mixed-type rejection. The latter type of rejection presented a small group ($n = 10$) and for statistical analysis these cases were combined with the humoral rejections. The presence of anti-HLA donor specific antibodies (DSA) were retrospectively measured at the pretransplant phase by Luminex single beads assay, as part of the PROCARE study (13, 14). DSA after transplantation were not routinely measured. If no serum donor-specific antibodies were present and/or C4d staining was negative or not stained for in the biopsy than the diagnosis of ABMR by histology (ABMRh, 34% of total ABMR cases) was made as described in detail before (15) and used in previous publications (16–18). The standard treatment protocol for TCMR consisted of high dose methylprednisolone (3 days of 1,000 mg per day intravenously) and T cell depletion by rabbit anti-thymocyte globulin or Alemtuzumab was added in cases of steroid-resistant rejection and/or vascular rejection. ABMR was treated with high dose methylprednisolone and intravenous immunoglobulins (1 gram/kg bodyweight) with additional plasmapheresis in early ABMR and in some selected cases Alemtuzumab as second-line treatment (15).

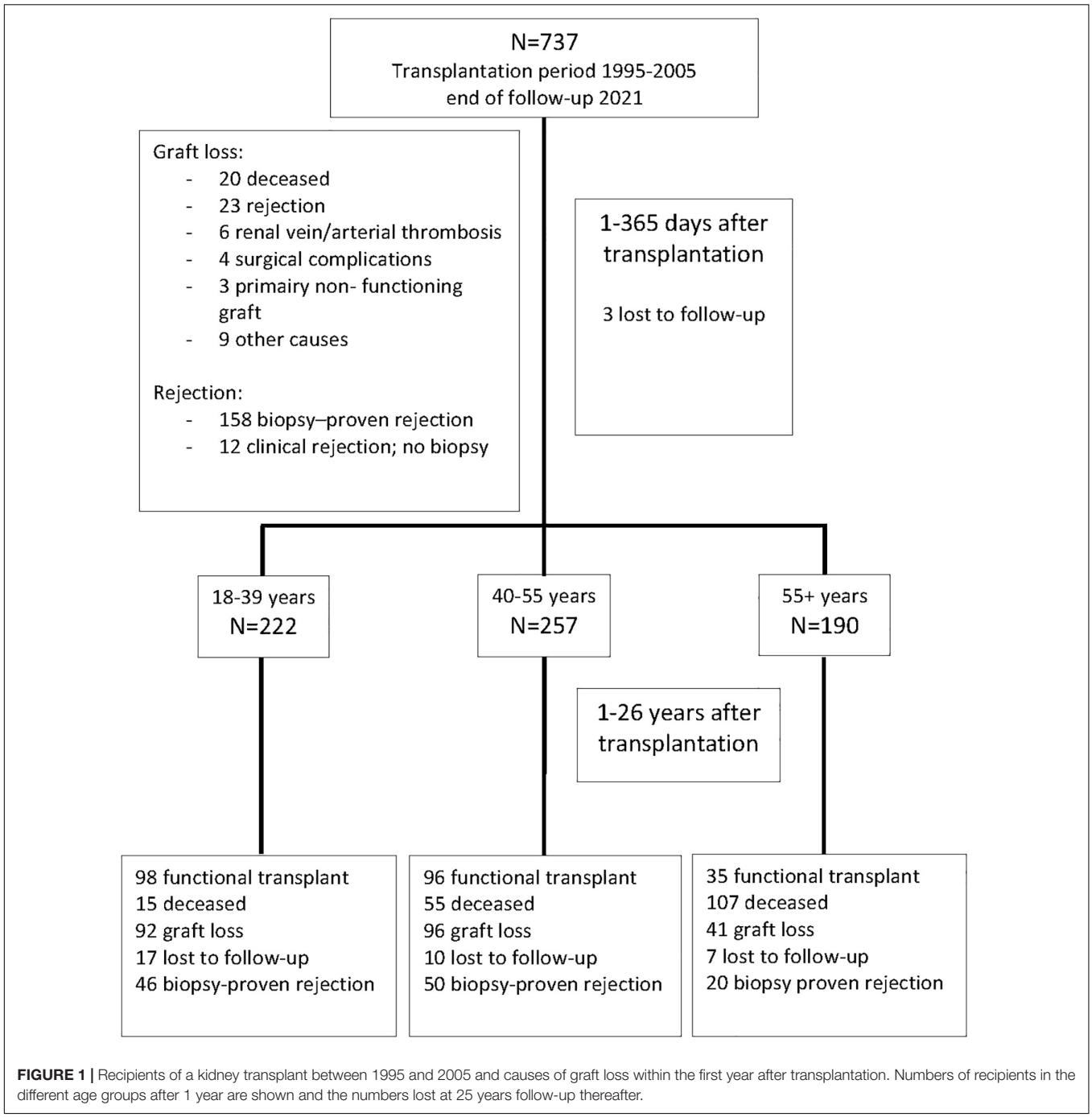


FIGURE 1 | Recipients of a kidney transplant between 1995 and 2005 and causes of graft loss within the first year after transplantation. Numbers of recipients in the different age groups after 1 year are shown and the numbers lost at 25 years follow-up thereafter.

For data analysis the outcome of the kidney biopsy was further categorized recurrence original kidney disease (e.g., IgA nephropathy, SLE-nephritis, C3-glomerulopathy), diagnosis of *de novo* kidney disease defined as a diagnosis of primary kidney disease which was not present before transplantation (e.g., amyloidosis, post-infection glomerulonephritis) and interstitial fibrosis with tubulus atrophy (IFTA) which category contains all biopsies without a classifying diagnosis other than the presence of IFTA as a sign of chronic renal damage. Underlying kidney disease of the recipients is shown in **Supplementary Table 1**.

In 38 recipients, a kidney biopsy was not performed although no obvious clinical diagnosis for their progressive deterioration of graft function was present. Reviewing the charts revealed that in less than 10% this was because of refusal of the recipient, a high risk for complication or end-stage renal disease at presentation. In most cases the treating physician considered a diagnosis of "chronic allograft nephropathy" and concluded that kidney biopsy would not alter treatment policy.

If graft failure occurred the diagnosis of the *for cause* kidney biopsy was used to categorize the type of graft failure. The

TABLE 1 | Clinical and demographic characteristics of recipients and kidney donors given for different age categories of recipients.

	18–39 years*n* = 242	40–55 years*n* = 277	>55 year*n* = 218	*p*-value
Median age recipient in years (IQR)	30 (25–35)	47 (43–51)	61 (57–65)	
Median age donor in years (IQR)	46 (34–55)	46 (38–55)	54 (39–61)	<0.001
Recipient male/female ratio	48/52%	47/53%	45/55%	0.9
Deceased/living donor kidney	37%/63%	64%/36%	73%/27%	0.07
-DBD type*	92%	80%	81%	
-DCD type*	8%	20%	19%	0.002
-Delayed graft function	59%	50%	40%	0.3
-Duration of delayed graft function median days (IQR)	12 (6–14)	10 (9–16)	15 (9–21)	0.3
Cold ischemia time in hours	9.8 ± 0.6	11.3 ± 0.6	11.7 ± 0.7	0.3
Retransplantation	23%	19%	11%	0.003
PRA at transplantation, means (SD)	10% (21.7)	8% (18.2)	5% (16.1)	<0.001
Total HLA mismatches, means (SD)	2.2 (1.4)	2.5 (1.6)	2.9 (1.6)	0.8
Follow-up in years, median (IQR)	14.6 (5.9–19.7)	14.9 (6.6–17.7)	11.2 (6.6–18.0)	<0.001
Recipients with anti-HLA DSA at time transplantation	24.5%	21.8%	18.8%	0.4
Induction therapy	15	24	18	0.9
- Anti-IL-2 receptor antibody	13	24	18	
- T cell depleting antibody	2	0	0	
Maintenance immune suppression				0.9
- Steroids	90.4%	92.2%	92.0%	
- Tacrolimus/ciclosporin	60.3%/38.6%	59.3%/37.5%	65.9/32.0%	
- MMF/azathioprine	69.7%/0.5%	75.6%/0.0%	70.7/0.0%	
- Sirolimus	8.4%	7.5%	9.2%	
- Other	4.5%	3.1%	3.2%	

**Type of deceased donor, by brain death (DBD) or cardiac death (DCD), given as % of total deceased donor kidneys, PRA, panel reactive antibodies; DSA, donor specific antibodies; SD, standard deviation; IQR, interquartile range; P-values were calculated with Kruskal-Wallis H-test for comparing multiple independent samples.*

other outcome categories were a clinical diagnosis of cause for graft failure (e.g., acute kidney injury related to contrast/drugs-associated nephropathy, sepsis, or hemorrhagic shock) and "unknown." The latter category contained all cases of graft failure in which no biopsy was performed and a clinical diagnosis for allograft failure could not be made.

Delayed graft function (DGF) was defined as the need for continuing dialysis after transplantation and duration of DGF was counted in days from transplantation to the last dialysis.

Within the first year after transplantation, the category "perioperative complications" was applied.

The cause of death of the recipients was documented and shown in **Supplementary Table 2**.

Statistical Analysis

Three age groups were made based on age at the time of transplantation: 18–39 years (young), 40–55 years (middle age), and > 55 years old (elderly) which roughly matched to tertiles of recipients age distribution and were considered clinically relevant age categories. Differences in patient, donor, and transplant characteristics were assessed by the Fisher's exact test for categorical variables and Mann-Whitney *U*-test for continuous variables. All *p*-values were 2-tailed.

Death censored graft loss and incidence of different causes of graft loss were assessed by Kaplan-Meier analysis with log-rank statistics for difference between strata. As all recipients had, by definition, a follow-up of 15 years, the causes of graft failure were specifically given for that point in time (**Table 1**). Univariate Cox proportional hazards analysis was used to identify clinical and demographic variables as given in **Table 1** for their association with rejection and graft survival. Variables with a *p*-value of < 0.1 were considered for further analysis by stepwise forward regression to calculate hazard ratios and corresponding confidence intervals. PH assumption of variables were tested by visual inspection of log-minus log graphs and further tested by assessment of time-dependency using the Cox regression with time-dependent covariate module in SPSS. All variables met the demands of PH unless stated otherwise. Interaction terms that met statistical significance ($p < 0.05$) were included in the multivariate model. Normal probability plots were made and presence of significant correlations was assessed. Absence of collinearity in the model covariates was formally assessed by calculating the variance inflation factor. Statistical analysis was performed with software IBM SPSS statistics 21.

RESULTS

Baseline Characteristics and Graft and Recipient Survival per Age Category

The clinical and transplant characteristics of recipients stratified according to their age category are given in **Table 1**. The uncensored all-cause graft survival curves for the different age groups are similar until 10 years post-transplantation (**Figure 2A**)

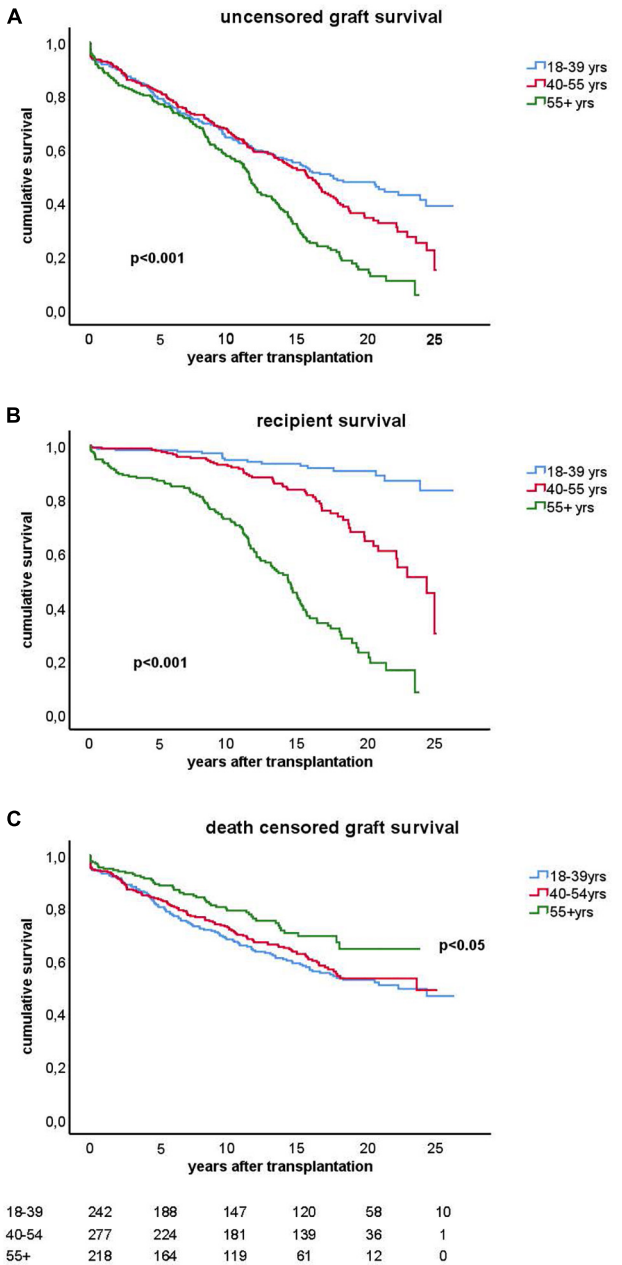


FIGURE 2 | Kaplan-Meier analysis of graft survival for different age groups. The top figure **(A)** shows uncensored graft survival, the middle figure **(B)** shows the loss of grafts because of death with functioning graft and the bottom figure **(C)** shows the graft survival censored for death. *P*-values < 0.05 obtained by pooled over strata in the upper and middle figure are shown. In the lower figure **(C)**, the *p*-value for difference by log rank test statistics comparing the elderly group with the young or middle age group is given. The numbers of recipients in follow-up at different time points after transplantation are shown below the figure.

after which death as cause of graft loss becomes a dominant factor (**Figure 2B**). Graft survival censored for death is significantly better for the elderly group as compared to the young and middle aged groups (**Figure 2C**) explaining the similar all-cause graft survival curves between age groups with the first 10 years after transplantation. Of note, the type of maintenance immune suppressive regimen (in particular ciclosporin or tacrolimus-based) was not associated with graft survival in the different age groups, in accordance with a previous analysis of the total PROCARE cohort (19). The majority of young recipients received a graft from a living donor as opposed to the elderly group and most of the deceased donor kidneys were from brain death donors. The incidence and duration of delayed graft function for deceased donor kidneys was similar for all age groups. Delayed graft function (and not duration) was associated with decreased graft survival (HR 1.5, CI 1.1–2.0, $p = 0.01$) but not recipient survival.

At 15 years after transplantation the frequency of death with a function graft ranges from 5.3% in the young group to 43% in the elderly group (**Table 2**). Within that period, rejection constitutes a major part of the known causes of graft loss censored for death in every age group; 53/89 (59%) in the young, 45/80 (56%) in the middle age, and 23/39 (58%) in the elderly group. IFTA is the second most frequent known cause of graft failure (respectively, 16, 21, and 20% in the young to elderly age category).

Figure 3A shows the relative contribution of causes of graft failure categorized in deceased with functioning graft, biopsy-proven, and clinical diagnosis of graft failure, and the number of "unknown causes" within in each time period after transplantation for the different age groups. Within the first year after transplantation the cause of graft failure was always identified by kidney biopsy and/or a clinical diagnosis (e.g., renal artery thrombosis, bleeding, or sepsis-related acute kidney injury) was made. Thereafter, the percentage of cases of graft failure categorized as "unknown cause" was variable per time period and age-category (**Figure 3A**) and on average 10% of the total number of graft losses (ranging from 0 to 19%).

Increased contribution of death to overall graft loss within the different time periods after transplantation and age categories ranged from 3 to > 80% (**Figure 3A**). For instance, follow-up beyond 15 years identified death with a functioning graft as a cause of graft loss in 82% in the elderly as opposed to 25% in the young group. As expected (20), malignancies, infection, and cardiovascular disease constituted the 3 main causes of death at follow-up. Relatively more infection-related death in the elderly group (17.8%) as compared to the young and middle age group (8.3% for both and *p*-value 0.08 compared to the young age group), and relatively more malignancies in the young age group (50 vs. 24.4% in the elderly age group, *p*-value 0.6) were noted (**Supplementary Table 1**).

Rejection Is a Major Cause for Graft Loss but Dependent on Age and Time After Transplantation

In all age categories, the major cause of graft loss other than death was rejection-related (see **Table 1** for 15 years follow-up and **Figure 2**). **Figure 3B** shows the relative contribution of causes of graft failure found by kidney biopsy within in each time period after transplantation for the different age groups. The risk for TCMR was clearly age and time after transplantation-related

TABLE 2 | Outcome at 15 years follow-up after transplantation for different categories of recipient age at time of transplantation.

	18–39 years*n* = 242	40–55 years*n* = 277	>55 year*n* = 218	*p*-value
Lost to follow-up*	14	6	9	ns
Median follow-up time at year 15 (IQR)	12.3 (6.0–15)	12.0 (6–15)	11.0 (5–15)	<0.001
Death with functioning graft	12 (5.3%)	36 (13.3%)	90 (43.1%)	<0.001
Number of graft loss other than death	95 (41.7%)	98 (36.2%)	47 (22.4%)	<0.001
Graft loss by:				
- Rejection	53 (23.2%)	45 (16.6%)	23 (11.0%)	<0.001
- IFTA	14 (6.1%)	17 (6.3%)	8 (3.8%)	ns
- Recurrence of original disease	8 (3.5%)	4 (1.5%)	1 (0.5%)	0.04
- Diagnosis *de novo* kidney disease	3 (1.3%)	2 (0.7%)	1 (0.5%)	ns
- Kidney injury/disease**	6 (2.6%)	6 (2.2%)	5 (2.4%)	ns
- Peri-operative complications	4 (1.7%)	5 (1.8%)	0 (0.0%)	ns
- Unknown	6 (2.6%)	18 (6.6%)	8 (3.8%)	ns
- Primary non-function	1 (0.4%)	1 (0.3%)	1 (0.5%)	ns

**Recipients lost to follow-up not included for calculation frequencies.*
***Events or diseases causing irreversible kidney injury leading to graft loss.*
ns, not significant ($p > 0.05$).

resulting in more TCMR-related graft loss in the young group (**Figure 3B**). After total follow-up, 23 cases in the young group had TCMR-related graft loss (9.5% of total young recipients included at time of transplantation) which was 17 cases (6.2%) and 7 cases (2.3%) in the middle age and elderly group, respectively. The incidence of TCMR became close to zero after 6–7 years for all patients. In the elderly group, graft loss because of TCMR was not observed any more after 5 years follow-up (**Figures 3B**, **4**). The percentage of TCMR episodes leading to graft failure was on average 22.7% but age-group dependent (young: 27.4%, middle age: 20.7% and elderly recipients: 17.0%, $p < 0.05$ for trend).

The cumulative risk for AMBR increased steadily until about 15 years after transplantation after which only very few new cases were observed (**Figure 4**). The presence of DSA at the time of transplantation (pretransplant DSA) was a significant risk factor for ABMR and the effect persisted for many years after transplantation. New cases of ABMR diagnosed after 15 years were only observed in the recipients with pretransplant DSA (**Figure 5**). The average annual incidence of ABMR in the period 1–15 years after transplantation was 1.1% (range 0.7–1.5%) and unaffected by age. Uni- and multivariate logistic regression analysis (**Table 3**) showed several known risk factors for TCMR such as recipient's age, cold ischemia time, positive PRA, and number of HLA mismatches. In a multivariate model, only the presence of DSA before transplantation showed a significant relation with the incidence of ABMR.

The percentage of biopsy-proven ABMR cases leading to graft loss at the end of follow-up was high (74.4%) and tended to be higher in the young recipient group (young: 83.7%, middle age: 69.0% and elderly recipients:68.4%, $p = 0.2$). The much higher risk for graft loss because of death in the elderly group obviously greatly reduced the impact of ABMR on graft survival. For instance, in the period 15–26 years after transplantation the relative and absolute number of cases with ABMR-related graft failure in the elderly group was significantly lower (4 cases; 5% of total graft loss) as compared to the younger group (13 cases; 27% of total graft loss, $p < 0.01$, **Figure 3B**). In other words, although the risk for ABMR-related graft loss is similar for all age categories, only 14 recipients in the elderly group (6.4%) had lost their graft because of ABMR after 1–26 years, compared to 15.3 and 12.8% in in the young and middle age group ($p = 0.01$).

As early TCMR may be a risk factor for later development of ABMR, the ABMR-free survival Kaplan-Meier curves were made for recipients with and without an episode of TCMR after transplantation. Interestingly, TCMR was associated with an increased incidence of ABMR diagnosed earlier after transplantation but survival lines converged after 10 years with overall no difference in the cumulative incidence of ABMR (**Figure 6**).

Interstitial Fibrosis and Tubular Atrophy-Related Graft Loss Is Independent of Recipients Age and Influenced by Previous T Cell-Mediated Rejection

The risk for graft loss with a biopsy-proven diagnosis of chronic damage was independent of age (**Figure 4**) and it became a relatively more frequent cause of graft loss long after transplantation (**Table 1** and **Figure 3B**). At 15 years follow up, the percentage of graft loss because of IFTA was 5.5% (**Table 1**). Beyond 15 years of follow up, ABMR and IFTA were the dominant, almost exclusive, causes of graft loss (**Figure 3B**).

A previous rejection may lead to IFTA and subsequent graft loss at longer follow-up (2). To test this hypothesis we made separate KM curves for IFTA-related graft loss for recipients with and without rejection. Only TCMR was significantly related to IFTA-related graft loss (**Figure 4**, bottom right figure) which was confirmed by logistic regression analysis (HR 2.3, $p = 0.008$). At maximal follow-up, 27 out of 369 recipients (7.3%) with no TCMR episode (60% of all IFTA-related graft loss) and 18 out of

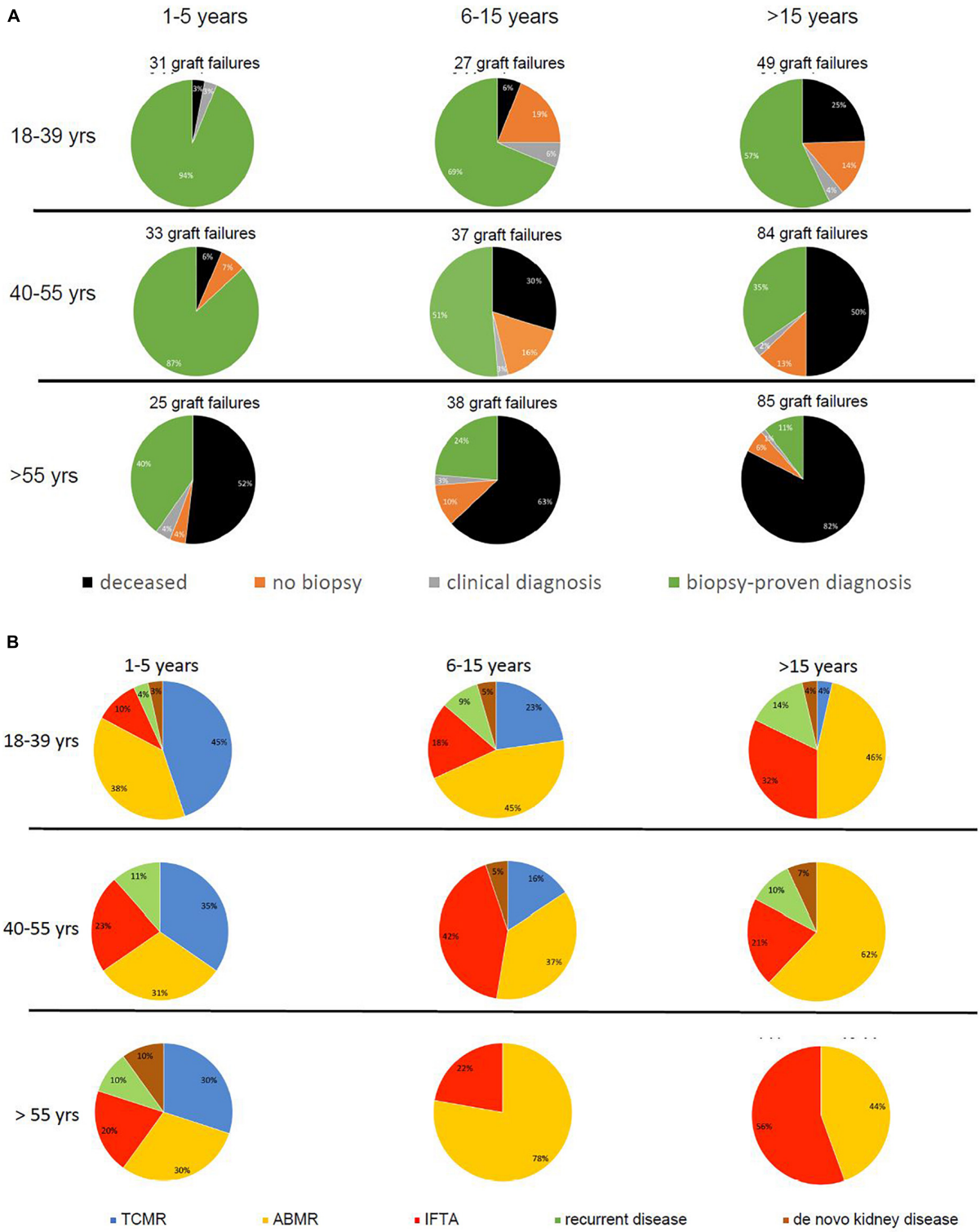


FIGURE 3 | Pie charts are given for causes of graft loss in different age groups in different time periods after transplantation starting from 1 year after transplantation. In part **(A)**, the categories of cause for graft loss represent death with functioning graft, unknown (no biopsy performed and no clinical diagnosis), a clinical diagnosis of kidney injury or disease, and kidney biopsy-based cause of graft loss. In part **(B)**, the category of kidney biopsy-based cause of graft loss is split into TCMR, ABMR, return original disease, and *de novo* kidney disease. The numbers of graft loss and recipients lost at follow-up within every post-transplantation period are shown above the pie charts. Every row of pie chart represents a recipient age category at the time of transplantation (18–39, 40–55, and > 55 years) and every column represents a time period after transplantation (1–5, 5–15, and > 15 years after transplantation).

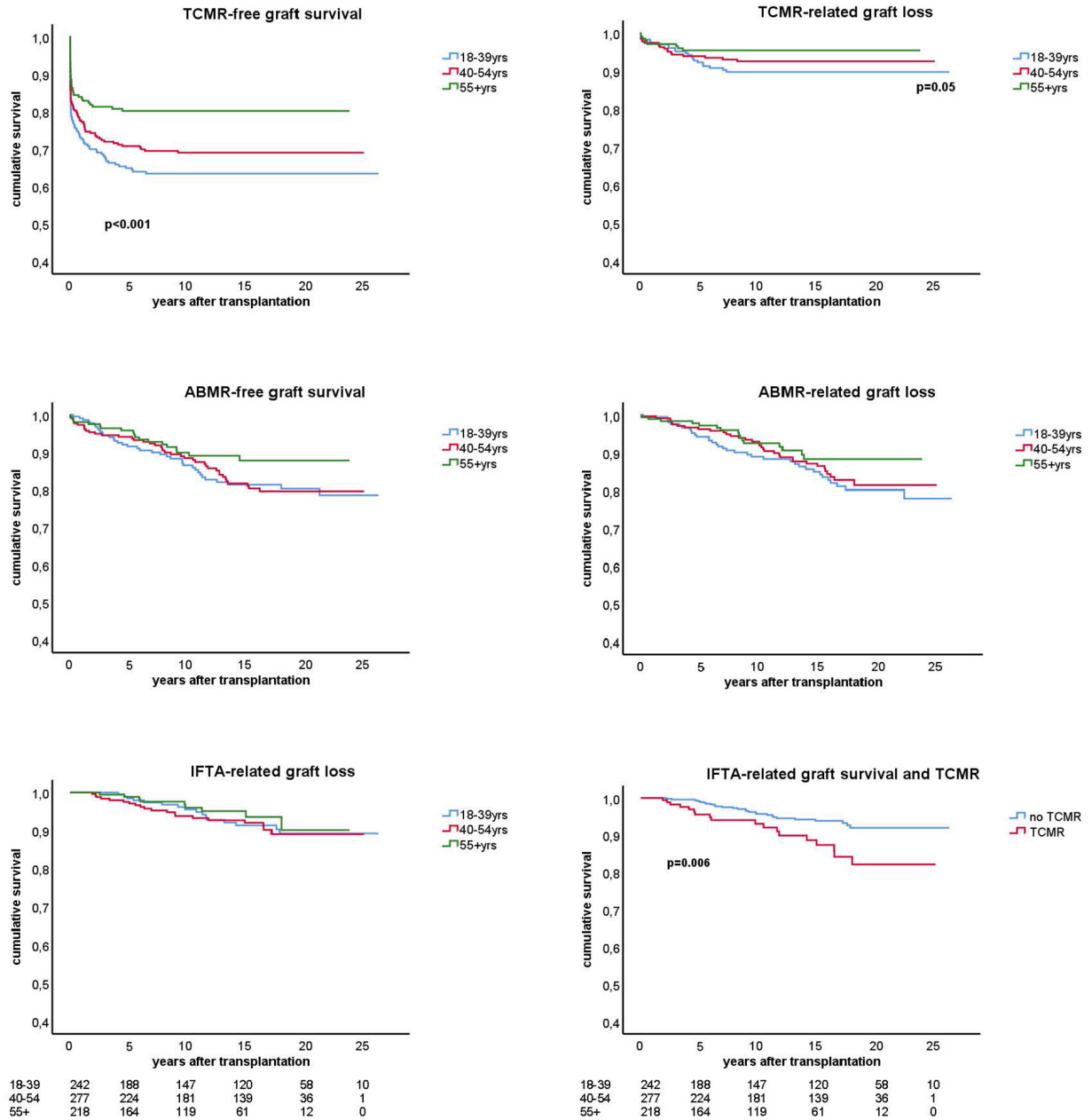


FIGURE 4 | Kaplan-Meier analysis of the antibody mediated (ABMR) and T cell mediated rejection (TCMR) free-survival and the ABMR and TCMR-related graft loss for different age groups. The lower panel right shows the interstitial fibrosis and tubular atrophy (IFTA) related graft loss with a subgroup analysis for recipients with and without previous TCMR. All analysis were done by censoring for death and lost at follow-up. Number of patients in follow-up per age stratum is shown below the graphs. Only *p*-values < 0.05 are shown in the figures and obtained by log rank test statistics pooled over strata (TCMR-free graft survival), comparing the young group with the other elderly group (TCMR-related graft loss), and pairwise over strata (TCMR and IFTA-related graft loss).

93 recipients (19.3%) with a previous TCMR had IFTA-related graft loss ($p = 0.001$).

Recurrence of Original Disease

Graft failure because of recurrent disease was relatively rare with 15 identified cases (2.0% of total recipients) with great diversity in biopsy diagnosis; IgA nephropathy ($n = 2$), autoimmune vasculitis ($n = 3$), diabetic nephropathy ($n = 3$), membranoproliferative glomerulonephritis ($n = 3$), thrombotic microangiopathy ($n = 1$), and focal segmental glomerulosclerose ($n = 3$). As expected based on the higher frequency of glomerulonephritis and glomerulopathy (**Supplementary Table 1**), recurrence of the original disease was predominantly noted in the young ($n = 8$, 3.3%) and middle age groups ($n = 6$, 2.2%) with only 1 case in the elderly group (0.5%) which is illustrated by **Figure 3B**.

TABLE 3 | Univariate and multivariate Cox regression analysis for risk of rejection.

	T-cell mediated rejection			Antibody mediated rejection		
	p-value	Hazard ratio	95% CI	*p*-value	Hazard ratio	95% CI
Univariate analysis						
Male sex recipient	0.99	1.00	0.751.34	0.81	1.06	0.62–1.84
Age recipient (per year)	0.001	0.98	0.97–0.99	0.13	0.99	0.97–1.04
Age donor (per year)	0.13	1.01	1.00–1.07	0.76	1.00	0.98–1.07
Deceased donor kidney	0.033	1.34	1.02–1.77	0.63	1.10	0.74–1.64
Previous transplant	0.56	1.12	0.82–1.59	0.21	1.35	0.89–2.20
Number of HLA mismatches	0.001	1.16	1.06–1.26	<0.001	1.26	1.11–1.43
PRA positive (>5%)	0.003	1.55	1.16–2.06	0.08	1.45	0.96–2.21
Cold ischemia time per hour	0.002	1.02	1.01–1.03	0.98	1.00	0.98–1.01
Pretransplant DSA present	0.38	1.15	0.83–1.59	<0.001	2.18	1.43–3.32
Multivariate analysis						
Age recipient (per year)	<0.001	0.96	0.97–0.99	–	–	–
Cold ischemia time per hour	<0.001	1.03	1.01–1.04	–	–	–
Pretransplant DSA present	–	–	–	<0.001	2.24	1.46–3.43
Number of HLA mismatches	<0.001	1.25	1.14–1.38	–	–	–
PRA positive (>5%)	0.02	1.41	1.06–1.89	–	–	–

Diagnosis of *de novo* Kidney Disease

De novo kidney disease was rarely encountered as a cause for graft failure and documented in 8 recipients (1.0% of total recipients); BKV nephropathy ($n = 2$), JC-virus nephropathy ($n = 1$), tubulo-interstitial nephritis ($n = 1$), cholesterol emboli ($n = 1$), diabetic nephropathy ($n = 2$), and anti-GBM disease in a recipient with Alport disease ($n = 1$).

DISCUSSION

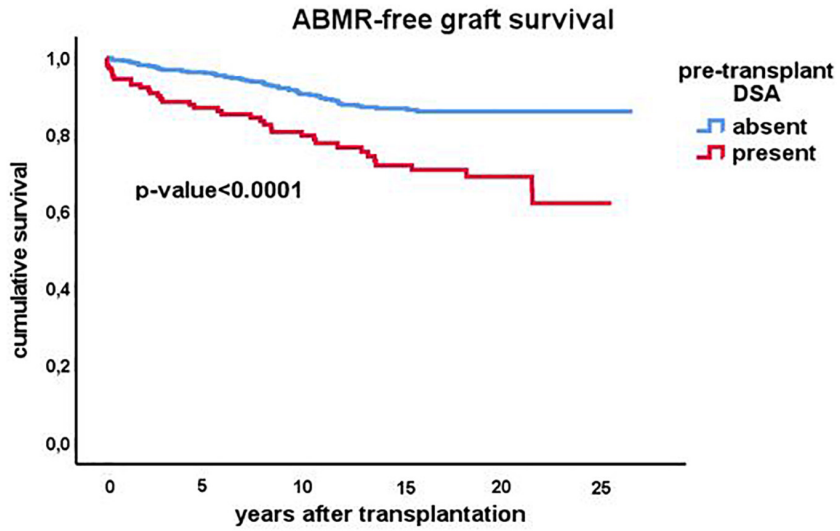
This analysis of a very long-term follow-up study of kidney transplant recipients up to 26 years is first in its kind to show that causes of graft failure are a function of post-transplantation time and recipient's age. The data obtained in this study indicate that TCMR is in particular contributing to graft loss in the young patients but the impact becomes negligible after 5 years post-transplantation and about 2 years earlier in the elderly recipients. The incidence of AMBR in for cause biopsies is remarkably constant in the period of 1–15 years after transplantation and not age-dependent.

After 15 years, there are very few new cases of ABMR and similar to TCMR, the ABMR-free survival curve flattens. A previous TCMR increases the incidence of ABMR shortly after transplantation but in the long run there is no influence of TCMR on the cumulative incidence of ABMR. Taken together the data suggest that a particular load of antigenic mismatches is required to develop ABMR, in accordance with recent studies on the association between the number of predicted indirect recognizable donor-derived HLA epitopes (PIRCHE) which can be presented by recipients HLA class II and long-term graft survival (21, 22). The cumulative incidence of ABMR is probably dependent on the intensity of the immune suppressive drug regimen and there are no data to support the hypothesis that after 15 years tolerance is achieved. However, the current data do imply that at least long after transplantation not only the risk of TCMR but also the risk of ABMR becomes very low. The latter seems to apply in particular to the group of recipients without the presence of DSA before transplantation. The data from this study suggests that pretransplant DSA cause an increase in the risk for ABMR which persists even many years after transplantation. The data from this cohort study emphasizes the important role of the anti-donor humoral response in (long-term) graft loss as was already postulated almost 20 years ago by Terasaki (23).

For clinical decision making, it is important to realize that death with functioning graft is a major competitive risk for long term causes of graft loss, in particular ABMR. Elderly recipients with a high burden of comorbidities will have a limited life span after transplantation although they may still benefit from kidney transplantation over continuing dialysis (24, 25). The mortality of recipients in the long term has improved over the decades but is still substantial in the elderly (20). Therefore, the *a priori* chance of losing the kidney graft because of ABMR in these vulnerable recipients is relatively small. In contrast, young patients have a substantial risk for graft loss because of either TCMR in the first couple of years and ABMR, thereafter. These recipients will have the greatest benefit of a well HLA-matched kidney allograft.

Recurrence of original kidney disease, newly diagnosed kidney disease, and clinically diagnosed causes of graft failure beyond 1 year post-transplantation are relatively infrequent causes of allograft failure.

The strength of our study is the unique long and close follow-up of the recipients with relative few lost to follow-up and a high score of *for-cause* kidney biopsies. However, we realize that such a prolonged observation period as in this study introduces many confounders which are difficult to account for. For instance, immune suppressive drug regimens have changed over time and within patients. In addition, it is unclear to what extent the result



DSA-	573	449	340	238	75	9
DSA+	160	106	80	61	28	1

FIGURE 5 | Kaplan-Meier analysis of the antibody mediated (ABMR) rejection free-survival for recipients with ($n = 159$) and without ($n = 573$) presence of pretransplant donor-specific antibodies against HLA (DSA). The *p*-value shown is obtained by comparing different strata with long rank test statistics.

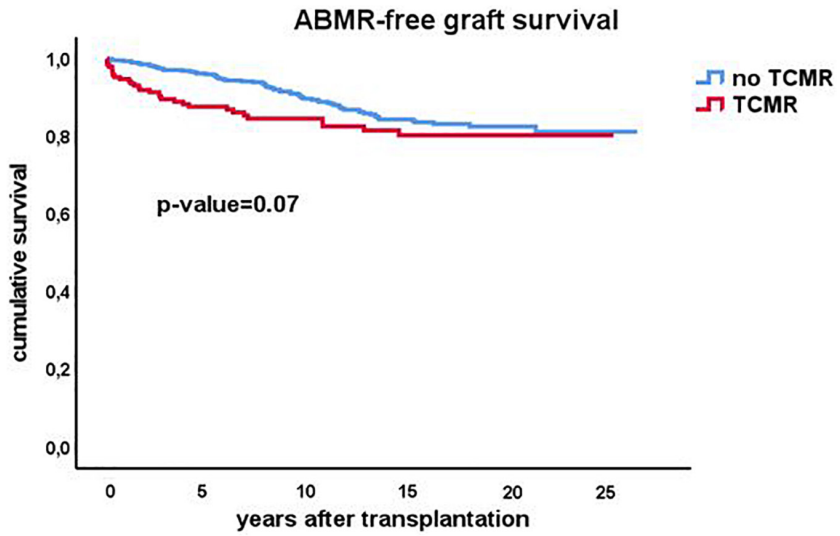


FIGURE 6 | Kaplan-Meier analysis of the antibody mediated (ABMR) rejection free-survival for recipients with ($n = 207$) and without ($n = 530$) a previous episode of TCMR. The *p*-value shown is obtained by comparing different strata with long rank test statistics.

of our center can be generalized, in particular, with respect to the incidence of death with function graft. In our center we tend to have a relative liberal transplantation policy with respect to the eligibility of patients with a high co -morbidity score (24, 26). However, the overall graft survival for deceased donors at 5 (72%) and 10 years (58%) observed in this study is very similar to the European data from the ERA-EDTA and the CTS registry obtained in the same era of transplantation (27). Moreover, death with functioning graft in the different age groups at different time intervals after transplantation is similar to the aforementioned CTS registry data.

In accordance with other studies, delayed graft function in the recipients receiving a deceased donor kidney negatively impacts the graft survival (28) and underlines the need for preventing this adverse event (29). Not only may it lead to delayed graft function to primary non-function of the kidney but it also impacts the long-term survival of kidneys which may be mediated by a significantly lower eGFR at 1-year post-transplantation (30).

Although we recognize that graft loss may have many contributing factors as recently demonstrated (12), this usually concerns renal hits that cause a transient or permanent decrease but not progressive loss of eGFR. Only when, for instance, pneumosepsis led to irreversible loss of graft function, with return to dialysis, this was registered as the cause for graft failure in our study. In all other cases, the kidney biopsy was performed because of a steadily declining graft function. The relative contribution of the category "unknown" was, on average, relatively small although quite variable per age group and time period. The impact on the overall results was judged as marginal, in particular, as no specific bias could be identified for not performing a diagnostic renal biopsy.

In summary, this study shows the impact of rejection on graft failure as a function of time-after-transplantation and age with death as a very strong competitive risk factor in the elderly recipients. For the younger recipients, ABMR in the long term is a dominant cause of graft failure, specifically in the group with pre-transplantation DSA. A plateau in the cumulative incidence of ABMR in the group without pretransplant DSA suggest that particular or total number of epitope mismatches are important determinants for the absolute risk for ABMR-related graft loss over a prolonged time of follow up.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Research Ethics Committee for Biobanks and the Medical Ethics Committee of the University Medical Center Utrecht. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MB participated in research design, writing of the manuscript, and data analysis. DR participated in research design and writing of the manuscript and provided analytical tool. MA and JK participated in data analysis and writing of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.842419/full#supplementary-material

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Graft survival differences in kidney transplants related to recipient sex and age

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Background: In recent years, there has been increasing interest in studying differences in recipient sex in renal disease treatment, access to renal replacement therapy, and subsequent outcomes. Our aim was to find out whether there are differences in outcomes after renal transplantation between female and male kidney transplant recipients in our series, particularly in adults under 60 years of age during long-term follow-up.

Methods: This was a retrospective study of our kidney transplant series (n = 1,101) to compare graft survival depending on the sex of the recipient in the entire series and patients < 60 years of age (n = 687) during long-term follow-up.

Results: We observed no association between recipient sex and graft survival throughout the series, regardless of recipient sex. However, adult female recipients under 60 years of age had lower graft survival than male recipients (p = 0.040). Pre-transplant sensitization (HR 2.438, p = 0.002) and donor age (HR: 1.021, p = 0.017) were the independent variables associated with graft failure.

Conclusion: Female recipients younger than 60 years of age had lower graft survival than male recipients, although there were no gender differences in graft or patient survival in the overall study population. Recipient sex *per se* was not related to graft failure, but the greater immunological risk in women and more frequent use of expanded criteria donors in female recipients under 60 years of age were the main factors related to their poorer graft survival. Further studies and new strategies are needed to identify these differences and develop the best approach to address them.

KEYWORDS

gender disparities, kidney transplantation, female recipients, graft survival, patient survival

Introduction

There is growing evidence that there are inequalities in various aspects of kidney disease, such as the burden of kidney disease, access to renal replacement therapies (RRT), or their subsequent development, in relation to the gender of the recipient (1–3). A higher burden of chronic kidney disease (CKD) and a higher proportion of pre-dialysis CKD have been described in women compared to men, but fewer women start renal replacement therapy, and female adult patients, as well as children, have been described as having poorer access to deceased and living donor transplants (1–3). Various biological, psychological, and socioeconomic aspects have contributed to gender differences in kidney disease recipients, but their detection appears to be more difficult than racial or economic aspects (4, 5).

Several authors and large registries have found no difference in survival between men and women after kidney transplantation, although the effect of the recipient's sex on graft survival has been disputed in several studies (1, 4, 6). The aim of this study was to find out whether there are differences in long-term graft survival and associated risk factors in female recipients, particularly in adult recipients younger than 60 years of age, for whom achieving a longer graft survival rate is critical to avoid the need for a new graft in the event of graft failure.

Materials and methods

Data source and study population

We performed a retrospective analysis on 1,101 adult deceased and living donor kidney transplant recipients at our hospital from January 2000 to January 2019. None of them was a multi-organ transplant. The allocation process during the period of analysis was not based on a computer algorithm but the independent decision of the nephrology staff. Patients gave signed consent for the use of their clinical and personal data for educational and research purposes. Data were taken from our kidney transplant database, which includes variables on donor and recipient demographics, kidney function, infections, cardiovascular disease, development of cancer, and graft failure or death and includes all patients transplanted since the start of our kidney transplant program. All data were extracted from each patient's medical records and entered into the database by trained personnel. The data were kept confidential in accordance with Spanish law and the Declaration of Helsinki.

Abbreviations: AR, acute rejection in the first 6 months after transplantation; BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; DBD, donation after brain death; DCD II, uncontrolled circulatory death donor (Maastricht II); DCD III, controlled circulatory death donor (Maastricht III); DGF, delayed graft function; DM, diabetes mellitus; ESRD, end-stage renal disease; HR, hazard ratio; HD, hemodialysis; HLA, human leukocyte antigen; HTN, high blood pressure; LD, living donor; PD, peritoneal dialysis; PRA, panel reactive antibody; PKD, polycystic kidney disease; < 60, adult younger than 60 years of age.

Variables analyzed

We analyzed the demographic characteristics of the recipients, such as sex, age, dialysis modality [preemptive transplantation, hemodialysis (HD), peritoneal dialysis (PD)], dialysis time (in months), re-transplantation, previous HLA sensitization, pre-transplant comorbidities such as hypertension (HTN), diabetes mellitus (DM), smoking habits or ischemic cardiomyopathy, body mass index (BMI), and end-stage renal disease (ESRD) etiology. Donor demographics such as sex, age, type of donor brain death donor (DBD), donor after circulatory death [DCD type II or type III, pediatric block or living donor (LD)], cerebrovascular death, HTN, and serum creatinine were analyzed. Duration of cold ischemia (hours), immunosuppressive treatment (tacrolimus), delayed graft function (DGF), acute rejection (AR) during the first 6 months diagnosed by biopsy, etiology of graft failure, and patient death were also studied.

Initially, we considered HLA sensitization in patients with PRA higher than 10% and, after 2012, in patients with anti-HLA higher than 1,500 MFI (Luminex techniques).

The immunosuppressive regimen included therapy with a standard dose of a calcineurin inhibitor (tacrolimus or cyclosporine), m-Tor inhibitors (sirolimus or everolimus), mofetyl mycophenolate, or sodium mycophenolate, and steroids in a decreasing dosage. Induction treatment with interleukin-2 receptor antagonist was used in non-extended criteria donors and low immunological risk patients. Thymoglobulin in reduced doses (two to three doses of 1.25 mg/kg every other day) was used for induction in the case of extended criteria donors with a high risk of DGF and a maximum cumulative dose of 6 mg/kg in those patients with high immunological risk (7).

Statistical analysis

Initially, a descriptive analysis of donor and recipient demographics and post-transplant variables was performed. Qualitative variables were described with absolute frequencies and percentages. Quantitative variables were summarized as the mean and standard deviation. A bivariate analysis to compare donor and recipient characteristics as well as the post-transplant variables, depending on the recipient sex, was performed. Associations between qualitative variables were evaluated by Fisher's exact test. Based on the recipient sex, the Student's *t*-test was used to compare the quantitative variables that were normally distributed, and the Mann-Whitney test was used for the non-normally distributed ones. Normality was tested

with the Shapiro-Wilk test and the Bartlett test. Graft survival according to recipient sex was calculated using the Kaplan-Meier method, and the null hypothesis was tested using the Log-rank test.

Additionally, Cox regression models were used to evaluate the time to graft failure depending on recipient and donor characteristics. The following were considered as possible explanatory variables in the model: sex, age, BMI, pre-transplant HLA-sensitization, donor sex and age, cardiovascular disease as the cause of donor death, cold ischemia time, and AR episodes in the first 6 months after transplantation. Goodness-of-fit of the models was evaluated by calculating their concordance, that is, the degree to which the models distinguish between patients at higher risk and lower risk (1 would indicate perfect discrimination; 0.5 would indicate discrimination close to chance). The score test was used to verify the proportional risk assumption (8). All the analyses were performed on the whole study population and younger recipients (under 60 years of age). We stratified our study population at 60 years of age, following other studies that consider differences in immune response and kidney transplant evolution at this age (9, 10).

R software version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria) was used to perform statistical analysis. *P*-values less than 0.05 (two-tailed) were considered statistically significant.

Results

Female recipients in the whole study population

The whole study population included 1,101 kidney transplant recipients with a mean follow-up of 7.2 ± 5.9 years (interquartile range: 2 – 11.1), of whom 435 (39.5%) were female recipients and 666 (60.5%) were male recipients.

With respect to ESRD origin, interstitial and polycystic kidney diseases were more prevalent in female recipients, while glomerular and vascular diseases were more prevalent in male recipients in the bivariate analysis (Table 1). Tobacco use was less frequent in female recipients. Pre-transplant sensitization was higher in women as well as the use of female donors and DBD. Induction treatment was less frequent in female recipients. No relevant differences were found in the rest of the analyzed variables. Kaplan-Meier curves showed no differences in graft or patient survival depending on recipient sex (Figure 1). The main cause of graft failure was chronic rejection in women and death with functioning graft in men (p = 0.014). The main causes of death were cardiovascular diseases and cancer (both of them more frequent in male recipients) and infectious diseases, with no statistical differences (Table 1). The incidence rate of graft failure and death was similar for female recipients (4.9%/year and 1.6%/year, respectively) and male recipients (4.8%/year and 1.9%/year, respectively). In Cox regression, the risk for graft failure increased by 2.7 times in sensitized patients, and the other factors related to graft failure were the occurrence of AR episodes, BMI, and donor age (Table 2). Recipient sex *per se* was not related to graft failure.

Female recipients younger than 60 years of age

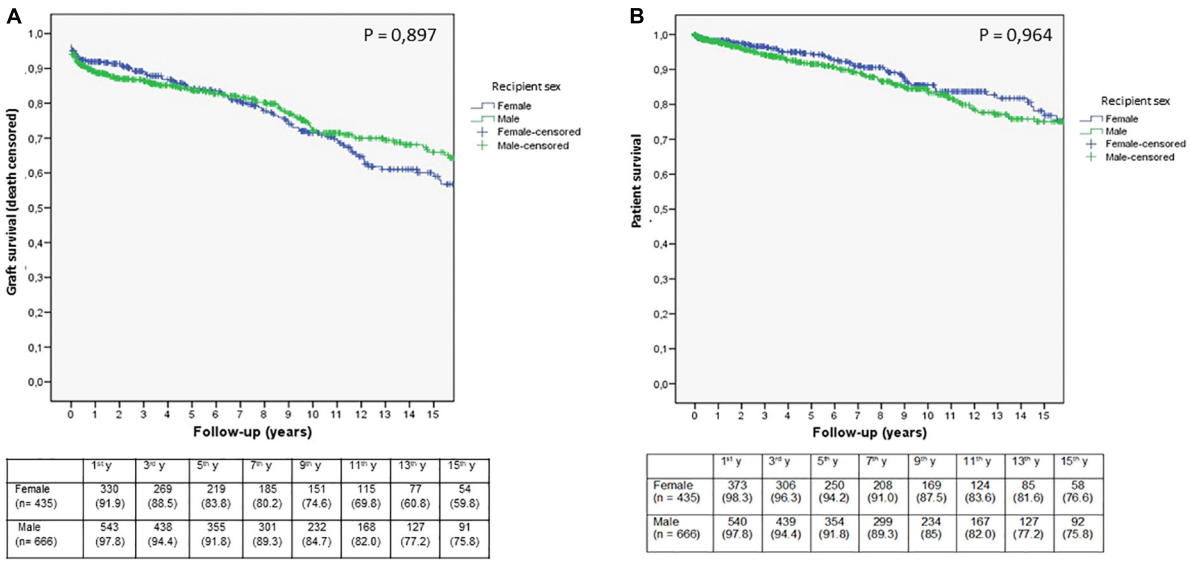
The distribution of patients depending on their age at the time of transplantation resulted as follows: 404 patients older than 60 years (37.1%) and 685 patients < 60 years (62.9%). Recipients < 60 years were 277 (40.4%) women and 408 (59.6%) men. In the bivariate analysis, no age differences were found between female and male recipients at the time of transplantation (Table 1). ESRD disease origin was different in female and male recipients, with similar results as in the whole study population. Smoking habits, as well as ischemic cardiomyopathy, were less prevalent in women than in men. HLA sensitization was more prevalent in women, without differences in the percentage of re-transplanted patients or the number of previous kidney transplants. Female recipients were transplanted more frequently with female, older donors or DBD. No differences were found depending on the type of donor, cold ischemia time, induction treatment, *de novo* or maintenance immunosuppressive treatment, DGF, or AR episodes. Graft function (measured by serum creatinine) was worse in female recipients than in male recipients only during the first 4 years of the follow-up ($p < 0.05$).

Female recipients showed a decrease in graft survival (death censored) (log-rank, p = 0.037) later in the follow-up period, from the seventh year onward (Figure 2A). The incidence rate of graft failure was 4.1% in women (3.1% in men) per year. The main cause of graft failure was chronic rejection, which was more common in female recipients, while primary non-function was more common in male recipients (p = 0.003) (Table 1). Recipient sex was not an independent risk factor for graft failure in the Cox analysis. HLA sensitization before transplantation resulted in a 2.5-fold higher risk of graft failure than in non-sensitized patients (p = 0.027), followed by donor age (HR 1.021, p = 0.017) (Table 2). We analyzed the effect of pre-transplant sensitization status and donor age on graft survival, and the worst graft survival was found in pre-transplant sensitized patients who received a graft from a donor older than 60 years ($p < 0.001$). No differences in graft survival were found between sensitized patients transplanted with grafts from younger donors and non-sensitized recipients transplanted with grafts from donors older than 60 years (p = 0.845). Patient survival was similar in both men and women (log-rank, p = 0.850) (Figure 2B), as was the incidence rate of patient death (0.9%/year). The main cause of death was

TABLE 1 Demographic characteristics of recipients, donors, and post-transplant variables depending on recipient sex in the whole study population and the adult recipients younger than 60 years.

	Global series (n = 1.101)			Patients younger than 60 years (n = 685)		
	Female (n = 435, 39.5%)	Male (n = 666, 60.5%)	P	Female (n = 277, 40.4%)	Male (n = 408, 59.6%)	P
Recipient demographics						
Age (years) (x ± DS)	53.2 ± 12.3	52.9 ± 13.2	0.81	46.3 ± 9.7	44.8 ± 10	0.051
ESRD etiology (%):						
Glomerular	86 (21.1)	176 (28.3)	<**0.001**	65 (24.6)	131 (33.8)	<**0.001**
Interstitial	79 (19.4)	59 (9.5)		48 (18.2)	41 (10.6)	
Vascular	36 (8.8)	108 (17.4)		20 (7.6)	50 (12.9)	
Polycystic	79 (19.4)	83 (13.3)		54 (20.5)	58 (14.9)	
Diabetic nephropathy	12 (2.9)	36 (5.8)		5 (1.9)	21 (5.4)	
Systemic	22 (5.4)	24 (3.9)		20 (7.6)	20 (5.2)	
Unknown	85 (20.9)	122 (19.6)		47 (17.8)	56 (14.4)	
Others	8 (2)	14 (2.3)		5 (1.9)	11 (2.8)	
Dialysis modality (%)			0.036	1 (0.4)		0.41
Preemptive transplantation	2 (0.5)	14 (2.2)		198 (74.7)	5 (1.3)	
Hemodialysis	323 (78.8)	506 (79.7)		66 (24.9)	315 (79.9)	
Peritoneal dialysis	85 (20.7)	115 (18.1)			74 (18.8)	
Months on dialysis (x ± DS)	44.9 ± 43.5	55.5 ± 232.3	0.29	44.9 ± 43.55	45.6 ± 43.4	0.60
HTN pre-Tx (%)	335 (85.5)	516 (87.6)	0.34	219 (84.9)	313 (83.7)	0.74
DM pre-Tx (%)	32 (8.3)	64 (10.0)	0.19	17 (6.6)	25 (6.7)	0.97
Tobacco use pre-Tx (%)	113 (30.3)	315 (55.5)	<**0.001**	88 (35.9)	195 (54)	<**0.001**
Ischemic cardiomyopathy pre-Tx (%)	19 (5.1)	49 (8.6)	0.053	7 (2.8)	23 (6.3)	0.057
BMI (x ± DS)	25.6 ± 5.2	25.4 ± 3.6	0.74	24.9 ± 5.6	24.9 ± 3.8	0.32
HLA sensitization pre-Tx (%)	78 (19.0)	27 (4.3)	<**0.001**	60 (22.6)	23 (5.9)	<**0.001**
Donor demographics						
Female (%)	220 (52)	268 (41.1)	<**0.001**	134 (49.6)	158 (39.4)	**0.011**
Age (years) (x ± DS)	53 ± 19.2	52.6 ± 19	0.56	46.5 (18.1)	44.3 (17.6)	**0.033**
Donor type (%):			0.45			0.61
DBD	380 (87.4)	592 (88.9)		235 (84.8)	349 (85.5)	
DCD type II	8 (1.8)	16 (2.4)		6 (2.2)	16 (3.9)	
DCD type III	15 (3.4)	24 (3.8)		10 (3.6)	13 (3.2)	
Pediatric block	22 (5.1)	20 (3.0)		17 (6.1)	18 (4.4)	
LD	10 (2.3)	13 (2.0)		9 (3.2)	12 (2.9)	
Cerebrovascular death (%)	279 (67.7)	386 (61.1)	**0.030**	165 (63.0)	199 (51.8)	**0.006**
HTN (%)	151 (37.0)	226 (36.2)	0.794	77 (29.5)	95 (24.8)	0.20
Serum creatinine (mg/dl)	0.9 ± 0.5	0.9 ± 0.41	0.82	0.90 ± 0.45	0.89 ± 0.36	0.93
Post-transplantation						
Cold ischemia time (hours)	18 ± 5.3	17.8 ± 5.6	0.58	17.8 ± 5.5	17.7 ± 5.6	0.58
Induction treatment (%)	257 (65.4)	434 (71.7)	**0.035**	145 (57.5)	238 (63.5)	0.16
Basiliximab (%)	88 (22.7)	148 (24.7)	0.49	55 (21.9)	90 (24.3)	0.56
Maintenance immunosuppression treatment (Tacrolimus) (%)	315 (77.2)	476 (77.3)	0.89	201 (76.7)	285 (74.4)	0.27
DGF (%)	116 (29.7)	180 (30.5)	0.83	67 (26.4)	104 (28.3)	0.65
AR (%)	55 (13.9)	74 (12.3)	0.50	40 (15.6)	47 (12.6)	0.29
Graf failure, causes:			**0.014**			**0.003**
Chronic rejection	68 (15.6)	56 (8.4)		51 (18.4)	33 (8.1)	
Death	51 (11.7)	89 (13.4)		21 (7.6)	30 (7.4)	
Primary non-function	22 (5.1)	48 (7.2)		8 (2.9)	25 (6.1)	
Acute rejection	5 (1.1)	7 (1.1)		4 (1.4)	4 (1)	
Recurrence of ESRD	5 (1.1)	10 (1.5)		4 (1.4)	7 (1.7)	
Virus BK nephropathy	0	4 (0.6)		0 (0)	3 (0.7)	
Others	9 (2.1)	11 (1.7)		5 (1.8)	4 (1)	
Death causes:			0.31			0.59
Infection	12 (2.7)	16 (2.4)		4 (1.4)	6 (1.4)	
Cardiovascular	16 (3.6)	31 (4.6)		7 (2.4)	14 (3.2)	
Cancer	11 (2.5)	30 (4.5)		7 (2.4)	11 (2.5)	
Others	12 (2.8)	9 (1.3)		7 (2.4)	3 (0.7)	

AR, acute rejection in the first 6 months after transplantation; BMI, body mass index; DBD, donation after brain death; DCD II, uncontrolled circulatory death donor (Maastricht II); DCD III, controlled circulatory death donor (Maastricht III); DGF, delayed graft function; DM, diabetes mellitus; ESRD, end-stage renal disease; HTN, high blood pressure; HLA, human leukocyte antigen; pre-Tx, pre-transplantation. Bold values denote statistical significance at the $p < 0.05$.



	1st y	3rd y	5th y	7th y	9th y	11th y	13th y	15th y
Female (n= 435)	330 (91.9)	269 (88.5)	219 (83.8)	185 (80.2)	151 (74.6)	115 (69.8)	77 (60.8)	54 (59.8)
Male (n= 666)	543 (97.8)	438 (94.4)	355 (91.8)	301 (89.3)	232 (84.7)	168 (82.0)	127 (77.2)	91 (75.8)

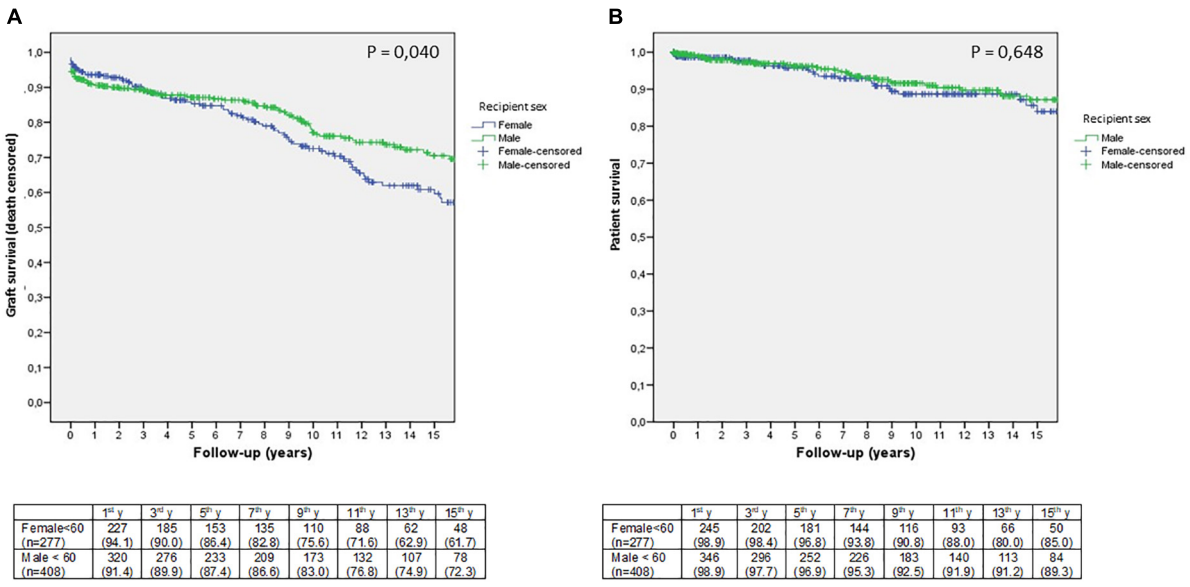
	1st y	3rd y	5th y	7th y	9th y	11th y	13th y	15th y
Female (n = 435)	373 (98.3)	306 (96.3)	250 (94.2)	208 (91.0)	169 (87.5)	124 (83.6)	85 (81.6)	58 (76.6)
Male (n = 666)	540 (97.8)	439 (94.4)	354 (91.8)	299 (89.3)	234 (85)	167 (82.0)	127 (77.2)	92 (75.8)

FIGURE 1
Kaplan-Meier estimates of death-censored graft **(A)** and patient survival **(B)** according to recipient sex in the whole study population. The number of patients at risk during follow-up is indicated in the table below the figures (n = 1,101).

TABLE 2 **Multivariate model to evaluate overall graft failure depending on recipients and donor variables in the whole study population (A) and in patients younger than 60-year series (B).**

	Estimated coefficients	Standard error	HR (CI 95%)	*P*
A				
Recipient sex (male)	−0.137	0.184	0.872 (0.608–1.25)	0.46
Recipient age (year)	−0.003	0.009	0.997 (0.979–1.015)	0.75
Body mass index (%)	0.056	0.021	**1.058** (1.014–1.103)	**0.008**
HTN pre-transplant (yes)	0.046	0.279	1.047 (0.606–1.81)	0.87
HLA sensitization pre-transplant (yes)	0.996	0.281	**2.708** (1.56–4.7)	<**0.001**
Donor sex (male)	−0.098	0.177	0.907 (0.641–1.283)	0.58
Donor age (year)	0.020	0.007	**1.02** (1.005–1.034)	**0.007**
Brain death (yes)	0.147	0.205	1.158 (0.774–1.732)	0.48
Cold ischemia time (hours)	0.002	0.019	1.002 (0.964–1.041)	0.93
Acute rejection (yes)	0.462	0.208	**1.587** (1.056–2.383)	**0.026**
B				
Recipient sex (male)	−0.335	0.229	0.701 (0.447–1.099)	0.12
Recipient age (year)	−0.011	0.012	0.989 (0.965–1.013)	0.36
Body mass index (%)	0.033	0.026	1.034 (1.983–1.087)	0.20
HTN pre-transplant (yes)	0.338	0.359	1.403 (0.694–2.836)	0.35
HLA sensitization pre-transplant (yes)	0.916	0.306	**2.5** (1.347–4.55)	**0.002**
Donor sex (male)	−0.181	0.217	0.834 (0.545–1.276)	0.40
Donor age (year)	0.020	0.009	**1.021** (1.004–1.038)	**0.017**
Brain death (yes)	−0.026	0.250	0.974 (0.597–1.59)	0.92
Cold ischemia time (hours)	0.012	0.024	1.012 (0.965–1.061)	0.63
Acute rejection (yes)	0.458	0.248	1.58 (0.972–2.57)	0.06

Concordance: 0.65. HTN, high blood pressure; HLA, human leukocyte antigen. Concordance: 0.653. HTN, hypertension; HLA, human leukocyte antigen. Bold values denote statistical significance at the $p < 0.05$.



	1st y	3rd y	5th y	7th y	9th y	11th y	13th y	15th y
Female<60 (n=277)	227 (94.1)	185 (90.0)	153 (86.4)	135 (82.8)	110 (75.6)	88 (71.6)	62 (62.9)	48 (61.7)
Male < 60 (n=408)	320 (91.4)	276 (89.9)	233 (87.4)	209 (86.6)	173 (83.0)	132 (76.8)	107 (74.9)	78 (72.3)

	1st y	3rd y	5th y	7th y	9th y	11th y	13th y	15th y
Female<60 (n=277)	245 (98.9)	202 (98.4)	181 (96.8)	144 (93.8)	116 (90.8)	93 (88.0)	66 (80.0)	50 (85.0)
Male < 60 (n=408)	346 (98.9)	296 (97.7)	252 (96.9)	226 (95.3)	183 (92.5)	140 (91.9)	113 (91.2)	84 (89.3)

FIGURE 2
Kaplan-Meier estimates of death-censored graft **(A)** and patient survival **(B)** according to recipient sex in adults younger than 60 years. The number of patients at risk during follow-up is indicated in the table below the figures (*n* = 685).

cardiovascular disease, followed by cancer, with no differences between female and male recipients.

Discussion

In this study, we found that there are differences in graft survival between women and men in patients younger than 60 years, unlike in the older population. This fact led us to consider the need to develop different strategies to improve outcomes in this high-risk population.

Numerous differences related to the gender of the recipient have been described in the medical literature. First, the prevalence of CKD is higher in women than in men (7), a fact that may favor next-generation CKD (1, 2, 11). However, although the prevalence is higher in women at all stages of CKD, access to RRT is lower in female patients (2). These differences are related to several factors: the different etiology of ESRD in men and women, the protective effect of estrogens, better adherence to treatment and healthier lifestyle, and poverty or lack of health literacy in women (2, 12, 13). Finally, unequal access to transplantation for women and girls has also been described (4).

We designed this study to investigate whether post-transplant outcomes differ among men and women in our single-center cohort of renal transplant recipients. The ratio of female to male transplanted patients in our series was similar to that described in other studies, with almost 40% of female patients and 60% of male patients, supporting the differential need for RRT according to the gender of the recipient (4). When analyzing our entire study population, we found differences in demographic factors depending on the sex of the recipient, such as the cause of ESRD, with interstitial and polycystic kidney disease being most common in female recipients, while glomerular and vascular disease were most common in male recipients. In addition, male recipients were more likely to smoke and have a history of ischemic cardiomyopathy, both factors associated with a poorer prognosis. However, in the overall study population, we did not find any differences in graft or patient survival.

Although we started the study including all patients in our series, our main interest was to investigate the differences in the under-60 population who were more likely to need another transplant in case of graft failure. In this selected population, we did not find differences in patient survival depending on the sex of the recipient, as described by other authors, but we did find that women under 60 years of age had a higher risk of graft failure during long-term follow-up compared to men. In this subgroup of patients, the distribution of the origin of ESRD, ischemic cardiomyopathy, and smoking habits were similar to the overall study population. Women in the group of patients younger than 60 years had a higher prevalence of pre-transplant HLA sensitization than in the whole series. As the proportion of re-transplanted patients was the same, previous blood transfusions and pregnancies were the most likely reason for sensitization in female recipients (5, 14). Sensitization has been described as the main reason for women's limited access to kidney transplantation (15, 16), and when women eventually receive a kidney transplant, it is also the main risk factor for graft failure, as we have shown in our study.

However, sensitization is not the only factor associated with poorer graft survival in women. Women received a higher proportion of grafts from female donors, older age, or DBD. Life expectancy is generally longer in women, and there is a high proportion of women among deceased donors in which cerebrovascular accidents are the most common cause of death (4). In addition, kidneys in women have a reduced nephron mass compared to those in men, which could be reduced even more in case of donors with extended criteria as a result of the aging process (17). On the contrary, the lower metabolic demand described for women, due to their smaller body size and weight, could mean a survival advantage for the graft over men in a donor with similar characteristics (4, 18–20). This approach may have been the main reason for the most frequent use of this type of donor in female recipients in our series. However, after analyzing these results, our experience shows that this theoretical survival advantage of women could reduce or even disappear in the case of donors with extended criteria, especially if the recipient has another strong risk factor for graft failure, such as HLA sensitization. Indeed, patients with pre-transplant HLA sensitization who received a graft from an old donor were those with a lower survival rate. So, in our experience, being a female recipient may have led to a negative bias in donor selection, negatively affecting prognosis, especially in younger women who have a higher percentage of HLA sensitization. Other factors such as DGF or AR were not associated with graft failure in recipients under 60 years of age. Induction treatment was used in a large proportion of patients in the entire series, not only in sensitized patients but also in patients at high DGF risk and low immunological risk with different doses. The high DGF risk might be the reason why induction was used more often in male recipients than in female recipients, but we would like to emphasize that the induction protocol was more intensive in sensitized patients than in patients in whom induction was used because of a high DGF risk.

This study has several limitations, mainly related to its monocentric and retrospective nature, although the data were collected prospectively by specially trained personnel. We presented data from our series, which included a large number of patients followed up over a long period of time, and this study allowed us to detect long-term differences between female and male recipients that might be underestimated by other studies or registries with a lower follow-up (6). However, the long follow-up time of the study is another factor that might increase the risk of bias, as differences only appear from the seventh year onward, so the results might be affected by many factors that we cannot even measure.

We do not have data on other gender differences described by other authors in female recipients, such as lower rates of pre-transplant medical screening, lower access to the waiting list, longer waiting list retention, or lower rates of kidney transplantation from deceased or living donors in women (4, 21–25). In our series, we found no differences in dialysis modality, although preemptive transplantation was less common in women.

The causes of differences related to recipient sex are multiple and complex, and we know less today about how to identify and resolve them. There are only a small number of studies, most of them monocentric, like ours, or focused on very specific issues (e.g., biological factors, personal finance, health literacy issues, and living donation) and rarely extended to other geographical locations or health systems (15, 20, 22–24, 26). In addition, it has already been described that health workers have limited ability to identify inequalities related to recipient sex, and these inequalities appear to be more difficult to identify than those related to financial issues, race or health literacy of kidney patients (22, 27, 28). In our study, we did not focus on financial issues because our public health system guarantees access to any treatment for the entire population, and difficulties in obtaining immunosuppressive treatment due to low income, as described for women in other countries or communities, are uncommon (14, 29, 30). We also ignore the impact of socio-cultural or financial problems on female patients' access to our health system, as described in other countries with health systems similar to ours (28, 31, 32).

However, our study has allowed us to learn about the state of this issue in our center, where deceased donors are our main source of kidneys, with a high proportion of donors with expanded criteria, and these results could be representative of other transplant programs with similar characteristics and allocation policies to ours. The most important question for us is how to improve graft survival in adult female recipients younger than 60 years. In our opinion, we need protocols that include pre-transplant and post-transplant measures. Before transplantation, sensitized patients should be enrolled in specific programs to achieve the best HLA compatibility, such as the National Priority Allocation System for Hypersensitized Patients based on virtual crossmatching, which has shown excellent results in cadaveric donor transplantation in Spain and the development of similar programs at the regional level (33). Desensitization treatments could facilitate access of sensitized patients to living donor transplant programs through strategies such as ABO-incompatible transplantation or exchange of kidney pairs (5). After transplantation, the use of specific immunosuppressive treatment protocols for patients at higher immunological risk, taking biopsies to detect humoral rejection at earlier stages, increasing the number of medical checks to detect poor adherence in suspected cases, or using low-nephrotoxic immunosuppressive treatments with their further evaluation must be considered to reduce graft failure (34). Other interventions such as reducing the use of older donor grafts in these sensitized patients would be desirable, but the time on dialysis could be prolonged and patient survival could be compromised while waiting for a graft with a standard risk profile (35). We would like to emphasize that there are no differences in patient survival according to the sex of the

recipient in long-term follow-up, in the whole study population and in the under-60 group, despite poorer graft survival in the latter. However, there is no doubt that we need to develop new strategies to improve long-term graft outcomes in the most vulnerable patient group, such as female recipients under 60 years of age because they have a higher proportion of pre-transplant HLA and are more likely to use expanded criteria donors compared to men. Part of the solution to this problem is to improve the living donor program at our center, as has been done in recent years. In addition, in recent months we have introduced a new computer system to optimize allocation based on an objective score, which will help us find the best recipient for each kidney transplant and avoid selection biases like this one based on the sex of the recipient.

In summary, no differences in graft and patient survival were found in the overall study population depending on the sex of the recipient, but the group of female recipients younger than 60 years had lower graft survival at longer follow-up than the male recipients. HLA sensitization and older donors were the main risk factors for poorer graft survival, with both factors being more pronounced in young female recipients. In female recipients younger than 60 years, strategies to improve outcomes are needed to avoid allocation bias and differences in graft survival depending on the gender of the recipient. These differences could be underestimated. Therefore, multicenter and high-quality studies are needed to improve our knowledge of this problem, find the best approach to avoid it, and, finally, improve our outcomes in long-term follow-up.

Data availability statement

The raw data supporting the conclusions 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. The patients/participants provided their written informed consent to participate in this study.

Author contributions

AS, EG, and JK participated in the research design, the writing of the manuscript, the performance of the research, and in the data analysis. SB, CC, and VE participated in the research design and the writing of the manuscript. JP and PM analyzed the data. BV, MG, and EC made the figures and revised the manuscript. AA drafted and revised the manuscript. All authors approved the final version of 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.

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25(OH)D-but not 1,25(OH)$_2$D–Is an independent risk factor predicting graft loss in stable kidney transplant recipients

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Background: Vitamin D deficiency (VDD) or vitamin D insufficiency is common in kidney transplant recipients (KTRs). The impact of VDD on clinical outcomes in KTRs remain poorly defined and the most suitable marker for assessing vitamin D nutritional status in KTRs is unknown so far.

Methods: We conducted a prospective study including 600 stable KTRs (367 men, 233 women) and a meta-analysis to pool existing evidence to determine whether 25(OH)D or 1,25(OH)$_2$D predicted graft failure and all-cause mortality in stable KTRs.

Results: Compared with a higher 25(OH)D concentration, a low concentration of 25(OH)D was a risk factor for graft failure (HR 0.946, 95% CI 0.912–0.981, p = 0.003), whereas 1,25 (OH)$_2$D was not associated with the study end-point graft loss (HR 0.993, 95% CI 0.977–1.009, p = 0.402). No association was found between either 25(OH)D or 1,25 (OH)$_2$D and all-cause mortality. We furthermore conducted a meta-analysis including 8 studies regarding the association between 25(OH)D or 1,25(OH)$_2$D and graft failure or mortality, including our study. The meta-analysis results were consistent with our study in finding that lower 25(OH)D levels were significantly associated with the risk of graft failure (OR = 1.04, 95% CI: 1.01–1.07), but not associated with mortality (OR = 1.00, 95% CI: 0.98–1.03). Lower 1,25(OH)$_2$D levels were not associated with the risk of graft failure (OR = 1.01, 95% CI: 0.99–1.02) and mortality (OR = 1.01, 95% CI: 0.99–1.02).

Conclusion: Baseline 25(OH)D concentrations but not $1,25(OH)_2D$ concentrations were independently and inversely associated with graft loss in adult KTRs.

KEYWORDS

kidney transplantation, all-cause mortality, graft loss, 25(OH)D, $1,25(OH)_2D$

Introduction

Graft and patient survival rates after kidney transplantation (KT) have improved over the past decade. The death-censored graft survival rate has increased steadily in adults and pediatric recipients (1). Although sequential improvements in short-term graft patency were achieved, the advances were not accompanied by similar progress in long-term graft survival (2). Thus, there is an urgent yet unmet medical need to implement comprehensive strategies to improve long-term outcomes.

Vitamin D deficiency (VDD) or vitamin D insufficiency is common and conflicting results concerning VDD-associated graft failure and mortality were described in kidney transplant recipients (KTRs). Many studies showed that VDD, measured as 25-hydroxyvitamin D [25(OH)D] or 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], relates to kidney dysfunction in renal disease and graft loss in KTRs. An animal study demonstrated that VDD reduces renal function, worsens renovascular morphological features, and aggravates moderate chronic kidney disease (CKD) (3). Epidemiologic research observed that VDD is associated with an increased risk of CKD progression (4–7), while vitamin D analogs in two small clinical trials provided some indication of renoprotective effects by reducing proteinuria (8–10).

Moreover, other clinical studies revealed that when comparing KTR patients with deficient and insufficient vitamin D levels to KTR patients with sufficient vitamin D levels, the latter had better graft survival and overall survival outcomes (11–13).

However, some clinical studies reported conflicting results that VDD, whether measured as 25(OH)D or $1,25(OH)_2D$, is not associated with patient survival and graft loss in KTRs (14–17). Also, a recent meta-analysis (18) showed that early vitamin D deficiency was associated with a higher mortality rate after KT; graft loss was unaffected. However, the vitamin D status of the studies included was assessed by 25(OH)D only, focused on vitamin D status right after transplantation, and these studies were not corrected for the varying methods of vitamin D measurement.

Given the increasing knowledge of widespread deficiency of vitamin D with 10% of cases in North America and>80% in part of Asia (18) and the lack of clarity as to whether VDD is associated with clinical outcomes in KTRs and the most suitable marker for assessing vitamin D nutritional status, we conducted a prospective study and a meta-analysis to pool existing evidence and the current observational study to determine whether 25(OH)D or $1,25(OH)_2D$ predicted graft failure and all-cause mortality in stable KTRs.

Materials and methods

Study population and design

This prospective cohort study comprises 600 KTRs who received a deceased kidney donation, which received a kidney transplant before October 15th, 2012, at the transplant clinic Charité-Mitte, Berlin, Germany. The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism." Exclusion criteria: patients with an acute infection, malignancy, acute rejection, acute myocardial infarction, pulmonary edema, or heart failure at blood sampling. Patients were followed up for graft loss and all-cause mortality for 3 years. Loss of graft function was defined as the need for renal replacement therapy based on the judgment of the treating physicians. The protocol was approved by the ethics committee of Charité University Hospital (approval number 2012−327) under the Declaration of Helsinki. After the patients' consent, clinical and laboratory data were collected. Patients' blood was collected at the beginning of the study.

Data sources and assays

Demographic data for recipients and donors (cold ischemia time, HLA mismatches, donor's age, panel reactive antibodies, recipient's age, sex, transplant survival, underlying kidney disease) were extracted from hospital records and the Euro-transplant records of the patients. Blood samples were collected from 600 KTRs during September and October 2012. EDTA was added to blood samples followed by centrifugation (4,500 rpm) for 20 min at 4°C, then plasma was collected and stored at −20°C until analysis. Laboratory parameters (including plasma 25(OH)D, $1,25(OH)_2D$, calcium, phosphorus, albumin, cholesterol, and creatinine) were measured by standardized laboratory techniques in the central clinical laboratory of the Charité Universitaetsmedizin Berlin, Germany.

Statistical analysis

Data are presented as median (interquartile range) and number (percentage) for normally distributed and nominal data. A p-value of less than 0.05 (two-tailed) was considered statistically significant. Statistical analyses were performed using SPSS 20.0 for Windows (SPSS Inc.) and STATA 14 (StataCorp, College Station, TX, USA).

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The blood levels of 25(OH)D and $1,25(OH)_2D$ were firstly analyzed using the area under the receiver operating characteristic (ROC) curve to obtain the optimal cut-off values, respectively (Supplementary Figure 1). Since the histograms of all parameters do not suggest multimodal distribution and the normality tests indicate normal distributions, plasma levels of all parameters in patients with 25(OH)D above or below the cutoff-value were compared using the independent *T*-test in Table 1. Furthermore,

TABLE 1 Patient characteristics of adult kidney transplant recipients (n = 600).

	All (n = 600)	25(OH)D ≤ 39.4 nmol/l	25(OH)D > 39.4 nmol/l	*P*
N	600	202	347	
Age at study entry (years)	55.0 (22.0)	56.0 (26.0)	54.0 (22.0)	0.742
Sex (female/male)	233 f/367 m	78 f/124 m	133 f/214 m	
Donor age (years)	52.0 (23.0)	50.0 (25.8)	53.5 (20.0)	0.231
Time on dialysis (months)	47.5 (59.0)	40.0 (54.0)	50.0 (56.0)	0.038
Time post-transplantation (months)	60.0 (77.0)	69.5 (86.0)	58.0 (70.0)	0.399
Cold ischemia time (hours)	9.8 (8.9)	9.8 (11.2)	10.4 (7.9)	0.420
eGFR (mL/min/1.73 m^2)	43.0 (26.0)	45.0 (24.0)	41.0 (26.8)	0.424
Hemoglobin (g/dl)	12.7 (2.5)	13.1 (2.8)	12.8 (2.6)	0.453
Plasma albumin (g/dl)	4.5 (0.5)	4.5 (0.6)	4.5 (0.4)	0.225
Plasma creatinine (mg/dl)	1.57 (0.74)	1.5 (0.7)	1.6 (0.8)	0.879
Total cholesterol (mg/dl)	219.0 (77.5)	217.0 (85.0)	220.0 (70.3)	0.520
HbA1c (%)	5.8 (0.8)	5.8 (0.8)	5.8 (0.8)	0.447
Plasma calcium (mmol/L)	2.5 (0.2)	2.5 (0.2)	2.4 (0.2)	0.007
Plasma phosphate (mmol/L)	0.8 (0.3)	0.9 (0.3)	0.8 (0.3)	0.804
Fasting blood glucose (mg/dl)	88.0 (32.3)	87.0 (29.8)	89.0 (34.5)	0.518
Urinary protein (mg/24 h)	166.5 (209.0)	163.0 (298.0)	169.0 (185.0)	0.173
Plasma iPTH (pg/ml)	85.8 (91.9)	90.2 (129.9)	81.0 (89.1)	0.001
Plasma 25(OH)D (nmol/L)	52.1 (43.6)	12.5 (20.0)	64.3 (31.1)	<0.001
Plasma $1,25(OH)_2D$ (pmol/L)	90.5 (72.8)	82.5 (75.3)	92.0 (71.5)	0.014
$1,25(OH)_2D/25(OH)D$ ($*10^{-3}$)	1.9 (2.3)	4.4 (3.8)	1.4 (1.2)	<0.001
Immunosuppressant				
Cyclosporin A	193 (36.4%)	74 (36.6%)	116 (33.4%)	
Tacrolimus	238 (44.9%)	81 (40.1%)	139 (40.1%)	
Everolimus	82 (15.5%)	31 (15.3%)	62 (17.9%)	
Combined medication	17 (3.2%)	6 (3.0%)	11 (3.2%)	
HLA mismatches				
HLA-A				
0 mismatch	233 (38.8%)	77 (38.1%)	137 (39.5%)	
1 mismatch	272 (45.3%)	90 (44.6%)	158 (45.5%)	
2 mismatches	95 (15.8%)	35 (17.3%)	52 (15.0%)	
HLA-B				
0 mismatch	174 (29%)	55 (27.2%)	101 (29.1%)	
1 mismatch	270 (45%)	89 (44.1%)	160 (46.1%)	
2 mismatches	156 (26%)	58 (28.7%)	86 (24.8%)	
HLA-DR				
0 mismatch	210 (35%)	70 (34.7%)	121 (34.9%)	
1 mismatch	289 (48.2%)	101 (50.0%)	167 (48.1%)	
2 mismatches	101 (16.8%)	31 (15.3%)	59 (17.0%)	

Values are presented as median (interquartile range) or n (%). eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; iPTH, intact parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25 dihydroxy vitamin D; HLA, human leukocyte antigens.

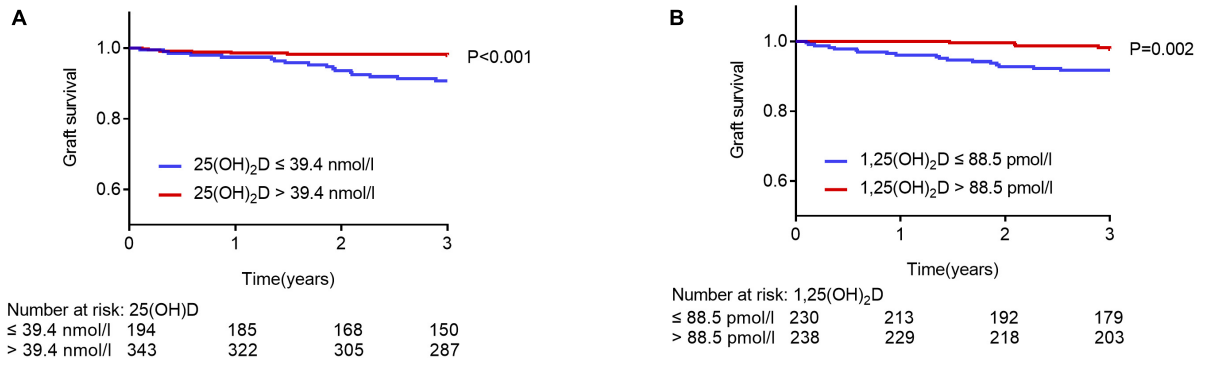

FIGURE 1
Graft survival rates in patients with higher and lower levels of plasma 25(OH)D or 1,25(OH)$_2$D. Kaplan-Meier curves, according to plasma 25(OH)D and 1,25 (OH)$_2$D for graft failure. Survival rates were compared with the log-rank test. **(A)** Blue lines, patients with plasma 25(OH)D levels of ≤ 39.4 nmol/l; red lines, patients with plasma 25(OH)D levels of > 39.4 nmol/l. **(B)** Blue lines, patients with plasma 1,25(OH)$_2$D levels of ≤ 88.5 pmol/l; red lines, patients with plasma 1,25(OH)$_2$D levels of > 88.5 pmol/l.

TABLE 2 Cox proportional hazards analysis of 25(OH)D or 1,25(OH)$_2$D and the relevant factors for graft loss in renal transplant recipients.

		25(OH)D			1,25(OH)$_2$D	
	HR	95% CI	*P*	HR	95% CI	*P*
Model A	0.968	0.951−0.985	<0.001	0.987	0.976−0.997	0.011
Model B	0.962	0.943−0.981	<0.001	0.985	0.974−0.996	0.007
Model C	0.959	0.933−0.985	0.002	0.983	0.969−0.998	0.024
Model D	0.946	0.912−0.981	0.003	0.993	0.977−1.009	0.402
Model E	0.947	0.911−0.985	0.006	0.989	0.969−1.009	0.274

Multiple proportional hazards regression analyses (Cox regression; enter). Patients were followed for graft loss for 3 years. Model A: crude. Model B: adjusted for patient age, sex, and donor age. Model C: as model B and additionally adjusted for cold ischemia time, urine protein, time on dialysis, plasma calcium, iPTH, hemoglobin. Model D: as model C and additionally adjusted for eGFR and plasma phosphate. Model E: as model D and additionally adjusted for cyclosporin A, tacrolimus, everolimus and mismatches of HLA-A, HLA-B, HLA-DR.

using these cut-off values, time-to-event analyses were performed with the log-rank test using the Kaplan-Meier method (Figure 1). In addition, multivariable-adjusted graft failure analyses were performed using Cox proportional hazards regression models (Tables 2, 3). Based on the following confounding factors: patient age, sex, donor age, cold ischemia time, urine protein, time on dialysis, eGFR, plasma calcium, phosphate, iPTH, hemoglobin, immunosuppressants and HLA mismatches, several models were built by adding 25(OH)D, 1,25(OH)$_2$D and then both, respectively, to analyze which is the independent risk factor for graft loss in KTRs. Pearson's correlation analysis was conducted to illustrate the correlation between the two forms of vitamin D and eGFR (Supplementary Figure 2). Furthermore, the 25(OH)D curves with hazard ratio were fitted for graft loss in the generalized additive model (GAM) (Figure 2).

For the association between 25(OH)D, or 1,25(OH)$_2$D and the risk of graft failure or mortality, we further conducted a meta-analysis to estimate the pooled estimates (HR). First, we searched PubMed electronically, using terms relating to vitamin D [e.g., vitamin D or 25-hydroxyvitamin D or 1,25-dihydroxyvitamin D or 1,25(OH)$_2$D or 25(OH)D], kidney transplantation (e.g., Kidney transplantation or renal transplantation or kidney transplant or renal transplant) and (graft failure or graft loss). The inclusion criteria of this meta-analysis were: original paper; follow-up studies with 1,25(OH)$_2$D or 25(OH)D data available; outcome included graft loss or mortality; published before December 31st, 2021; the language of the study was English. Exclusion criteria were: animal study or review paper.

After screening the papers, we included seven studies that met the inclusion criteria for our meta-analysis to calculate the pooled HR of the association between 1,25(OH)$_2$D or 25(OH)D and the risk of graft failure or mortality. Fixed effects models were used for studies without significant heterogeneity, while random-effects models were used for studies with significant heterogeneity. RevMan Manager version 5.4 software[1] was used for the present meta-analysis.

1 http://www.cc-ims.net/revman

TABLE 3 Cox proportional hazards analysis of potential bio-marker predicted graft failure of renal transplant patients forward likelihood ratio model (*n* = 600).

	HR	95% CI	*P*
Sex	0.151	0.037−0.620	0.009
Urinary protein	1.001	1.000−1.001	<0.001
Plasma phosphate	58.424	10.572−322.880	<0.001
Plasma hemoglobin	0.598	0.424−0.842	0.003
Plasma 25(OH)D	0.943	0.912−0.975	<0.001

25(OH)D, 1,25(OH)$_2$D + patient age, sex, donor age, cold ischemia time, urine protein, time on dialysis, eGFR, Ca, PO4, PTH, and Hb being the independent variable.

Results

Study population

The baseline characteristics for 600 KTRs consisting of 233 men and 367 women aged 20–87 years are shown in Table 1, which contains both clinical and laboratory parameters. After 3 years of follow-up, 38 patients showed graft failure, and 65 died in this observation period.

Association of vitamin D status and all-cause mortality

In order to separate the study population, $1,25(OH)_2D$ and 25(OH)D were firstly analyzed using ROC analysis for all-cause mortality to obtain the cut-off values [$1,25(OH)_2D$ 83.5 pmol/l; 25(OH)D 52.35 nmol/l]. No association was found between two forms of vitamin D and all-cause mortality using the Kaplan-Meier survival curve [$1,25(OH)_2D$, $p = 0.385$; 25(OH)D, $p = 0.616$; data do not show]. Furthermore, univariable Cox regression analysis indicated that none of the two forms of vitamin D is associated with all-cause mortality [$1,25(OH)_2D$, HR 1.001, 95% CI 0.996−1.006, $p = 0.688$; 25(OH)D, HR 1.004, 95% CI 0. 998−1.011, $p = 0.154$; data do not show].

Association of vitamin D status and graft loss, and eGFR

We separated the study population with optimal cut-off values of $1,25(OH)_2D$ and 25(OH)D, respectively, using a ROC analysis for graft loss (Supplementary Figure 1). In a Kaplan-Meier survival curve in patients after kidney transplantation, 25(OH)D concentrations above 39.4 nmol/l were significantly associated with graft loss (Figure 1, $p < 0.001$, log-rank test), as well as $1,25(OH)_2D$ concentrations above 88.5 pmol/l were associated with graft loss ($p = 0.002$, log-rank test). On the contrary, the ratio of $1,25\ (OH)_2D$ and 25(OH)D was not associated with graft loss ($p = 0.531$, log-rank test, data do not show).

We analyzed the impact of $1,25(OH)_2D$, as well as 25(OH)D in KTRs using Cox regression models by gradually adding confounding factors (Table 2). In univariable Cox analyses, both $1,25(OH)_2D$ and 25(OH)D were significantly associated with graft loss (Model A, HR 0.987, 95% CI 0.976−0.997, $p = 0.011$; HR 0.968, 95% CI 0.951−0.985, $p < 0.001$). The association between 25(OH)D and graft loss remained significant after adjustment for known confounders factors (Model D, HR 0.946, 95% CI 0.912−0.981, $p = 0.003$). However, 1,25 (OH)2D was no longer significantly correlated to graft loss after adding an adjustment for eGFR and plasma phosphate (Model D, HR 0.993, 95% CI 0.977−1.009, $p = 0.402$). In addition, we build model E based on model D and additionally adjusted for cyclosporin A, tacrolimus, everolimus and mismatches of HLA-A, HLA-B, HLA-DR, and the association between 25(OH)D and graft loss remained significant (model E, HR 0.947, 95% CI 0.911−0.985, $p = 0.006$). Moreover, we also performed Cox regression models using the forward likelihood ratio method. This approach likewise showed that a low concentration of plasma 25(OH)D (Table 3, HR 0.943, 95% CI 0.912−0.975, $p < 0.001$) was a risk factor for graft failure, whereas again, 1,25 (OH)2D concentrations were not associated with the study end-point graft loss.

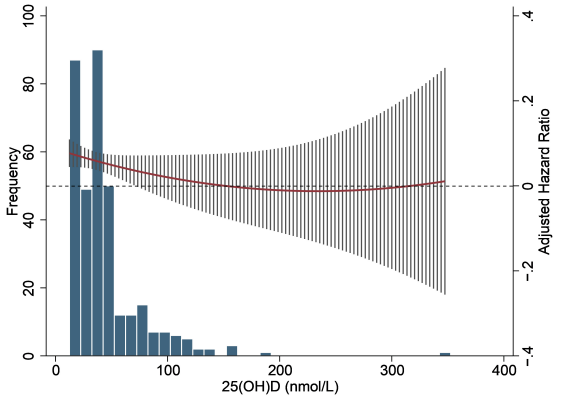

FIGURE 2
Estimated spline for the hazard ratio of graft failure with 25(OH)D in renal transplant recipients. Y axis represents spline smooth functions. Tick marks in X axis indicate distribution of observations. Dashed lines designate 95% confidence interval for smoothing functions.

Supplementary Figure 2 showed that eGFR was positively correlated with $1,25(OH)_2D$ plasma concentration but not with 25(OH)D plasma concentration in KTRs. Furthermore, Figure 2 reveals that graft loss hazard ratios (HRs) were inversely associated with 25(OH)D in a non-linear model.

Meta-analysis

We performed a meta-analysis to assess the association between $1,25(OH)_2D$ or 25(OH)D and graft failure or mortality. Lower 25(OH)D levels were significantly associated with the risk of graft failure (OR = 1.04, 95% CI: 1.01−1.07) but not associated with mortality (OR = 1.00, 95% CI: 0.98−1.03) (Figure 3). Lower $1,25(OH)_2D$ levels were not associated with the risk of graft failure (OR = 1.01, 95% CI: 0.99−1.02) and mortality (OR = 1.01, 95% CI: 0.99−1.02) (Figure 3).

Discussion

We performed an observational cohort study and meta-analysis to investigate the effect of low 25(OH)D and $1,25(OH)_2D$ levels measured in stable KTRs on graft failure and all-cause mortality. This meta-analysis is the first to summarize the available evidence on the long-term outcomes of 25(OH)D and $1,25(OH)_2D$ in KTRs head-to-head in one study. The cohort study and meta-analysis results were consistent in finding that baseline 25(OH)D concentrations were independently and inversely associated with graft loss regardless of known confounding factors, whereas 25(OH)D concentrations were not associated with mortality in adult KTRs. In addition, no association of $1,25(OH)_2D$ with mortality and graft failure was found in adult KTRs.

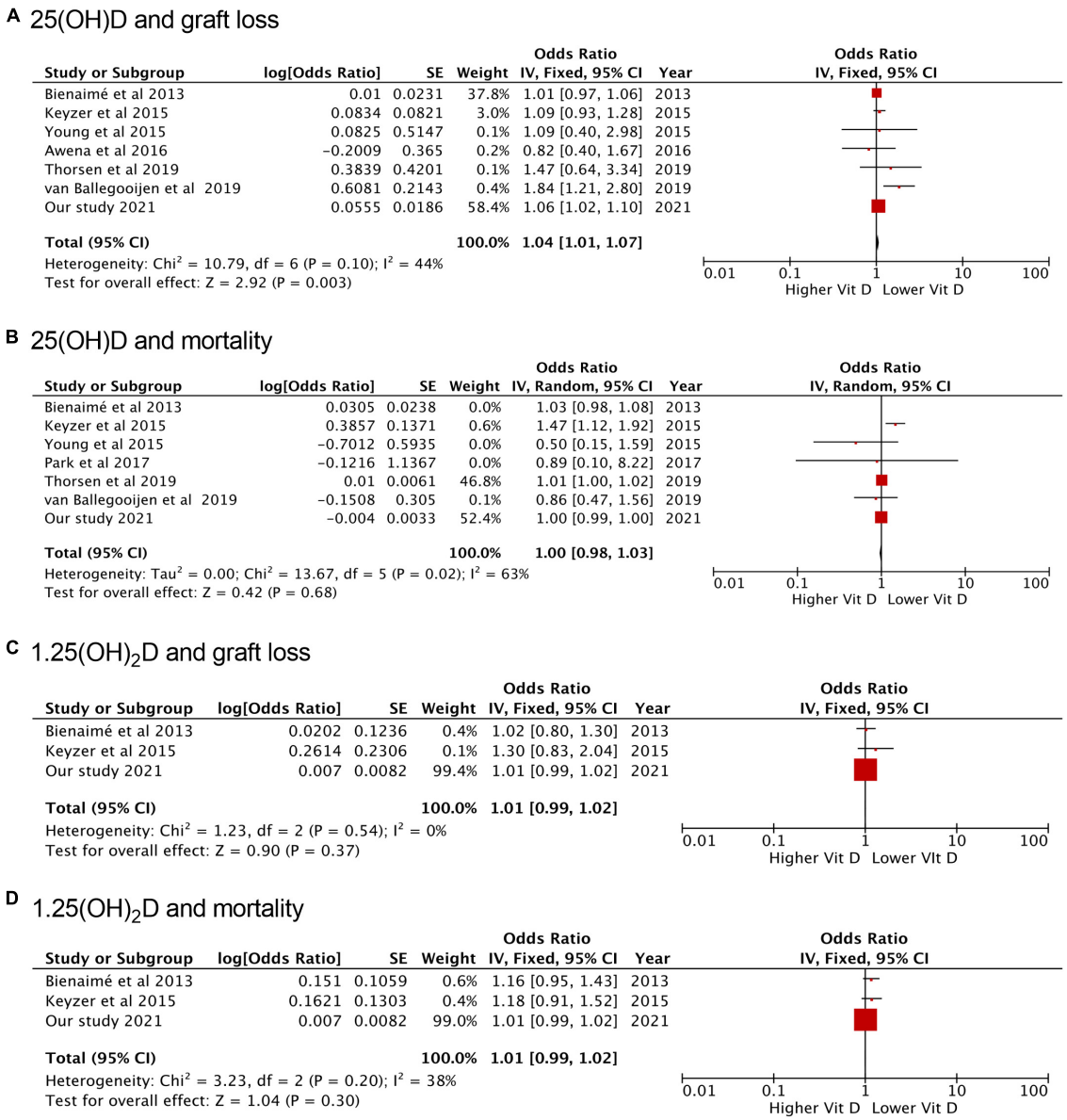

FIGURE 3
Forest plots of the included studies evaluating the association between vitamin D and death and graft loss. **(A)** 25(OH)D and graft loss; **(B)** 25(OH)D and mortality; **(C)** 1,25$(OH)_2$D and graft loss; **(D)** 1,25$(OH)_2$D and mortality. SE, standard error; IV, inverse-variance; CI, confidence interval.

The patient characteristics indicated that the cohort study population was representative of a typical European post-transplant cohort (19). We observed that the incidence rate of renal graft loss was 6.33% (38/600) after a follow-up of 3 years, in line with the average in other centers (2). Therefore, our results are generally applicable. In addition to our cohort study, the meta-analysis included seven clinical studies published on the prognostic impact of vitamin D levels in KTRs with comparable patient characteristics to the study population in our study (Table 4) (11–17).

Our cohort study and meta-analysis consistently showed that 25(OH)D concentrations were associated with increased graft loss rates but not mortality. Although some of the previous clinical studies (11–13) suggested a relationship between vitamin D levels and all-cause mortality, we did not find any benefit in patient survival from higher concentrations of 25(OH). This discrepancy may be due to the small sample size of previous studies. They were most likely underpowered for this end-point. Hence, our metanalysis is helpful by combining the evidence from all published studies, including our data, and indicates no mortality effect of either 25(OH)D or 1,25$(OH)_2$D.

Our finding that 25(OH)D is associated with graft loss fits very well with published preclinical studies in the field. VDD deficiency was linked to an impaired kidney function in a CKD animal model (3) and associated with increased graft failure risk in experimental KTRs (11–13). It has been confirmed that vitamin D analogs inhibit kidney fibrosis with potential renoprotective activity in a cyclosporine-induced rat model of CKD (20). Renal vitamin D receptor binding to nuclear response elements is reduced in rats with incipient renal failure (21).

TABLE 4 Information clinical studies analyzing the correlation between vitamin D levels and survival in renal transplant recipients.

	Region	Time and journal	*N*	Sex (M/F %)	Indicator	Cutoff	Blood collection time	Follow	Results
Bienaime et al. (14)	France	J Am Soc Nephrol. 2013	634	58.7/41.3	25(OH)D; $1,25(OH)_2D$	No	3 months	48.6 months (median)	Low 25(OH)D or 1,25(OH)D concentrations 3 months after transplantation did not predict early death or graft loss.
Thorsen et al. (11)	Norway	Clinical transplantation, 2019	762	67.6/32.4	25(OH)D	30 nmol/L and 50 nmol/L	10 week after transplantation	82 months (median)	Long-term graft and patient survival were better in recipients with vitamin D sufficiency 10 weeks post-transplant compared with those with vitamin D deficiency and insufficiency.
Keyzer et al. (12)	Netherlands	The Journal of Clinical Endocrinology and Metabolism, 2015	435	51/49	25(OH)D; $1,25(OH)_2D$	No	6 years (median) after transplantation	7 years (median)	Low 25(OH)D is independently associated with an increased risk of all-cause mortality and 25(OH)D $<$ 12 ng/ml with a rapid eGFR decline in stable KTR. The association of low $1,25(OH)_2D$ with mortality or graft failure depends on renal function.
Kwon et al. (15)	Korea	Medicine (Baltimore). 2015	410	63.9/36.1	25(OH)D	10 ng/mL	Within 2 weeks before kidney transplantation	7.3 years (median)	25(OH)D deficiency was not significantly associated with patient mortality and graft failure.
van Ballegooijen et al. (13)	Netherlands	Nephrol Dial Transplant. 2020	461	53.1/46.9	25(OH)D	50 nmol/L	At transplantation	6.1 years (median)	Combined vitamins D and K deficiency are highly prevalent and are associated with increased mortality and graft failure risk compared with high vitamins D and K status.
Le Fur et al. (16)	France	Transpl Int. 2016	444	60.6/39.4	25(OH)D	10 and 30 ng/mL	At transplantation	12 months	25(OH)D deficiency was not significantly associated with patient–graft survival.
Park et al. (17)	Korea	Korean J Intern Med. 2017	164	70.70/29.3	25(OH)D	20 ng/mL	Within 2 week before kidney transplant	24.8 months	The frequencies of allograft failure and patient death did not significantly differ between patients with the low and high vitamin D.
Our study	Germany		600	61.2/38.8	25(OH)D; $1,25(OH)_2D$	No	average 7 years after transplantation	3 years	25(OH)D- but not $1,25(OH)_2D$- is an independent risk factor predicting graft loss in stable renal transplant recipients

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Moreover, the administration of vitamin D is beneficial in experimental transplantation models. $1,25(OH)_2D$ administration prevented acute rejection and prolonged survival in the ACI to Lewis rat renal transplant model (22) and also prevented chronic allograft nephropathy in the Fisher 344 to Lewis rat renal transplantation model (23). A close association between VDD and graft loss was also reported in liver transplant recipients. Martucci et al. (24) showed that after orthotopic liver transplantation, incomplete graft recovery was associated with lower vitamin D on postoperative day (POD) 28 (OR: 0.84; CI 95%: 0.73−0.97; $P = 0.014$), indicating that the value of vitamin D on POD28 had a strong association with graft function.

The deficiency of 25(OH)D, but not $1,25(OH)_2D$, in blood circulation is an independent risk factor for diminished allograft survival in our cohort study and meta-analysis. It may be that the stability of 25(OH)D in the circulation makes it more representative of the patient's vitamin D status. It is known that 25(OH)D has a higher affinity for vitamin D's transport protein than $1,25(OH)_2D$ but a lower affinity for specific VDR (25). Because of these features, 25(OH)D is considered a transfer form rather than a biological effector *in vivo*. Compared to $1,25(OH)_2D$, 25(OH)D in the peripheral circulation usually has a higher concentration, lower affinity, and a longer half-life (26) describing better the average vitamin status and or vitamin D substitution than $1,25(OH)_2D$ and may thus be better linked to outcomes such as graft loss.

The association between low $1,25(OH)_2D$ and graft failure was seen in our cohort and was dependent on renal function. This association is not unexpected since the 1α-hydroxylase of the kidney, i.e., the enzyme that converts 25(OH)D into $1,25(OH)_2D$, is damaged simultaneously with the deterioration of renal function.

$1,25(OH)_2D$ is identified as a VD form with relatively short half-life that is also influenced by adrenocorticotropic hormone (27) and sex hormone (28). Furthermore, $1,25(OH)_2D$ has been shown to reduce glomerulosclerosis and urinary albumin excretion in progressive glomerular damage (25). Furthermore, it has been demonstrated that $1,25(OH)_2D$ has anti-proliferative properties for glomeruli (29) and has a renal protective effect by targeting podocytes (30, 31). Therefore, in the case of chronic kidney damage, it makes little sense to correlate graft loss with $1,25(OH)_2D$ concentrations alone; instead, it is more reasonable to adjust the association of low $1,25(OH)_2D$ concentrations with graft failure and mortality for impaired renal function (12). This could be why 25(OH)D, rather than $1,25(OH)_2D$, is a better marker of VD status in this patients cohort and can independently predict graft loss.

Given the high prevalence of vitamin D deficiency (VDD) in renal transplant patients and that VDD is treatable, a target concentration of 25(OH)D needs to be determined. The generalized additive model (GAM) (see **Figure 2**) shows that in the patients with 25(OH)D levels below 150 nmol/L, the hazard ratio decreases with an increase of 25 (OH)D levels in stable KTRs. A recent guideline suggested that vitamin D exceeding 250 nmol/L is deemed a "risk" of vitamin D toxicity (32). In **Figure 2**, the curve of 25(OH)D with hazard ratio in generalized additive model (GAM), the levels of 310 nmol/L is above zero, and the curve's trend increases with a numerical increase of 25(OH)D. Since only a very small number of patients had levels of 25(OH)D that exceed the level of possible adverse effects, we cannot make any firm conclusions if an unlimited increase in 25(OH)D may increase the risk ratio of graft failure and at what precise concentration of 25(OH)D this may occur. Nowadays, no clear criteria have been applied to define the deficiency/insufficiency status (33). The bone centered guidelines recommend a target 25(OH)D concentration of at least 20 ng/mL (50 nmol/L), while the guidelines that focus on the pleiotropic effect of vitamin D recommend a higher threshold of 25(OH)D concentration of 30 ng/mL (75 nmol/L). Despite that, it seems evident that most KTRs have moderately or even severely decreased levels of native vitamin D (34, 35). Vitamin D deficiency is a widespread global health problem (36). Since 74% of KTR's sample was below 30 ng/mL (75 nmol/L) in our cohort, 25(OH)D Vitamin D deficiency/insufficiency in KTRs is more frequent and severe than in the general population. Most KTRs require an additional replenishment of vitamin D. The optimal target interval of vitamin D levels in the healthy general population may not be appropriate for KTRs, and the 25 (OH)D level must be much higher than 50 nmol/mL effectively improve the clinical outcome of KTRs.

Several limitations of our cohort study warrant consideration:

1. Although we have eliminated several potential known confounding factors, we cannot exclude the possibility of remaining confounding, as our study had an observational character.

2. Since barely any patients with the levels of 25(OH)D exceed the level of possible adverse effects [i.e., 25(OH)D levels>250 nmol/L], we do not know what concentration of 25 (OH) D may have an adverse effect.

This meta-analysis has several strengths and limitations:

1. All included studies were observational studies, and 25% were retrospective.

2. The studies assessed 25(OH)D levels at different time points, from 2 weeks before transplant to 6 months after. In addition, the use of different cut-off values may increase heterogeneity among studies.

3. Although early VDD was confirmed as a risk factor for inferior outcomes, studies focusing on the effect of vitamin D supplements on transplant outcomes are lacking.

4. Finally, just total but not the bioactive free form of 25(OH)D was measured. This, however, might be especially important in patients with CKD (33, 37–40).

In conclusion, 25(OH)D was independently associated with graft loss in adult KTRs. The lower optimal range of 25(OH)D to prevent renal graft loss in KTRs seems to be 50 nmol/L. The association of low $1,25(OH)_2D$ with graft failure depends on renal function. Vitamin D deficiency/insufficiency in KTRs is widespread and might be a preventable risk factor for graft loss.

Data availability statement

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

Ethics statement

The protocol was approved by the Ethics Committee of Charité University Hospital (approval number 2012−327) under the Declaration of Helsinki. The patients/participants provided their written informed consent to participate in this study.

Author contributions

BH initiated this study, finalized and critically reviewed the manuscript, and approved the final version. SZ wrote the manuscript and conducted the literature search of the meta-analysis. SL drafted the **Figure 2**. YY drafted the **Figure 3**, performed the data extraction, quality assessment, and statistical analysis of the meta-analysis. SL, C-FH, CC, and ZW edited the manuscript and collected data. ZZ, BK, and BH reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1141646/full#supplementary-material

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Risk factors for BK virus infection in DCD donor kidney transplant recipients

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Background: BK virus infection after kidney transplantation can negatively impact the prognosis of patients. However, current risk factor analyses primarily focus on BK virus nephropathy, while BK viruria and BK viruria progressing to BK viremia receive less attention. This study aims to analyze the risk factors associated with BK viruria and BK viruria progressing to BK viremia in recipients of donation after cardiac death (DCD), with the goal of facilitating early intervention.

Methods: Donor characteristics and clinical data of recipients before and after transplantation were evaluated, and logistic univariate and multivariate analyses were performed to determine the risk factors associated with BK viruria and the progression of BK viruria to BK viremia. Additionally, machine learning techniques were employed to identify the top five features associated with BK viruria evolving into BK viremia.

Results: During a median follow-up time of 1,072 days (range 739–1,418), 69 transplant recipients (15.6% incidence rate) developed BK viruria after transplantation, with 49.3% of cases occurring within 6 months post-transplantation. Moreover, 19 patients progressed to BK viremia. Donor age [OR: 1.022 (1.000, 1.045), $p = 0.047$] and donor procalcitonin (PCT) levels [0.5–10 ng/ml; OR: 0.482 (0.280, 0.828), $p = 0.008$] were identified as independent risk factors for BK viruria. High BK viruria [OR: 11.641 (1.745, 77.678), $p = 0.011$], recipient age [OR: 1.106 (1.017, 1.202), $p = 0.018$], and immunoinduction regimen [ATG; OR: 0.063 (0.006, 0.683), $p = 0.023$] were independent risk factors for BK viruria progressing to BK viremia. Machine learning analysis confirmed the importance of high BK viruria, recipient age, and immunoinduction regimen (ATG) in predicting the progression of BK viruria to BK viremia.

Conclusion: The development and progression of BK virus in DCD kidney transplant recipients is influenced by multiple factors. Early intervention and treatment could potentially extend the lifespan of the transplanted organ.

KEYWORDS

kidney transplantation, BK viruria, BK viremia, risk factors, donation after cardiac death, machine learning

Background

The BK virus is a common resident in healthy individuals and typically reactivates when the immune system is weakened (1, 2). After a BK virus infection, kidney transplant patients commonly develop BK viruria, which may later progress to BK viremia and BKVN (3–6). BKVN is a significant cause of graft failure, affecting up to 10% of kidney transplant recipients and resulting in graft loss in up to 50% of those affected (7). Presently, there is no effective treatment plan for BK virus infection, and management primarily involves reducing immunosuppressive dosages and relying on autocellular immunity to achieve antiviral effects (8).

The screening of risk factors for BK virus infection mainly focuses on BKVN and BK viremia. A meta-analysis has summarized the risk factors for BK viremia and BKVN (9). It has been found that deceased donors are an independent risk factor for BK viremia. However, there are limited studies on the risk factors of BK viruria and BK viruria evolving into BK viremia in the context of DCD (10). BK viruria has been reported to progress to BK viremia in around 33% of cases, and high levels of BK viruria can be employed as a screening tool for BK viremia and BKVN in kidney transplant recipients (11). Moreover, persistent BK viruria can be used as an early marker of BKVN development (12). Therefore, early detection of BK viruria and clinical intervention for those at a high risk of BK viruria progressing to BK viremia are essential to control the progression of the disease as much as possible.

Traditional logistic regression has long been the primary method for investigating risk factors associated with BK virus infection (13, 14). However, machine learning techniques have shown promise in predicting complex clinical data (15). For example, recent research has applied machine learning to proteomic analysis of extracellular vesicles to identify potential biomarkers of BK viruria and BK viremia (16). However, there are no studies that have yet used machine learning to explore the risk factors associated with BK virus infection using clinical data of donors and recipients. Therefore, this study aims to conduct a retrospective study at a single center. Specifically, the study will first employ traditional logistic regression to identify potential risk factors for BK viruria and BK viruria evolving into BK viremia. Thereafter, a random forest model will be used to determine the importance ranking of potential risk factors for developing BK viremia, as a validation of the potential risk factors identified by traditional logistic regression.

Materials and methods

Patient groups

This study enrolled 353 kidney transplant recipients who received kidneys from deceased donors in the Organ Transplantation Department of Renmin Hospital of Wuhan University from November 2018 to September 2021. Inclusion criteria required single kidney transplantation from organ donation after cardiac death (DCD), and all DCD donors were Maastricht III. Exclusion criteria included allocation of donor kidneys from other hospitals, combined heart-kidney or liver-kidney transplantation, death within 1 month after surgery and living kidney transplantation. The flow chart of case screening is shown in Figure 1. Recipients were followed up until May 2023. The study was approved by the Ethics Committee of Renmin Hospital of Wuhan University. The kidney transplant patients were classified into two groups: the control group and the BK viruria group. It is important to note that all patients with BK viremia had previously developed BK viruria. As a result, the BK viruria group was further divided into a control group and a BK viremia group based on whether or not BK viruria had progressed to BK viremia.

BKV surveillance protocol

In order to track BK virus (BKV) DNA levels in urine and blood, we employed quantitative polymerase chain reaction (qPCR) at regular intervals. Specifically, we conducted monthly monitoring in the first year post-transplant, every 3 months in the second year, and annually thereafter until the fifth year.

Diagnostic criteria

The diagnostic criteria for acute kidney injury (AKI) followed the guidelines recommended by the Kidney Disease: Improving Global Outcomes (KDIGO), which included an increase in serum creatinine (sCr) level $\geq$ 26.5 μmol/l (0.3 mg/dl) within 48 h or a known or presumed increase in sCr to 1.5 times or more of the baseline value within the past 7 days (17). Delayed graft function (DGF) was defined as the need for dialysis within 1 week after kidney transplantation (18). Due to the lower limit of quantitation was 10^3 copies/ml. BK viruria was defined as the detection of BKV DNA load in urine $\geq 10^3$ copies / ml, and high BK viruria was defined as the detection of BKV DNA load in urine $\geq 10^7$ copies/ml (19). BK viremia was defined as the detection of BKV DNA load in blood $\geq 10^3$ copies/ml (20).

Data collection

The collection of clinical data involves both donor pre-donation data and recipient clinical data. Donor data include: (1) Clinical data such as gender, age, BMI and blood group; (2) Comorbidities and primary disease; (3) Relevant laboratory test indicators such as terminal albumin, terminal urea, terminal serum creatinine, terminal eGFR, terminal hemoglobin, terminal urine protein, terminal procalcitonin (PCT), and terminal hematuria sputum culture. Recipient data included: (1) Clinical data such as gender, age, BMI, and blood group; (2) Type matching data including PRA, HLA mismatch number; (3) Dialysis data including preoperative dialysis mode and duration; (4) Comorbidities and primary disease; (5) Postoperative laboratory examination indicators such as BKV DNA load in urine, BK viruria time, BKV DNA load in blood, and BK viremia time; (6) Hospitalization information including length of hospitalization (LOH) and DGF; (7) Immunosuppressant use such as Immunosuppressive and induction regimen.

Statistical analysis

The statistical analysis was performed utilizing the Statistical Package for Social Sciences (SPSS), version 25.0 (SPSS Inc., Chicago, IL, USA), and R4.2.1. The continuous variables were determined using

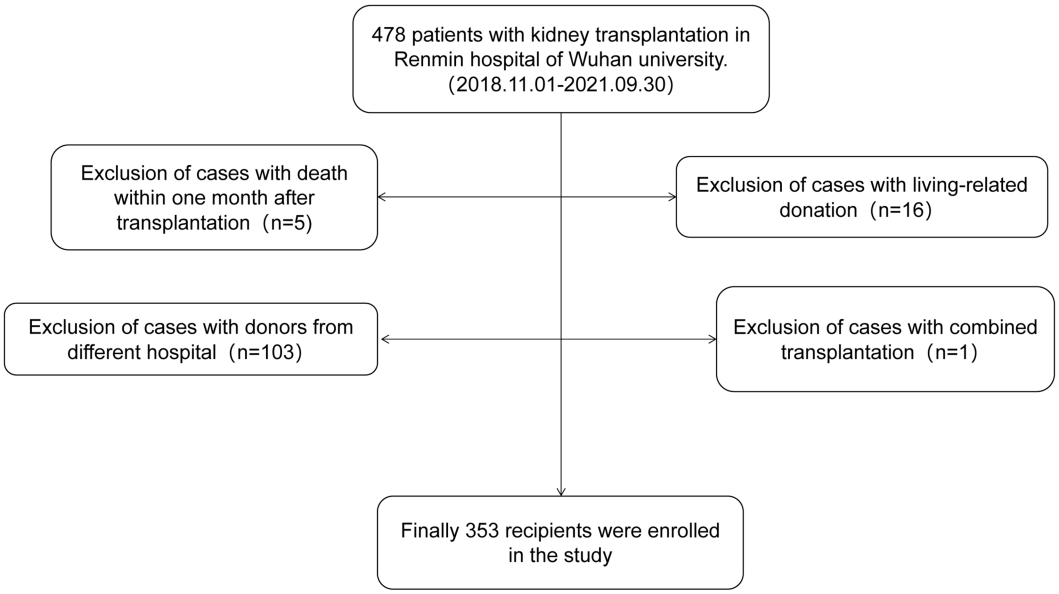

FIGURE 1
Flowchart of patient selection in the present study.

either an independent t-test or Mann–Whitney U test and presented as means ± SD or medians with interquartile ranges, respectively. Categorical variables were evaluated using the Chi-squared or Fisher's exact tests and expressed as numbers (percentages). Initially, a univariate logistic proportional risk regression model was fitted for BK viruria in the entire renal transplant population. Subsequently, variables with $p < 0.1$ were included in a multivariate logistic proportional risk regression model. In addition, a single-factor logistic proportional risk regression model for BK viremia was fitted for the population with BK viruria, and variables with $p < 0.1$ were included in a multivariate logistic proportional risk regression model. All statistical tests and confidence intervals were two-sided, and $p < 0.05$ were considered to be statistically significant. To validate the results obtained through logistic regression, a machine learning approach was used to assess the importance of potential risk factors for the progression of BK viruria to BK viremia. Categorical variables were treated in the dataset of 69 BK viruria cases by creating dummy variables, and the number of variables was reduced by lasso regression and 10-fold cross-validation, with the final number of variables determined by the lambda with the minimum mean square error. We used the random forest model for model fitting, with the dependent variable set to whether the patient progressed to BK viremia and the independent variables set to features other than the dependent variable, ranked in importance using Gini importance for the independent variables.

Results

Comparison of donor and recipient data between BK viruria group and control group

This study analyzed a total of 353 kidney transplant recipients, of which 284 were free of BK virus infection, and 69 had BK viruria after the transplant surgery. All donors provided DCD kidneys, and the transplantation was carried out using a single kidney from a donor with the same blood group as that of the recipient. The immunosuppressive maintenance regimen used by 97.7% of the recipients was Tacrolimus + Mycophenolate mofetil + Glucocorticoid (TAC + MMF + GC), and 37.1% of the recipients received Antihuman thymocyte globulin (ATG) for immune induction. When compared to the control group, the BK viruria group had a higher donor age (58.00 [47.00, 63.00] vs. 53.00 [44.00, 60.00], $p = 0.01$) and a greater proportion of donor pre-donor PCT at 0.5-10 ng/ml (40.6 vs. 58.1%, $p = 0.013$). No statistical differences were observed in other comparisons. Please refer to Table 1 for detailed data.

Analysis of risk factors for BK viruria

The association between BK viruria and various variables was analyzed using a univariate logistic proportional risk regression model. Variables with a value of p less than 0.1 were included in the multivariate logistic proportional risk regression model. The univariate analysis identified certain variables with $p < 0.1$, such as donor age, donor diabetes (yes), and donor PCT (0.5–10 ng/ml). After including these variables in the multivariate analysis, it was found that donor age [OR: 1.022 (1.000, 1.045), $p = 0.047$] and donor PCT [0.5–10 ng/ml; OR: 0.482 (0.280, 0.828), $p = 0.008$] were independent predictors of BK viruria. Table 2 provides detailed information about the analysis.

Comparison of donor and recipient data between BK viremia group and control group

After stratifying the 69 patients with BK viruria into control and BK viremia groups based on whether they developed BK viremia or not, we observed that the BK viremia group had a higher recipient age

TABLE 1 Demographics and clinical characteristics of donors (kidneys) and recipients.

Characteristic	All, *n* = 353	Control, *n* = 284	BK viruria, *n* = 69	*p*-value
Donor (kidneys) characteristic				
Age, year	54.00 [45.00, 61.00]	53.00 [44.00, 60.00]	58.00 [47.00, 63.00]	0.01
Male, *n*	304 (86.1)	245 (86.3)	59 (85.5)	1
Blood group, *n*				0.791
A	124 (35.1)	103 (36.3)	21 (30.4)	
B	75 (21.2)	58 (20.4)	17 (24.6)	
AB	34 (9.6)	27 (9.5)	7 (10.1)	
O	120 (34.0)	96 (33.8)	24 (34.8)	
BMI, kg/m^2	22.86 [20.76, 24.49]	22.86 [20.96, 24.49]	22.49 [20.76, 25.35]	0.651
Cause of death, *n*				0.357
Cerebral hemorrhage	165 (46.7)	126 (44.4)	39 (56.5)	
Cerebral infarction	94 (26.6)	78 (27.5)	16 (23.2)	
Cerebral trauma	32 (9.1)	29 (10.2)	3 (4.3)	
Brain tumor	33 (9.3)	27 (9.5)	6 (8.7)	
Others	29 (8.2)	24 (8.5)	5 (7.2)	
Hypertension, *n*	149 (42.2)	115 (40.5)	34 (49.3)	0.234
Diabetes, *n*	28 (7.9)	19 (6.7)	9 (13.0)	0.133
Al, g/l	35.60 [33.42, 38.20]	35.60 [34.00, 38.31]	35.30 [32.80, 37.30]	0.151
Urea, mmol/l	8.60 [6.15, 13.42]	8.20 [6.01, 13.31]	10.30 [6.48, 13.42]	0.127
sCr, μmol/l	59.00 [41.00, 83.00]	58.00 [40.00, 83.75]	60.00 [45.00, 79.00]	0.784
eGFR, ml/min	108.24 [87.24, 129.00]	111.22 [82.20, 131.20]	101.06 [92.00, 114.66]	0.141
Hemoglobin, g/l	106.00 [95.00, 123.00]	106.00 [95.00, 124.00]	106.00 [95.00, 121.00]	0.798
Urine protein, n	134 (38.0)	111 (39.1)	23 (33.3)	0.456
AKI, *n*	50 (14.2)	43 (15.1)	7 (10.1)	0.382
PCT, ng/ml				0.013
<0.5 or >10	160 (45.3)	119 (41.9)	41 (59.4)	
0.5–10	193 (54.7)	165 (58.1)	28 (40.6)	
Culture, n	181 (51.3)	145 (51.1)	36 (52.2)	0.974
Recipient characteristic				
Age, year	42.76 ± 10.51	42.77 ± 10.59	42.74 ± 10.25	0.982
Male, *n*	251 (71.1)	205 (72.2)	46 (66.7)	0.448
Blood group, *n*				0.88
A	120 (34.0)	99 (34.9)	21 (30.4)	
B	78 (22.1)	61 (21.5)	17 (24.6)	
AB	38 (10.8)	31 (10.9)	7 (10.1)	
O	117 (33.1)	93 (32.7)	24 (34.8)	
BMI, kg/m^2	21.48 [19.23, 23.67]	21.48 [19.51, 23.66]	21.61 [18.67, 23.92]	0.625
Dialysis modalities, *n*				0.702
No	33 (9.3)	25 (8.8)	8 (11.6)	
Hematodialysis	59 (16.7)	47 (16.5)	12 (17.4)	
Peritoneal dialysis	255 (72.2)	208 (73.2)	47 (68.1)	
Both	6 (1.7)	4 (1.4)	2 (2.9)	
Dialysis time, mth	12.00 [5.00, 28.00]	12.00 [6.00, 30.00]	8.00 [3.00, 24.00]	0.06
Hypertension, *n*	319 (90.4)	258 (90.8)	61 (88.4)	0.698

(Continued)

TABLE 1 (Continued)

Characteristic	All, *n* = 353	Control, *n* = 284	BK viruria, *n* = 69	*p*-value
Diabetes, *n*	25 (7.1)	21 (7.4)	4 (5.8)	0.84
Hepatitis B, *n*	58 (16.4)	46 (16.2)	12 (17.4)	0.953
Transplantation etiology, *n*				0.317
Chronic glomerulonephritis	265 (75.1)	213 (75.0)	52 (75.4)	
Hypertensive nephrosclerosis	16 (4.5)	15 (5.3)	1 (1.4)	
Polycystic kidney disease	14 (4.0)	13 (4.6)	1 (1.4)	
Diabetic nephropathy	17 (4.8)	13 (4.6)	4 (5.8)	
Others	41 (11.6)	30 (10.6)	11 (15.9)	
HLAmm, *n*	4.00 [3.00, 5.00]	4.00 [3.00, 5.00]	4.00 [3.00, 5.00]	0.922
PRA, *n*				1
<10%	257 (72.8)	207 (72.9)	50 (72.5)	
≥10%	96 (27.2)	77 (27.1)	19 (27.5)	
DGF, *n*	46 (13.0)	38 (13.4)	8 (11.6)	0.845
LOH, d	20.00 [18.00, 23.00]	20.00 [18.00, 23.00]	20.00 [19.00, 22.00]	0.433
Immunosuppressive regimen, *n*				1
CsA + MMF + GC	8 (2.3)	6 (2.1)	2 (2.9)	
TAC + MMF + GC	345 (97.7)	278 (97.9)	67 (97.1)	
Immunoinduction regimen, n				0.254
Basiliximab	222 (62.9)	174 (61.3)	48 (69.6)	
ATG	131 (37.1)	110 (38.7)	21 (30.4)	

BMI, body mass index; Al, terminal Albumin; Ur, terminal Urea; sCr, terminal serum creatinine; eGFR, terminal estimated glomerular filtration rate; Hemoglobin, terminal Hemoglobin; Urine protein, terminal Urine protein was present; AKI, Acute Kidney Injury; PCT, procalcitonin; Culture, terminal hematuria sputum culture status any one is positive; PRA, panel reactive antibodies; HLA mm, HLA mismatch; DGF: Delayed Graft Function; LOH: The length of post-kidney transplant hospitalization; ATG, Antihuman thymocyte globulin; TAC, Tacrolimus; CsA, Cyclosporin A; MMF, Mycophenolate mofetil; GC, Glucocorticoid.

TABLE 2 Univariate and multivariate analysis of BK viruria.

Variable	Single-factor analysis, OR (95% CI)	*p*-value	Multiple-factor analysis, OR (95% CI)	*p*-value
Donor age, year	1.023 (1.001,1.046)	0.037	1.022 (1.000,1.045)	0.047
Donor diabetes(yes)	2.092 (0.902,4.852)	0.085		
Donor PCT (0.5 ~ 10 ng/ml)	0.493 (0.288,0.841)	0.01	0.482 (0.280,0.828)	0.008

OR, odds ratio; 95% CI, 95% confidence interval; PCT: procalcitonin.

(47.68 ± 7.34 vs. 40.86 ± 10.63, $p = 0.012$), a higher proportion of high BK viruria (89.5 vs. 54.0%, $p = 0.014$), and a lower proportion of ATG use (5.3 vs. 40.0%, $p = 0.012$), as compared to the control group. However, no significant differences were observed in the remaining donor-recipient profiles, and detailed donor-recipient profiles can be found in Table 3.

Analysis of risk factors for the evolution of BK viruria to BK viremia

A univariate analysis of BK viremia was performed, revealing variables with *p*-values below 0.1, including donor age, donor diabetes (yes), high BK viruria, recipient age, and immunoinduction regimen (ATG). Following inclusion of these variables in a multivariate analysis, it was determined that high BK viruria [OR: 11.641 (1.745, 77.678), $p = 0.011$], recipient age [OR: 1.106 (1.017, 1.202), $p = 0.018$], and immunoinduction regimen [ATG; OR: 0.063 (0.006, 0.683), $p = 0.023$] were independent predictors of BK viremia. Table 4 provides detailed data regarding the analysis.

Time distribution of BK virus infection in kidney transplant recipients

The study revealed that BK viruria had an incidence of 15.6%, with a median follow-up period of 1,072 days (range 739–1,418). Figure 2A displays the time distribution of 69 kidney transplant recipients with BK viruria, with a median follow-up time of 185 days (range 82–387). The majority of BK viruria cases occurred within the first 6 months following kidney transplantation, accounting for nearly 49.3% of cases. Over time, the incidence of BK viruria decreased, but there was a rebound observed 2 years after surgery. Furthermore, the study reported a BK viremia incidence of 5.4%. BK viremia was discovered either simultaneously with or after BK viruria, and among kidney transplant

TABLE 3 Demographic and clinical characteristics of donor (kidney) and recipient of BK viruria evolving into BK viremia.

Characteristic	BK viruria, *n* = 69	Control, *n* = 50	BK viremia, *n* = 19	*p*-value
Donor (kidneys) characteristic				
Age, year	58.00 [47.00, 63.00]	56.00 [45.00, 63.75]	62.00 [52.00, 63.00]	0.211
Male, *n*	59 (85.5)	44 (88.0)	15 (78.9)	0.568
Blood group				0.107
A	21 (30.4)	13 (26.0)	8 (42.1)	
B	17 (24.6)	10 (20.0)	7 (36.8)	
AB	7 (10.1)	6 (12.0)	1 (5.3)	
O	24 (34.8)	21 (42.0)	3 (15.8)	
BMI, kg/m^2	22.87 ± 3.42	22.77 ± 3.56	23.13 ± 3.10	0.698
Cause of death, *n*				0.643
Cerebral hemorrhage	39 (56.5)	27 (54.0)	12 (63.2)	
Cerebral infarction	16 (23.2)	11 (22.0)	5 (26.3)	
Cerebral trauma	3 (4.3)	2 (4.0)	1 (5.3)	
Brain tumor	6 (8.7)	6 (12.0)	0 (0.0)	
Others	5 (7.2)	4 (8.0)	1 (5.3)	
Hypertension, *n*	34 (49.3)	23 (46.0)	11 (57.9)	0.54
Diabetes, *n*	9 (13.0)	4 (8.0)	5 (26.3)	0.106
Al, g/l	35.25 ± 3.61	35.16 ± 3.99	35.50 ± 2.42	0.736
Urea, mmol/l	10.30 [6.48, 13.42]	10.30 [6.55, 14.04]	10.30 [6.32, 11.76]	0.677
sCr, μmol/l	60.00 [45.00, 79.00]	62.00 [45.50, 82.00]	60.00 [45.50, 72.00]	0.648
eGFR, ml/min	101.06 [92.00, 114.66]	100.81 [91.76, 114.91]	106.50 [97.72, 110.61]	0.424
Hemoglobin, g/l	106.00 [95.00, 121.00]	105.50 [95.00, 119.25]	106.00 [97.00, 121.50]	0.364
Urine protein, *n*	46 (66.7)	34 (68.0)	12 (63.2)	0.924
AKI, n	7 (10.1)	7 (14.0)	0 (0.0)	0.203
PCT, ng/ml				0.665
<0.5 or>10	41 (59.4)	31 (62.0)	10 (52.6)	
0.5–10	28 (40.6)	19 (38.0)	9 (47.4)	
Culture, *n*	36 (52.2)	23 (46.0)	13 (68.4)	0.163
Recipient characteristic				
Age, year	42.74 ± 10.25	40.86 ± 10.63	47.68 ± 7.34	0.012
Male, *n*	46 (66.7)	33 (66.0)	13 (68.4)	1
Blood group, *n*				0.107
A	21 (30.4)	13 (26.0)	8 (42.1)	
B	17 (24.6)	10 (20.0)	7 (36.8)	
AB	7 (10.1)	6 (12.0)	1 (5.3)	
O	24 (34.8)	21 (42.0)	3 (15.8)	
BMI, kg/m^2	21.38 ± 3.31	21.39 ± 3.40	21.36 ± 3.12	0.978
Dialysis modalities, *n*				0.415
No	8 (11.6)	5 (10.0)	3 (15.8)	
Hematodialysis	12 (17.4)	7 (14.0)	5 (26.3)	
Peritoneal dialysis	47 (68.1)	36 (72.0)	11 (57.9)	
Both	2 (2.9)	2 (4.0)	0 (0.0)	
Dialysis time, mth	8.00 [3.00, 24.00]	7.50 [3.25, 18.25]	12.00 [4.00, 24.00]	0.505
Hypertension, *n*	61 (88.4)	45 (90.0)	16 (84.2)	0.803

(Continued)

TABLE 3 (Continued)

Characteristic	BK viruria, *n* = 69	Control, *n* = 50	BK viremia, *n* = 19	*p*-value
Diabetes, *n*	4 (5.8)	4 (8.0)	0 (0.0)	0.488
Hepatitis B, *n*	12 (17.4)	9 (18.0)	3 (15.8)	1
Transplantation etiology, *n*				0.127
Chronic glomerulonephritis	52 (75.4)	35 (70.0)	17 (89.5)	
Hypertensive nephrosclerosis	1 (1.4)	1 (2.0)	0 (0.0)	
Polycystic kidney disease	1 (1.4)	0 (0.0)	1 (5.3)	
Diabetic nephropathy	4 (5.8)	4 (8.0)	0 (0.0)	
Others	11 (15.9)	10 (20.0)	1 (5.3)	
HLAmm, *n*	4.00 [3.00, 5.00]	4.00 [3.00, 5.00]	4.00 [3.00, 4.00]	0.572
PRA, *n*				1
<10%	50 (72.5)	36 (72.0)	14 (73.7)	
≥10%	19 (27.5)	14 (28.0)	5 (26.3)	
DGF, *n*	8 (11.6)	8 (16.0)	0 (0.0)	0.152
LOH, d	20.00 [19.00, 22.00]	20.00 [19.00, 22.00]	19.00 [18.00, 20.00]	0.2
Immunosuppressive regimen, *n*				0.478
CsA + MMF + GC	2 (2.9)	1 (2.0)	1 (5.3)	
TAC + MMF + GC	67 (97.1)	49 (98.0)	18 (94.7)	
Immunoinduction regimen, *n*				0.012
Basiliximab	48 (69.6)	30 (60.0)	18 (94.7)	
ATG	21 (30.4)	20 (40.0)	1 (5.3)	
High BK viruria	44 (63.8)	27 (54.0)	17 (89.5)	0.014

BMI, Body Mass Index; Al, terminal Albumin; Ur, terminal Urea; sCr, terminal Serum creatinine; eGFR, terminal Estimated glomerular filtration rate; Hemoglobin, terminal Hemoglobin; Urine protein, terminal Urine protein was present; AKI, Acute Kidney Injury; PCT, procalcitonin; Culture, terminal hematuria sputum culture status any one is positive; PRA, panel reactive antibodies; HLA mm, HLA mismatch; DGF: Delayed Graft Function; LOH: The length of post-kidney transplant hospitalization; ATG, Antihuman thymocyte globulin; TAC, Tacrolimus; CsA, Cyclosporin A; MMF, Mycophenolate mofetil; GC, Glucocorticoid.

TABLE 4 Univariate and multivariate analysis of BK viruria evolving into BK viremia.

Variable	Single-factor analysis, OR (95% CI)	*p*-value	Multiple-factor analysis, OR (95% CI)	*p*-value
Immunoinduction regimen(ATG)	0.083 (0.01,0.675)	0.083	0.063 (0.006,0.683)	0.023
Donor age, year	1.042 (0.993,1.094)	0.094		
Donor diabetes(yes)	4.107 (0.969,17.413)	0.055		
High BK viruria	7.241 (1.511,34.705)	0.013	11.641 (1.745,77.678)	0.011
Recipient age	1.077 (1.01,1.145)	0.018	1.106 (1.017,1.202)	0.018

OR, odds ratio; 95% CI, 95% confidence interval; ATG, Antihuman thymocyte globulin.

patients with BK viremia, 63.2% were diagnosed within 3 months after BK viruria, with a median follow-up time of 26 days (range 0–119). Figure 2B illustrates the time interval between the diagnosis of BK viruria and BK viremia in 19 kidney transplant recipients.

Application of machine learning in assessing the evolution of BK viruria to BK viremia

We further used machine learning approaches to analyze the importance of potential risk factors for the evolution of BK viruria to BK viremia. We used a lasso regression approach for data dimensionality reduction, and when the minimum mean square error of λ was 0.065, the variables were reduced to eight, namely: recipient transplantation etiology (polycystic kidney disease), recipient blood group (O), recipient DGF, recipient age, high BK viruria, recipient immunoinduction regimen, donor diabetes and donor age. The variables were screened as shown in Figures 3A,B. Furthermore, we utilized the random forest model to identify the top five variables for predicting BK viremia, as illustrated in Figures 4A,B. It is noteworthy that the potential risk factors obtained by conventional logistic regression were among the top five variables.

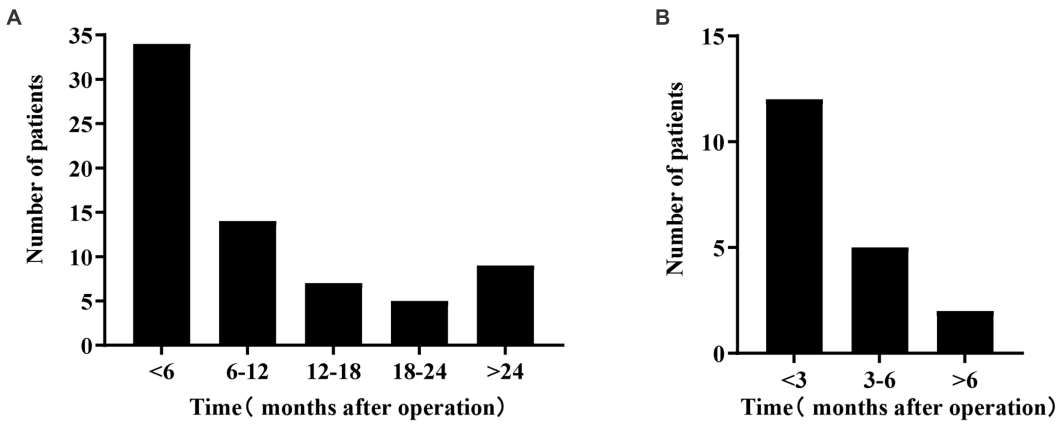

FIGURE 2
(A) Shows the time distribution of BK viruria after kidney transplantation. The horizontal axis represents the time after kidney transplantation, and the vertical axis represents the number of patients. This graph shows the highest incidence within 6 months of the transplant; it decreases over time and then increases again 2 years later. **(B)** Shows the time distribution of BK viremia after BK viruria. The horizontal axis shows the time after BK viruria, and the vertical axis shows the number of patients. This graph shows that BK viremia occurs mainly within 3 months of diagnosis of BK viruria.

Discussion

BK virus is commonly present in the kidneys of most adults. However, in kidney transplant patients who receive immunosuppressive therapy for an extended period of time, the virus can become reactivated. The process starts with lysis of renal tubule cells, leading to urinary excretion of the BK virus. The virus then replicates in interstitial cells and penetrates the peritubular endothelial barrier to enter the bloodstream, resulting in BK viremia. Once the virus reaches the allograft, it attacks renal tubular epithelial cells, causing interstitial fibrosis and leading to the development of BKVN. This condition can ultimately result in renal graft degeneration and transplant failure. A study has shown that approximately 33% of patients with BK viruria progress to BK viremia and subsequently to BKVN without any intervention (7).

In this study conducted at a single center, 69 kidney transplant recipients developed BK viruria, of whom 19 also developed BK viremia. The rate of progression to BK viremia was 27.5%, which was slightly lower than the 33% reported in other studies. No BKVN was identified as no kidney biopsy was performed. Similar to findings from other centers, the incidence of BK viruria at our center was highest within the first 6 months post-transplantation, followed by a declining trend. However, there was a subsequent increase in incidence observed at the 2-year post-transplantation mark (21–23). The study findings indicate that the majority of BK viremia cases (63.2%) occurred within the first 3 months following BK viruria. As there are currently no specific antiviral therapies for BK virus-related diseases, kidney transplant patients typically rely on reducing immunosuppressant doses and changing immunotherapy regimens. Although this approach can increase the risk of chronic rejection, early detection and intervention of BK viruria and BK viremia are beneficial in reducing the incidence of BKVN.

Prior research has identified several potential risk factors associated with postoperative BK virus infection in kidney transplant patients, including recipient age, deceased donor, tacrolimus regimen and male recipient (6, 9, 24). While previous research has established that deceased donors are a risk factor for BK virus infection, few studies have examined the specific risk factors associated with DCD. In this study, all included kidney donors were DCD donors, and tacrolimus regimen is used by 97.7% of the population. Our analysis revealed that donor age may be an independent risk factor for BK viruria [OR: 1.022 (1.000, 1.045), $p = 0.047$]. Deceased donors are typically older, and in this study, the average donor age was 54.00 [45.00, 61.00] years. The percentage of ECD is as high as 42.8%. Notably, the age of donors in the control group was significantly lower than that in the BK viruria group (53.00 [44.00, 60.00] vs. 58.00 [47.00, 63.00], $p = 0.01$). Advanced donor age is often indicative of poor kidney quality, and it may be a contributing factor to BK virus infection. The biomarker PCT, which is linked to bacterial infection and inflammation, has been found to be a useful predictor of AKI in critically ill patients (25, 26). Our study revealed that the PCT range of 0.5 to 10 ng/ml before donation from deceased donors had a protective effect against BK viruria infection [OR: 0.482 (0.280, 0.828), $p = 0.008$], but not against the progression of BK viruria to BK viremia. Since DCD donors have longer Intensive Care Unit (ICU) stays, bacterial infections may still occur despite efforts to avoid sepsis. The PCT range of 0.5 to 10 ng/ml may reflect this phenomenon. Bacterial infections may activate the immune system, which could inhibit BK virus replication. Moreover, DCD donors often receive multiple antibiotics, and it is worth exploring whether the antiviral properties of these antibiotics inhibit the growth of microorganisms that promote BK virus transmission.

Following kidney transplantation, some patients may experience progression from BK viruria to BK viremia as a result of BK virus infection. The early identification of high-risk factors for developing BK viremia is of particular importance. Through traditional logistic regression analysis, we identified three independent risk factors, including recipient age [OR: 1.106 (1.017, 1.202), $p = 0.018$], high BK viruria [OR: 11.641 (1.745, 77.678), $p = 0.011$], and the immunoinduction regimen [ATG; OR: 0.063 (0.006, 0.683), $p = 0.023$]. Consistent with prior literature, recipient age and high BK viruria were found to be independent risk factors for the development of BK viremia (20, 27–31). For example, one study found that the BKPyV urine assay that best distinguished between positive and negative BK

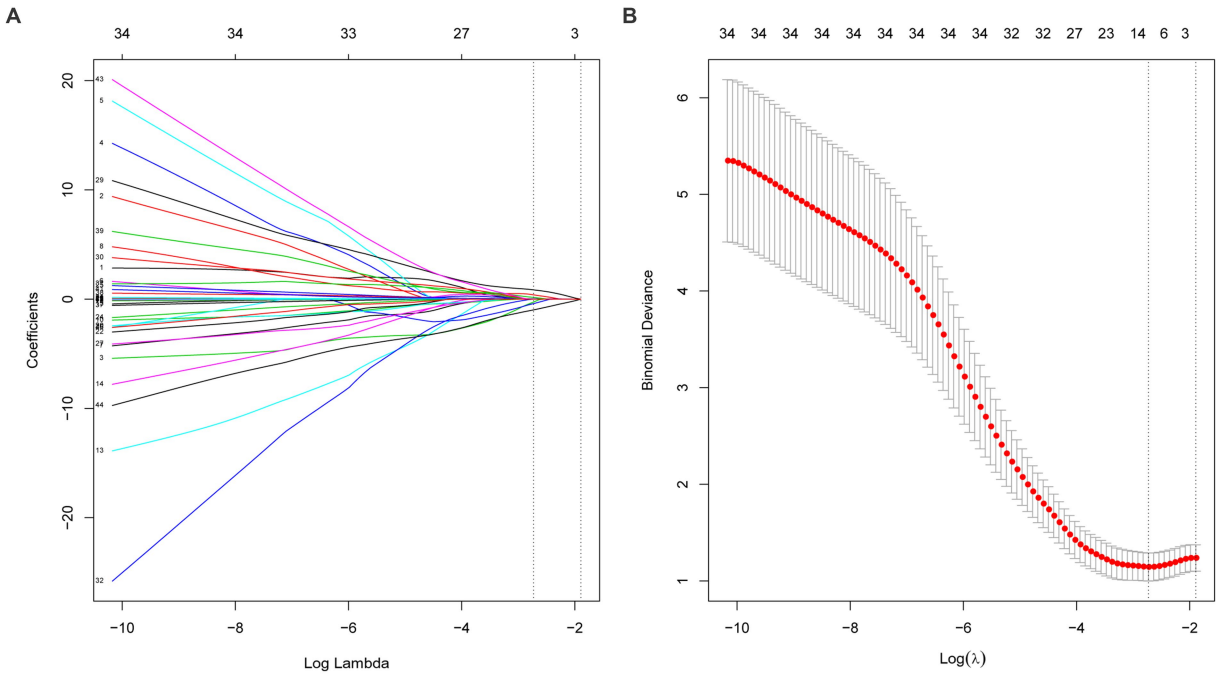

FIGURE 3
(A,B) Show the variable selection process of lasso regression. When the minimum mean square error of λ was 0.065, the variables were reduced to eight.

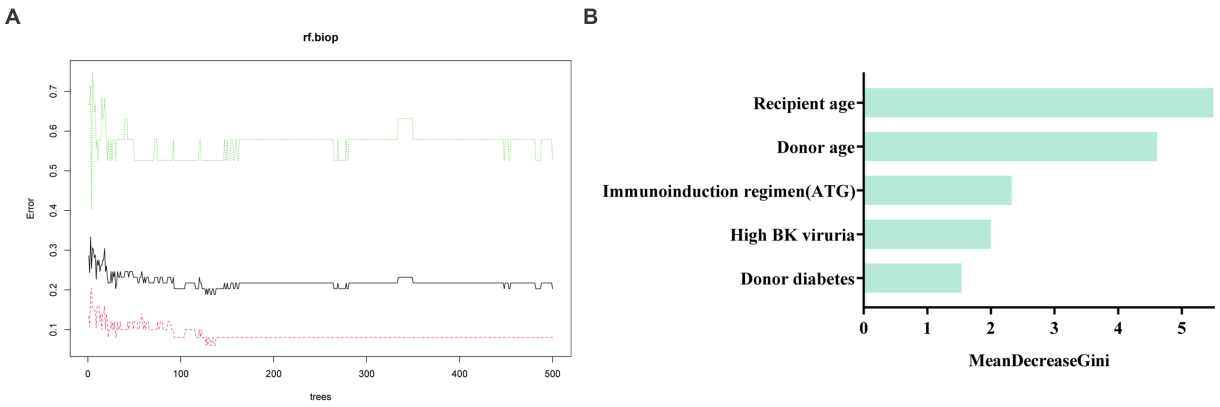

FIGURE 4
(A) shows the training process of the random forest model. The optimal number of trees of the random forest model is 127. **(B)** Displays the results of the Mean Decrease Gini importance analysis, which ranks the importance of features after random forest screening. The analysis identified recipient age, donor age, immunoinduction regimen (ATG), high BK viruria, and donor diabetes as the top five important factors in descending order.

viremia was 6.71 log10 copies/ml [AUC = 0.953, $p < 0.001$ (30)]. Another single-center study from Thailand also reported the positive effect of urinary BK viral load in predicting BK viremia (31). In contrast to previous studies, our results suggest that the use of ATG as an immune induction method is protective against the progression of BK viruria to BK viremia, as compared to the use of basiliximab. Typically, ATG has a stronger immunosuppressive effect than basiliximab, which has a higher risk of infection. For instance, a clinical study of low-risk living kidney transplants found that immune induction with ATG was a risk factor for BK viremia infection (32). However, a different study came to a different conclusion, stating that the occurrence of BK viremia was not related to the mode of immunosuppression induction (33). To reduce the incidence of rejection after renal transplantation, our center routinely performs postoperative immune induction with basiliximab. However, for recipients who receive high-risk donor kidneys, we switch the immune induction method to ATG. Additionally, a tacrolimus-based triple suppression regimen is used for immunosuppressive maintenance in 97.7% of our population. The difference in the study population may also account for the variation in results, as all of our study participants received DCD donor kidneys, with 42.8% of them being ECD donors.

In addition, we used a machine learning approach to assess important variables associated with the progression of BK viruria to

BK viremia. The results showed that recipient age, high BK viruria and immunoinduction regimen (ATG) appeared in the top five variables of the random forest model. Thus when patients present with BK viruria, close attention to such patients and timely intervention may be helpful in the progression of BK virus.

This study aims to identify risk factors associated with BK virus infection and the progression of BK viruria to BK viremia, using a machine learning approach to evaluate the significance of potential variables in predicting such progression. These factors are critical in our study population and can aid clinicians in making informed decisions. However, certain limitations must be considered. Firstly, in order to obtain complete clinical data, we excluded donor kidneys from other hospitals, which may slightly underestimate the actual incidence of BK virus infection. Secondly, the results of our study may not be generalizable to other centers, as all participants were recipients of DCD donor kidneys, close to half of which belonged to ECD, and the majority of recipients were receiving a tacrolimus-dominant triple immunosuppressant postoperatively. Therefore, the difference in the study population may affect the results. Thirdly, we acknowledge that there is a sample size problem in single-center studies, and therefore, further expansion of the sample size is needed to validate our findings.

Conclusion

BK virus infection in kidney transplant recipients is influenced by multiple factors related to both the donor and the recipient, particularly in the context of DCD. Identifying and screening high-risk groups for BK virus infection and implementing early intervention and treatment can help prolong the lifespan of the transplanted organ.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Renmin Hospital of Wuhan University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JZ and TQ designed the study. YL and CK carried out data collection and analyzed the data. TW, YZ, and HH made the figures. YL, HH, and CK drafted and revised the paper. 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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Prediction models for the recipients' ideal perioperative estimated glomerular filtration rates for predicting graft survival after adult living-donor kidney transplantation

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Introduction: The impact of the perioperative estimated glomerular filtration rate (eGFR) on graft survival in kidney transplant recipients is yet to be evaluated. In this study, we developed prediction models for the ideal perioperative eGFRs in recipients.

Methods: We evaluated the impact of perioperative predicted ideal and actual eGFRs on graft survival by including 1,174 consecutive adult patients who underwent living-donor kidney transplantation (LDKT) between January 2008 and December 2020. Prediction models for the ideal perioperative eGFR were developed for 676 recipients who were randomly assigned to the training and validation sets (ratio: 7:3). The prediction models for the ideal best eGFR within 3 weeks and those at 1, 2, and 3 weeks after LDKT in 474 recipients were developed using 10-fold validation and stepwise multiple regression model analyzes. The developed prediction models were validated in 202 recipients. Finally, the impact of perioperative predicted ideal eGFRs/actual eGFRs on graft survival was investigated using Fine–Gray regression analysis.

Results: The correlation coefficients of the predicted ideal best eGFR within 3 weeks and the predicted ideal eGFRs at 1, 2, and 3 weeks after LDKT were 0.651, 0.600, 0.598, and 0.617, respectively. Multivariate analyzes for graft loss demonstrated significant differences in the predicted ideal best eGFR/actual best eGFR within 3 weeks and the predicted ideal eGFRs/actual eGFRs at 1, 2, and 3 weeks after LDKT.

Discussion: The predicted ideal best eGFR/actual best eGFR within 3weeks and the predicted ideal eGFRs/actual eGFRs at 1, 2, and 3weeks after LDKT were independent prognostic factors for graft loss. Therefore, the perioperative predicted ideal eGFR/actual eGFR may be useful for predicting graft survival after adult LDKT.

KEYWORDS

cross-validation, estimated glomerular filtration rate, graft survival, living-donor kidney transplantation, prediction model

1. Introduction

Donor and recipient characteristics, operative factors, postoperative complications, and immunosuppressive drugs may affect graft function after living-donor kidney transplantation (LDKT). Specifically, donor and recipient characteristics, including donor age, recipient sex, and donor estimated glomerular filtration rate (eGFR), graft pathological features, and anti-human leukocyte antigen (HLA) donor-specific antibodies (DSAs) affect postoperative graft function (1–3). Postoperative graft function is also affected by operative factors and postoperative complications, including laparoscopic nephrectomy, warm ischemia time, urological and vascular complications, and rejection (4–7). Calcineurin inhibitors can cause nephrotoxicity and lead to a low eGFR (8, 9). Furthermore, postoperative graft function is considered a good predictor of graft survival. Studies have investigated the impact of the eGFR on graft survival at 1 year after kidney transplantation (KT) (2, 10–16). However, the effect of the perioperative eGFR on graft survival in LDKT is yet to be investigated. Previously, perioperative graft function was stratified and evaluated based on slow or delayed graft function (17, 18). Several studies have developed prediction models for recipients' eGFRs at 1–5 years after KT (19–21). However, to our knowledge, no study has reported the development of prediction models for ideal eGFRs during perioperative LDKT. Therefore, we investigated the impact of perioperative actual eGFRs on graft survival in adult LDKT. Additionally, we developed prediction models for recipients' ideal eGFRs during the perioperative period to investigate the impact of predicted ideal eGFRs on graft survival.

2. Materials and methods

2.1. Study design

This single-center retrospective cohort study was approved by the Nagoya Daini Red Cross Hospital's Institutional Review Board (Aichi, Japan; approval number: 1504) and was conducted following the principles of the Declaration of Helsinki. The study included 1,174 consecutive adult patients who underwent LDKT between January 2008 and December 2020. First, the impacts of the actual best eGFR within 3 weeks after LDKT and actual eGFRs at 1, 2, and 3 weeks and at 1, 3, 6, and 12 months after LDKT on graft survival were investigated in 1174 recipients. Second, prediction models were developed for the ideal best eGFR within 3 weeks and ideal eGFRs at 1, 2, and 3 weeks after LDKT. We developed prediction models based on 676 ideal recipients selected from 1,174 recipients. Finally, the impact of the predicted ideal best eGFR/actual best eGFR within 3 weeks and the predicted ideal eGFRs/actual eGFRs at 1, 2, and 3 weeks after LDKT on graft survival was investigated in 1174 recipients. This study was reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

2.2. Participants

This study included all consecutive recipients who underwent LDKT at our hospital between January 2008 and December 2020. The recipients were followed up until August 2021. Additionally, we excluded recipients with an immunosuppressive regimen using iscalimab in clinical trials (two recipients) (Figure 1). All donor and recipient data were retrospectively collected from the medical records and analyzed anonymously; therefore, the requirement for informed consent was waived by the Nagoya Daini Red Cross Hospital's Institutional Review Board (Aichi, Japan; approval number: 1504).

2.3. Living donors

Living donors were selected according to the guidelines for living kidney donors in Japan (22). The laterality of the kidney for donor nephrectomy was determined using the results of technetium-99 m diethylene triamine pentaacetic acid (Tc-99m DTPA). A difference in Tc-99m DTPA ≥10% between the right and left kidneys indicated a nephrectomy of the inferior side. In contrast, a discrepancy in Tc-99m DTPA of <10% indicated left nephrectomy. Furthermore, data on donor characteristics, surgical outcomes, and perioperative complications were collected and analyzed.

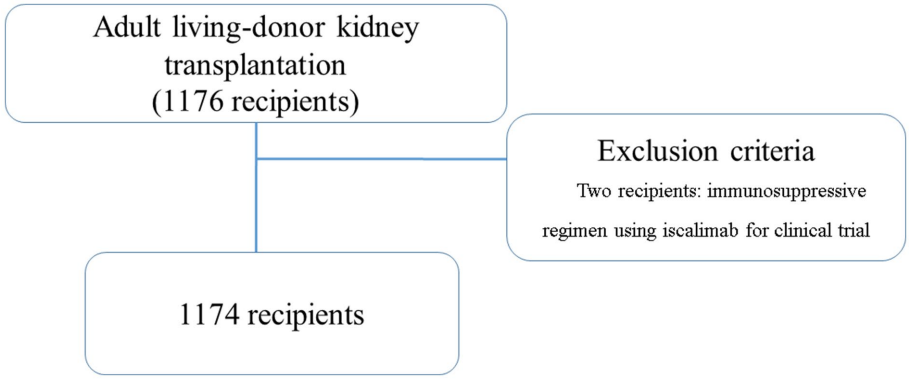

FIGURE 1
Patient flowchart.

2.4. Recipients

LDKTs were performed following the Istanbul Declaration. The recipients stayed at the hospital for 3 weeks after LDKT. After discharge, postoperative recipient assessments were performed fortnightly for the first 3 months and subsequently monthly at our hospital and local hospitals. Protocol biopsies were performed at 1 h after reperfusion as a baseline, and at 1 month after KT.

Data on donor and recipient characteristics and operative outcomes, actual eGFR after LDKT, graft survival, and recipient mortality were collected. These data were used to analyze the impact of the actual best eGFR within 3 weeks after LDKT as well as actual eGFRs at 1, 2, and 3 weeks and at 1, 3, 6, and 12 months after LDKT on graft survival. Additionally, to develop the prediction models for the ideal best eGFR within 3 weeks and ideal eGFRs at 1, 2, and 3 weeks after LDKT, data on donor and recipient characteristics and operative outcomes; perioperative adverse events; best eGFR within 3 weeks after LDKT; and actual eGFRs at 1, 2, and 3 weeks after LDKT were collected and analyzed. Furthermore, prediction models were developed for recipients with ideal graft conditions within 1 month after LDKT. Recipients who received grafts from donors with intraoperative adverse events; those who received transplanted grafts with arterial reconstruction or ligation of the thin upper pole artery; and those who experienced perioperative adverse events, conversion of the immunosuppressive regimen, recurrence of nephritis, calcineurin inhibitor toxicity, and rejection within 1 month were excluded from the development of the prediction models for ideal eGFRs within 3 weeks. The detailed reasons for excluding 498 recipients from the development of the prediction models are presented in Supplementary Table S1. Data on donor and recipient characteristics, predicted ideal eGFR/actual eGFR, graft survival, and recipient mortality were collected to investigate the impact of the predicted ideal eGFR/actual eGFR on graft survival.

2.5. Immunosuppressive protocols

For the ABO-compatible KT, basiliximab, steroids, calcineurin inhibitors (i.e., cyclosporin, tacrolimus, or extend-release tacrolimus), and an antimetabolite or mammalian target of rapamycin inhibitor (i.e., mycophenolate mofetil, mizoribine, or everolimus) were administered for induction and maintenance therapy. Desensitization was performed using rituximab or splenectomy, double-filtration plasmapheresis, and plasmapheresis for the ABO-incompatible KT. Basiliximab, steroids, and calcineurin inhibitors (i.e., cyclosporin, tacrolimus or extend-release tacrolimus), and mycophenolate mofetil were administered for induction and maintenance therapy. Regarding the preformed-DSA KT, desensitization was performed using rituximab, double-filtration plasmapheresis, plasmapheresis, or intravenous immunoglobulin administration. Furthermore, basiliximab, steroids, calcineurin inhibitors (i.e., tacrolimus or extend-release tacrolimus), and mycophenolate mofetil were administered for induction and maintenance therapy.

2.6. Statistical analysis

Statistical analyzes of donor and recipient characteristics were performed using the Kruskal–Wallis test and the chi-square or Fisher's exact test for continuous and categorical variables, respectively. An estimation equation model was constructed to predict the eGFR. The independent variables used in the estimation equation were initially tested for collinearity in advance, and factors with collinearity were excluded to prevent overfitting the model. Subsequently, estimation equations were constructed on the training set and confirmed using the validation set. The patients were randomly categorized into two groups in a 7:3 ratio, of whom 474 and 202 were assigned to the training and validation sets, respectively (Figure 2).

A linear regression prediction model was constructed using the eGFR as the dependent variable of the training set to establish an equation for estimating the eGFR. Subsequently, a stepwise method with 10-fold validation was used to limit the variables to be included in the model, and the estimation accuracy was evaluated. The R and R-squared values were used to estimate the accuracy. Finally, the constructed estimation equations were evaluated for their accuracy on the validation set.

A Fine–Gray competing risk regression model was used to determine the prognostic factors for graft loss. The proportional hazard assumption was confirmed using a log–log plot for the Fine–Gray competing risk regression model. No interaction effects between the variables were found in the models using the interaction items. Covariates with a p-value <0.05 in the univariate logistic regression analysis were used in the multivariate logistic regression analysis. Statistical significance was set at 0.05 (two-sided). All analyzes were performed using the Statistical Package for the Social Sciences (Version 24.0, IBM Japan Ltd., Tokyo, Japan) and R version 4.0.3 (R Core Team [2020], Vienna, Austria).

3. Results

3.1. Study population

Overall, 1,176 adult LDKTs were performed at our hospital during this study, of which two LDKTs were excluded, and the remaining 1,174 recipients were included. The 1,174 recipients were followed up between January 2008 and August 2021 (median observation period: 77.0 [interquartile range, 45.0–117.0] months) and were included in the final analysis.

3.2. Recipient results

3.2.1. Descriptive data concerning donors and recipients

The characteristics of donors and recipients are presented in Table 1. Using the Fine–Gray competing risk regression model, recipients were presented in the following three groups: recipients with functioning grafts (1,059 patients), graft loss (73 patients), and death with functioning grafts (42 patients). Regarding donor characteristics, significant differences were observed in donor age ($p = 0.002$); donation to first-degree relative recipients ($p = 0.001$); preoperative comorbidities ≥1 (hypertension: blood pressure > 140/90 mmHg or treatment with blood pressure-lowering medications; dyslipidemia: low-density lipoprotein cholesterol level > 140 mg/dL, triglyceride level > 150 mg/dL, high-density lipoprotein cholesterol level < 40 mg/dL, or treatment of dyslipidemia;

FIGURE 2
Flowchart of the development of the prediction models for ideal eGFRs using 10-fold validation. Overall, 676 ideal recipients were selected to develop the prediction models. The recipients were randomly categorized into two groups in a 7:3 ratio (474 and 202 recipients for the training and validation sets, respectively). In the 474 recipients, 10-fold validation and stepwise multiple regression model analyzes were used to develop prediction models for ideal eGFRs, while the developed prediction models were validated in 202 patients. eGFR, estimated glomerular filtration rate.

glucose intolerance: impaired fasting glycemia, impaired glucose tolerance, or diabetes mellitus without insulin treatment; and obesity: body mass index [BMI] >30 kg/m^2) ($p = 0.015$); preoperative systolic blood pressure ($p = 0.003$); preoperative diastolic blood pressure ($p = 0.023$); preoperative hemoglobin A1c level ($p = 0.039$); preoperative BMI ($p = 0.027$); preoperative urine albumin/creatine ratio ($p = 0.014$); and baseline biopsy findings at 1 h after transplantation (presence of interstitial fibrosis, tubular atrophy, arteriolosclerosis, or glomerulosclerosis based on the 2018 Banff classification) ($p = 0.048$) (23).

Regarding the recipient characteristics, significant differences were observed in recipient age ($p < 0.001$); cause of end-stage renal disease ($p = 0.019$); follow-up period ($p < 0.001$); transplantation from first-degree relative donors ($p = 0.001$); preoperative flow cytometry B cell crossmatch positivity ($p = 0.010$); dialysis vintage ($p < 0.001$); HLA-AB mismatch ($p = 0.010$); calcineurin inhibitor administration at KT ($p < 0.001$); calcineurin inhibitor administration at the best eGFR within 3 weeks after KT ($p < 0.001$); calcineurin inhibitor administration at 1, 2, and 3 weeks after KT ($p < 0.001$, $p < 0.001$, $p < 0.001$, respectively); mycophenolate mofetil, mizoribine, or everolimus administration at transplantation ($p = 0.049$); actual best eGFR within 3 weeks ($p = 0.039$); actual eGFRs at 1 and 2 weeks and 3, 6, and 12 months ($p = 0.038$, $p = 0.013$, $p = 0.025$, $p < 0.001$, and $p < 0.001$, respectively); recurrence of nephritis ($p = 0.013$); *de novo* DSA ($p < 0.001$); rejection ($p = 0.007$); and recipient death ($p < 0.001$).

3.2.2. Operative outcomes of the donor and recipients

The operative outcomes of the donors and recipients are presented in Table 2. In the donor operation, significant differences were observed in donor nephrectomy operation time ($p = 0.020$) and operation methods ($p < 0.001$). In the recipient operation, significant differences were observed in cold ischemia time ($p < 0.001$), delayed graft function ($p < 0.001$), and occurrence of arterial thrombosis ($p = 0.001$), lymphocele ($p < 0.001$), incisional hernia ($p = 0.007$), and severe pneumonia ($p = 0.001$).

3.2.3. Causes of graft loss and death with functioning grafts

Graft loss was identified in 73 recipients (30, 18, 10, 8, 5, 1, and 1 cases of rejection, allograft nephropathy, infection, recurrent nephritis, cardiac events, arterial thrombosis, and unknown cause, respectively). Death with functioning grafts was observed in 42 recipients (13, 10, 4, 3, 3, and 9 cases of malignant diseases, cardiovascular diseases, accidents, infectious diseases, cerebrovascular diseases, and other causes, respectively).

3.2.4. Impact of actual eGFR on graft loss

The results of the univariate Fine–Gray competing risk regression model for graft loss are presented in Supplementary Table S2. Significant differences were observed in male recipient ($p = 0.032$); preformed DSA ($p = 0.013$); preoperative desensitization (preoperative rituximab administration or splenectomy, preoperative double-filtration plasmapheresis, plasmapheresis, or intravenous immunoglobulin, $p = 0.028$); actual eGFR at 6 months after LDKT ($p = 0.003$); actual eGFR at 12 months after LDKT ($p < 0.001$); and donor age ($p = 0.048$). Table 3 and Supplementary Tables S3A–H show the graft loss risk of the actual best eGFR within 3 weeks after LDKT and actual eGFRs at 1, 2, and 3 weeks and at 1, 3, 6, and 12 months after LDKT adjusted for male recipient, preformed DSA, preoperative desensitization, and donor age using the multivariate Fine–Gray competing risk regression model. Significant differences were observed in the actual eGFRs at 6 and 12 months after LDKT ($p = 0.015$, hazard ratio [HR]: 0.946, 95% confidence interval [CI]: 0.904–0.989, $p < 0.001$; HR: 0.937, 95% CI: 0.907–0.967).

TABLE 1 Donor and recipient characteristics.

		Functioning graft	Graft loss	Death with functioning grafts	*p*-value
		n = 1,059	*n* = 73	*n* = 42	
Donor					
Donor age (years, SD)		58.6 (10.0)	60.3 (10.0)	63.4 (8.2)	**0.002**
Donor sex (male, %)		390 (36.8)	27 (37.0)	14 (33.3)	0.898
Donation to first-degree relative recipients (%)		487 (46.0)	43 (58.9)	10 (23.8)	**0.001**
Smoking history (%)		474 (44.8)	34 (46.6)	14 (33.3)	0.320
Preoperative comorbidities ≥1 (%)		764 (72.1)	60 (82.2)	37 (88.1)	**0.015**
	Hypertension (%)	300 (28.3)	34 (46.6)	22 (52.4)	**<0.001**
	Dyslipidemia (%)	610 (57.6)	46 (63.0)	32 (76.2)	**0.041**
	Glucose intolerance (%)	284 (26.8)	27 (37.0)	13 (31.0)	0.151
	Obesity—body mass index ≥30 kg/m^2 (%)	5 (0.5)	2 (2.7)	0	**0.045**
Donor preoperative systolic blood pressure (mmHg, SD)		122.8 (14.5)	127.3 (13.3)	127.0 (11.8)	**0.003**
Donor preoperative diastolic blood pressure (mmHg, SD)		73.7 (10.8)	75.9 (10.3)	76.7 (11.3)	**0.023**
Donor preoperative total cholesterol level (mg/dL, SD)		211.6 (37.0)	206.0 (32.6)	217.2 (33.7)	0.264
Donor preoperative triglyceride level (mg/dL, SD)		139.3 (87.1)	137.2 (79.5)	152.1 (90.4)	0.581
Donor preoperative low-density lipoprotein cholesterol level (mg/dL, SD)		123.3 (30.8)	122.9 (28.8)	126.6 (29.2)	0.704
Donor preoperative high-density lipoprotein cholesterol level (mg/dL, SD)		63.0 (16.3)	50.3 (15.9)	64.3 (24.4)	0.454
Donor preoperative fasting glucose level (mg/dL, SD)		99.3 (12.5)	99.5 (12.8)	97.1 (9.7)	0.597
Donor preoperative 75-g oral glucose tolerance test results—blood glucose level at 2 h after glucose administration (mg/dL, SD)		131.2 (36.3)	139.5 (49.4)	133.6 (34.3)	0.358
Donor preoperative HbA1c level (%, SD)		5.7 (0.4)	5.8 (0.4)	5.8 (0.3)	**0.039**
Donor preoperative body mass index (kg/m^2, SD)		22.7 (2.8)	23.2 (3.3)	23.8 (2.7)	**0.027**
Donor preoperative eGFR (mL/min/1.73 m^2, SD)		73.4 (12.7)	73.7 (13.5)	72.6 (18.9)	0.382
Donor preoperative split kidney function on Tc-99m DTPA scintigraphy (%, SD)		48.0 (3.7)	48.3 (3.6)	47.2 (3.3)	0.239
Preoperative urine albumin/Cr ratio (mg/g Cr, SD)		9.4 (11.6)	15.8 (24.4)	11.3 (10.4)	**0.014**
Baseline biopsy findings at 1 h after transplantation (%)		582 (55.4)	37 (51.4)	29 (74.4)	**0.048**
Recipient					
Recipient age (years, SD)		48.6 (13.7)	46.7 (14.9)	60.9 (8.9)	**<0.001**
Recipient sex (male, %)		658 (62.1)	53 (72.6)	28 (66.7)	0.177
Cause of end-stage renal disease	Diabetes mellitus (%)	197 (18.6)	13 (17.8)	15 (35.7)	**0.019**
	Glomerulonephritis (%)	414 (39.1)	31 (42.5)	12 (28.6)	
	Hypertension (%)	78 (7.4)	6 (8.2)	2 (4.8)	
	Polycystic kidney disease (%)	88 (8.3)	0	6 (14.3)	
	Others (%)	282 (26.6)	23 (31.5)	7 (16.7)	
Recipient body mass index (kg/m^2, SD)		22.4 (3.7)	23.0 (4.4)	22.4 (3.7)	0.515
Recipient follow-up period (months, SD)		74.4 (44.5)	101.8 (37.6)	66.5 (40.1)	**<0.001**
Transplantation from first-degree relative donors (%)		487 (46.0)	43 (58.9)	10 (23.8)	**0.001**
Preoperative flow cytometry T cell crossmatch (positive, %)		37 (3.5)	2 (2.7)	2 (4.8)	0.851
Preoperative flow cytometry B cell crossmatch (positive, %)		94 (8.9)	14 (19.2)	6 (14.3)	**0.010**
Dialysis vintage (months, SD)		73.7 (392.7)	30.8 (46.9)	89.0 (195.0)	**<0.001**
Preoperative ejection fraction on ultrasonographic cardiography (%)		61.9 (7.6)	61.8 (8.7)	53.5 (15.7)	0.580
Preoperative ventricular wall motion asynergy on ultrasonographic cardiography (%)		125 (11.8)	11 (15.3)	10 (23.8)	0.053
Preoperative sensitization—transfusion, pregnancy, transplantation (%)		437 (41.3)	25 (34.2)	18 (42.9)	0.481
HLA-AB mismatch (SD)		2.4 (1.0)	2.3 (0.9)	2.9 (0.9)	**0.010**
HLA-DR mismatch (SD)		1.4 (0.6)	1.3 (0.5)	1.6 (0.6)	0.056
Preoperative PRA class I (positive, ≥5%, %)		153 (14.4)	12 (16.4)	4 (9.5)	0.589
Preoperative PRA class II (positive, ≥5%, %)		86 (8.1)	4 (5.5)	1 (2.4)	0.298
Preformed DSA (%)		70 (6.6)	10 (13.7)	3 (7.1)	0.073
	Preoperative flow cytometry T cell crossmatch after desensitization for preformed DSA (positive, %)	10 (14.3)	0	1 (33.3)	0.292

(Continued)

TABLE 1 (Continued)

		Functioning graft	Graft loss	Death with functioning grafts	*p*-value
		n = 1,059	*n* = 73	*n* = 42	
	Preoperative flow cytometry B cell crossmatch after desensitization for preformed DSA (positive, %)	56 (80.0)	7 (77.8)	2 (66.7)	0.850
	ICFA class I after desensitization for preformed DSA (positive, %)	3 (5.9)	0	0	0.940
	ICFA class II after desensitization for preformed DSA (positive, %)	4 (7.8)	0	0	0.919
ABO-incompatible transplantation (%)		346 (32.7)	28 (38.4)	18 (42.9)	0.253
Preoperative desensitization (preoperative rituximab administration or splenectomy, preoperative double-filtration plasmapheresis, plasmapheresis, or IVIG, %)		393 (37.1)	36 (49.3)	19 (45.2)	0.073
Calcineurin inhibitor administration at kidney transplantation	TAC (%)	193 (18.2)	22 (30.1)	14 (33.3)	**<0.001**
	CsA (%)	370 (34.9)	42 (57.5)	24 (57.1)	
	TACER (%)	496 (46.8)	9 (12.3)	4 (9.5)	
Calcineurin inhibitor administration at best eGFR within 3 weeks after kidney transplantation	TAC (%)	192 (18.1)	21 (28.8)	14 (33.3)	**<0.001**
	CsA (%)	369 (34.8)	42 (57.5)	24 (57.1)	
	TACER (%)	498 (47.0)	10 (13.7)	4 (9.5)	
Calcineurin inhibitor administration at 1 week after kidney transplantation	TAC (%)	191 (18.0)	20 (27.4)	14 (33.3)	**<0.001**
	CsA (%)	373 (35.2)	43 (58.9)	24 (57.1)	
	TACER (%)	495 (46.7)	10 (13.7)	4 (9.5)	
Calcineurin inhibitor administration at 2 weeks after kidney transplantation	TAC (%)	192 (18.1)	21 (28.8)	14 (33.3)	**<0.001**
	CsA (%)	371 (35.1)	43 (58.9)	24 (57.1)	
	TACER (%)	495 (46.8)	9 (12.3)	4 (9.5)	
Calcineurin inhibitor administration at 3 weeks after kidney transplantation	TAC (%)	182 (17.6)	18 (26.5)	13 (32.5)	**<0.001**
	CsA (%)	359 (34.7)	41 (60.3)	23 (57.5)	
	TACER (%)	495 (47.8)	9 (13.2)	4 (10.0)	
MMF, MZ, or EVR administration at transplantation	MMF (%)	842 (79.5)	68 (93.2)	34 (81.0)	**0.049**
	MZ (%)	32 (3.0)	2 (2.7)	2 (4.8)	
	EVR (%)	185 (17.5)	3 (4.1)	6 (14.3)	
Conversion of immunosuppressive regimen within 1 month (%)		13 (1.2)	2 (2.7)	0	0.406
Actual best eGFR within 3 weeks (mL/min/1.73 m^2, SD)		58.0 (16.2)	55.2 (22.4)	53.5 (15.7)	**0.039**
Actual eGFR at 1 week (mL/min/1.73 m^2, SD)		50.2 (15.1)	47.7 (21.7)	45.0 (15.4)	**0.038**
Actual eGFR at 2 weeks (mL/min/1.73 m^2, SD)		49.6 (14.5)	46.9 (21.1)	44.7 (12.1)	**0.013**
Actual eGFR at 3 weeks (mL/min/1.73 m^2, SD)		48.8 (14.2)	45.5 (20.1)	45.7 (11.1)	0.055
Actual eGFR at 1 month (mL/min/1.73 m^2, SD)		47.4 (13.1)	44.0 (18.1)	45.9 (15.5)	0.060
Actual eGFR at 3 months (mL/min/1.73 m^2, SD)		45.2 (12.1)	41.4 (19.1)	42.9 (14.0)	**0.025**
Actual eGFR at 6 months (mL/min/1.73 m^2, SD)		45.2 (11.5)	38.2 (16.9)	41.7 (11.7)	**<0.001**
Actual eGFR at 12 months (mL/min/1.73 m^2, SD)		45.0 (11.8)	36.3 (14.7)	41.5 (12.3)	**<0.001**
Trough levels of calcineurin inhibitor	TAC at best eGFR (ng/mL)	11.1 (4.5)	12.1 (7.5)	11.6 (3.9)	0.797
	TAC at 1 week (ng/mL)	11.1 (3.8)	12.6 (4.5)	12.2 (3.7)	0.100
	TAC at 2 weeks (ng/mL)	10.5 (2.9)	9.8 (3.7)	12.0 (3.1)	0.080
	TAC at 3 weeks (ng/mL)	9.8 (2.6)	9.5 (3.1)	10.9 (3.0)	0.324
	CsA at best eGFR (ng/mL)	262.3 (109.4)	273.5 (99.9)	284.4 (102.5)	0.386
	CsA at 1 week (ng/mL)	268.6 (102.3)	292.0 (95.8)	287.7 (95.5)	0.179
	CsA at 2 weeks (ng/mL)	252.6 (95.0)	227.2 (96.8)	292.5 (129.3)	0.175
	CsA at 3 weeks (ng/mL)	235.7 (86.7)	284.1 (123.8)	229.8 (88.7)	0.087
	TACER at best eGFR (ng/mL)	7.4 (2.7)	7.9 (3.5)	7.5 (2.1)	0.811
	TACER at 1 week (ng/mL)	7.8 (3.0)	8.3 (3.5)	10.3 (8.6)	0.812
	TACER at 2 weeks (ng/mL)	7.5 (2.3)	8.9 (3.4)	7.3 (0.8)	0.452
	TACER at 3 weeks (ng/mL)	7.5 (2.0)	8.5 (1.7)	7.4 (2.8)	0.232
Pathological findings at protocol biopsy at 1 month after kidney transplantation (%)	Recurrence of nephritis (%)	4 (4.3)	2 (3.1)	1 (2.6)	**0.013**
	Calcineurin inhibitor toxicity (%)	81 (8.7)	7 (10.9)	2 (5.3)	0.617

(Continued)

TABLE 1 (Continued)

		Functioning graft	Graft loss	Death with functioning grafts	*p*-value
		n = 1,059	*n* = 73	*n* = 42	
De novo DSA (%)		103 (11.0)	22 (36.7)	4 (11.8)	< **0.001**
Rejection (pathological and clinical, %)		30 (2.8)	7 (9.6)	2 (4.8)	**0.007**
Graft survival period (months, SD)		74.4 (44.5)	72.3 (40.1)	66.5 (40.1)	0.592
Recipient death (%)		0	10 (13.7)	42 (100.0)	**<0.001**

Cr, creatine; CsA, cyclosporine A; DSA, donor-specific anti-human leukocyte antigen antibody; eGFR, estimated glomerular filtration rate; EVR, everolimus; HbA1c, hemoglobin A1c; HLA, human leukocyte antigen; ICFA, immunocomplex capture fluorescence analysis; IVIG, intravenous immunoglobulin; MMF, mycophenolate mofetil; MZ, mizoribine; PRA, panel reactive antibody; SD, standard deviation; TAC, tacrolimus; TACER, extended-release tacrolimus; Tc-99m DTPA, technetium-99 m diethylene triamine pentaacetic acid.
Preoperative flow cytometry B cell crossmatch after desensitization for preformed DSA became false positive when rituximab was administered. ICFA classes I and II were examined for recipients who received rituximab. The bold font indicates statistically significant results.

TABLE 2 Donor and recipient operative outcomes.

		Functioning graft	Graft loss	Death with functioning graft	*p*-value
		n = 1,059	*n* = 73	*n* = 42	
Donor operation					
Kidney laterality (left, %)		973 (91.9)	67 (91.8)	37 (88.1)	0.683
Kidney weight (g, SD)		117.2 (42.2)	183.5 (41.1)	182.1 (45.3)	0.217
Warm ischemia time (s, SD)		139.9 (69.4)	148.3 (72.0)	146.6 (45.8)	0.321
Operating time (min, SD)		208.4 (95.7)	218.1 (45.5)	214.1 (51.9)	**0.020**
Operation blood loss (mL, SD)		34.5 (12.7)	44.6 (13.5)	72.6 (18.9)	0.426
Adverse events	Arterial injury (%)	1 (0.1)	0	0	0.947
	Venous injury (%)	2 (0.2)	0	0	0.897
	Open conversion (%)	3 (0.3)	0	0	0.849
	Intraoperative bleeding (%)	2 (0.2)	0	0	0.897
	Subcapsular hematoma (%)	2 (0.2)	0	0	0.897
	Bowel injury (%)	1 (0.1)	0	0	0.947
Operation methods of donor nephrectomy	Hand-assisted laparoscopic (%)	1,011 (95.5)	64 (87.7)	34 (81.0)	**<0.001**
	Non-hand-assisted retroperitoneoscopic (%)	35 (3.3)	4 (5.5)	5 (11.9)	
	Open (%)	13 (1.2)	5 (6.8)	3 (7.1)	
Recipient operation					
Cold ischemia time (min, SD)		95.5 (39.0)	109.3 (47.8)	116.2 (43.5)	**<0.001**
Arterial reconstruction or ligation of thin upper pole artery (%)		300 (28.3)	20 (27.4)	16 (38.1)	0.378
Recipient perioperative adverse events	Delayed graft function (%)	0	0	1 (2.4)	**<0.001**
	Surgical site infection (%)	12 (1.1)	2 (2.7)	1 (2.4)	0.403
	Arterial thrombosis (%)	0	1 (1.4)	0	**0.001**
	Arterial stenosis (%)	2 (0.2)	0	0	0.897
	Urine leakage (%)	10 (0.9)	0	1 (2.4)	0.442
	Ureteral necrosis (%)	2 (0.2)	0	0	0.897
	Ureteral stenosis (%)	3 (0.3)	1 (1.4)	0	0.283
	Lymphocele (%)	9 (0.8)	5 (6.8)	0	**<0.001**
	Incisional hernia (%)	3 (0.3)	2 (2.7)	0	**0.007**
	Postoperative bleeding requiring reoperation (%)	14 (1.3)	1 (1.4)	2 (4.8)	0.187
	Gastrointestinal bleeding or perforation (%)	2 (0.2)	0	0	0.897
	Colon perforation (%)	3 (0.3)	0	0	0.849
	Severe pneumonia (%)	0	1 (1.4)	0	**0.001**

SD, standard deviation. The bold font isndicates statistically significant results.

TABLE 3 Multivariate Fine–Gray competing model analysis for graft loss adjusted for male recipient, preformed DSA, preoperative desensitization, and donor age.

	p-value	Hazard ratio	95% confidence interval	
			Lower limit	Upper limit
Actual best eGFR within 3 weeks after transplantation (mL/min/1.73 m^2)	0.930	1.001	0.980	1.022
Actual eGFR at 1 week after transplantation (mL/min/1.73 m^2)	>0.999	1.000	0.978	1.022
Actual eGFR at 2 weeks after transplantation (mL/min/1.73 m^2)	0.850	1.002	0.978	1.027
Actual eGFR at 3 weeks after transplantation (mL/min/1.73 m^2)	0.810	0.996	0.967	1.027
Actual eGFR at 1 month after transplantation (mL/min/1.73 m^2)	0.530	0.990	0.960	1.022
Actual eGFR at 3 months after transplantation (mL/min/1.73 m^2)	0.470	0.986	0.947	1.025
Actual eGFR at 6 months after transplantation (mL/min/1.73 m^2)	**0.015**	0.946	0.904	0.989
Actual eGFR at 12 months after transplantation (mL/min/1.73 m^2)	**<0.001**	0.937	0.907	0.967

DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate. The bold font indicates statistically significant results.

TABLE 4 Coefficients in the validation set.

	Model	R	R-squared
Best eGFR within 3 weeks after transplantation	4	0.651	0.423
eGFR at 1 week after transplantation	7	0.600	0.360
eGFR at 2 weeks after transplantation	7	0.598	0.358
eGFR at 3 weeks after transplantation	7	0.617	0.380

eGFR, estimated glomerular filtration rate. The bold font indicates statistically significant results.

3.2.5. Development of eGFR prediction models

Details of the recipients who met the inclusion and exclusion criteria for developing prediction models are presented in Supplementary Tables S4, S5. For developing prediction models, recipients with conversion of the immunosuppressive regimen, recurrence of nephritis, calcineurin inhibitor toxicity, rejection, operative adverse events in donor and recipient operations, and arterial reconstruction or ligation of the thin upper pole artery were excluded, and those with these factors were not identified as recipients for prediction models.

The donor and recipient characteristics and operative outcomes for the training and validation sets are presented in Supplementary Tables S6, S7. Significant differences were identified in donor sex ($p = 0.042$) and preoperative flow cytometry T cell crossmatch ($p = 0.033$). Supplementary Table S8 presents the training set results using 10-fold cross-validation for the ideal best eGFR within 3 weeks after LDKT. Model 4 had the best R and R-squared values (0.646 and 0.418, respectively). The best prediction model for the ideal best eGFR within 3 weeks after LDKT is presented in Supplementary Table S9. Additionally, the R and R-squared values in the validation set were 0.651 and 0.423, respectively (Table 4). Supplementary Table S10 presents the training set results using 10-fold cross-validation for the predicted ideal eGFR at 1 week after LDKT. Model 7 had the best R and R-squared values (0.573 and 0.328, respectively). Supplementary Table S11 shows the best prediction model for the predicted ideal eGFR at 1 week after LDKT, and the R and R-squared values in the validation set were 0.600 and 0.360, respectively (Table 4). Supplementary Table S12 shows the training set results using 10-fold cross-validation for the predicted ideal eGFR at 2 weeks after LDKT. Model 7 had the best R and R-squared values (0.619 and 0.383, respectively). Furthermore, the best-estimated model for the predicted ideal eGFR at 2 weeks after LDKT is presented in Supplementary Table S13. The R and R-squared values in the validation set were 0.598 and 0.358, respectively (Table 4). Supplementary Table S14 presents the training set results using 10-fold cross-validation for the predicted ideal eGFR at 3 weeks after LDKT, and model 7 had the best R and R-squared values (0.693 and 0.480, respectively). The best-estimated model for the predicted ideal eGFR at 3 weeks after LDKT is presented in Supplementary Table S15. Furthermore, the R and R-squared values in the validation set were 0.617 and 0.380, respectively (Table 4).

3.2.6. Impact of predicted ideal and actual eGFRs on graft loss

Supplementary Figures S1A–D shows the association between the perioperative predicted ideal and actual eGFRs.

The results of the univariate Fine–Gray competing risk regression model for graft loss are presented in Supplementary Table S16. Significant differences were observed in male recipient ($p = 0.032$); preformed DSA ($p = 0.013$); preoperative desensitization ($p = 0.028$); predicted ideal best eGFR/actual best eGFR within 3 weeks after LDKT ($p < 0.001$); predicted ideal eGFRs/actual eGFRs at 1, 2, and 3 weeks after LDKT ($p = 0.045$, $p = 0.008$, and $p < 0.001$, respectively); and donor age ($p = 0.048$). Table 5 and Supplementary Tables S17A–D show the graft loss risk of the predicted ideal best eGFR/actual best eGFR within 3 weeks after LDKT and predicted ideal eGFRs/actual eGFRs at 1, 2, and 3 weeks after LDKT adjusted for male recipient, preformed DSA, preoperative desensitization, and donor age using the multivariate Fine–Gray competing risk regression model. Additionally, significant differences were identified in the predicted ideal best eGFR/actual best eGFR within 3 weeks after LDKT and the predicted ideal eGFRs/actual eGFRs at 1, 2, and 3 weeks after LDKT ($p < 0.001$, HR: 1.496, 95% CI: 1.225–1.826; $p = 0.006$, HR: 1.309, 95% CI: 1.079–1.588; $p = 0.002$, HR: 1.323, 95% CI: 1.105–1.584; and $p < 0.001$, HR: 1.452, 95% CI: 1.240–1.699, respectively). In

TABLE 5 Multivariate Fine–Gray competing model analysis for graft loss adjusted for male recipient, preformed DSA, preoperative desensitization, and donor age.

	p-value	Hazard ratio	95% confidence interval	
			Lower limit	Upper limit
Predicted ideal best eGFR/actual best eGFR within 3 weeks after transplantation	**<0.001**	1.496	1.225	1.826
Predicted ideal eGFR/actual eGFR at 1 week after transplantation	**0.006**	1.309	1.079	1.588
Predicted ideal eGFR/actual eGFR at 2 weeks after transplantation	**0.002**	1.323	1.105	1.584
Predicted ideal eGFR/actual eGFR at 3 weeks after transplantation	**<0.001**	1.452	1.240	1.699

DSA, donor-specific anti-human leukocyte antigen antibody; eGFR, estimated glomerular filtration rate. The bold font indicates statistically significant results.

Supplementary Tables S17A–D, in addition to the significant differences in predicted ideal eGFRs/actual eGFRs, significant differences were observed in male recipient, preformed DSA, and donor age.

4. Discussion

This study suggests that the actual eGFRs at 6 and 12 months after LDKT could be an independent risk factor for graft loss, although the actual eGFR within 3 months after LDKT does not seem to be a risk factor. However, the predicted ideal best eGFR/actual best eGFR within 3 weeks after LDKT and the predicted ideal eGFRs/actual eGFRs at 1, 2, and 3 weeks after LDKT might be independent prognostic factors for graft loss.

In this work, as LDKTs were performed between Asian (Japanese) recipients and donors, the race composition differed from those reported previously, and the BMI values of the recipients and donors were lower than those reported in previous works from different countries (24–26). In Japan, LDKT is limited between relatives. This may have contributed to the higher rate of ABO-incompatible KTs (33.4%) found in this study than those reported in previous studies on LDKT, although the rate of preformed-DSA KTs (7.1%) was similar to those reported in previous studies on LDKT (27, 28). The desensitization protocols for ABO-incompatible and preformed-DSA KTs were similar to those of previous reports, although those for preformed-DSA KT have not been established (28–30). However, the *de novo* DSA, rejection, graft loss, and death with functioning graft rates during the median observation period of 77.0 months were 11.0, 3.3, 6.2, and 3.6%, respectively. Interestingly, these results are similar to those of previous reports from other countries (31–33). In this study, the routine hospital stay at our institution after the transplantation was 3 weeks, which might be longer than that in other countries (34, 35). This might have facilitated a more in-depth investigation of post-LDKT graft function.

The eGFR at 1 year after KT could be a prognostic factor for graft loss (2, 10–17). However, no studies to date have investigated the impact of eGFR within 1 year of KT. This study is the first to examine the effects of actual eGFRs within 1 year on graft loss. Using multivariate Fine–Gray competing model analysis, actual eGFRs at 6 and 12 months after LDKT, preformed DSA, and male recipient were shown to be independent prognostic factors for graft loss. In previous studies, the graft survival of recipients with preformed DSAs was worse than that of those without because of antibody-mediated rejection (AMR). However, desensitization was performed to prevent acute AMR (28, 29, 31). Although many clinical studies on desensitization using intravenous immunoglobulin, rituximab, plasmapheresis, and imlifidase have been conducted to improve the graft survival of recipients with preformed DSAs, no desensitization regimen for preformed DSAs has been established (36–39). Here, the recipients with preformed DSAs were desensitized using rituximab, plasmapheresis, and intravenous immunoglobulin administration. However, these desensitization procedures were ineffective in improving graft survival. Consistent with previous studies, male recipient was also found to be an independent prognostic factor for graft loss (40, 41). This study is novel because it investigated the impact of the actual eGFRs on graft loss within 1 year after LDKT. Moreover, no studies have investigated the impact of the actual eGFRs at 1, 2, and 3 weeks and 1, 3, and 6 months after LDKT on graft survival (2, 10–15). Therefore, this study revealed that actual eGFRs within 3 months after LDKT could not be an independent prognostic factor for graft loss.

Notably, the prediction models for ideal eGFRs at 1, 2, and 3 weeks after LDKT and the ideal best eGFR within 3 weeks were developed using 10-fold cross-validation and stepwise multiple regression model analysis. Ideal KTs were selected by excluding problematic donors and recipients during the perioperative period. Finally, data from 676 recipients were used to develop prediction models for ideal eGFRs during this period. This study is novel because no studies to date have investigated prediction models for ideal eGFRs during the perioperative period. During the development of the prediction models, the trough levels of tacrolimus and extended-release tacrolimus were separately presented, as they were found to be significantly different when the same dose was administered in a previous study (42).

Overfitting of the model was prevented using 10-fold cross-validation (43, 44). The predicted ideal best eGFR/actual best eGFR within 3 weeks after LDKT and predicted ideal eGFRs/actual eGFRs at 1, 2, and 3 weeks after LDKT were obtained in 1174 recipients to investigate the impact of ideal eGFRs/actual eGFRs on graft loss. In the multivariate Fine–Gray competing model analysis, covariates that were independent prognostic factors in the univariate Fine–Gray competing model analysis were used as follows: male recipient; preformed DSA; preoperative desensitization; donor age; predicted best eGFR/actual best eGFR within 3 weeks after LDKT; and predicted ideal eGFRs/actual eGFRs at 1, 2, and 3 weeks after LDKT. In the multivariate Fine–Gray competing model analysis, in addition to the predicted ideal best eGFR/actual best eGFR within 3 weeks after LDKT and predicted ideal eGFRs/actual eGFRs at 1, 2, and 3 weeks after LDKT, male recipient, preformed DSA, and donor age were independent prognostic factors for graft loss (3, 28, 29, 31, 40, 41). In this analysis, male recipient and preformed DSA were the prognostic factors for graft loss, similar to those indicated by the multivariate analyzes for the impact of actual eGFRs on graft loss. Additionally, donor age was found to be an independent prognostic factor for graft

loss. This result is consistent with those of previous studies indicating that graft loss may occur more frequently when the graft is transplanted from elderly donors owing to donor age-related graft nephrosclerosis (3, 41, 45). Furthermore, the graft loss risk of the predicted ideal eGFRs/actual eGFRs adjusted with the multivariate Fine–Gray competing model analysis using male recipient, preformed DSA, and donor age as factors was significant. These results show that the graft loss risk increases as the predicted ideal eGFR/actual eGFR increases. Moreover, this implies that when recipients receive actual eGFRs that are lower than those of the predicted ideal eGFR, the graft survival may be worse than that of recipients who obtained better eGFRs than the predicted ideal eGFR. Although actual eGFRs at 1, 2, and 3 weeks after LDKT were not significant predictors for graft loss, those at 6 and 12 months after LDKT were significant predictors. This implies that we cannot predict graft loss based on the actual eGFRs at 1, 2, and 3 weeks after KT. The *P*-values in the multivariate Fine–Gray competing model analysis for graft loss, adjusted for male recipient, preformed DSA, preoperative desensitization, and donor age, decreased as the time after KT passed. This may imply that the widening disparities of the actual eGFR after KT between the graft loss and non-graft loss groups did not contribute to graft loss prediction until 6 months after KT. However, it may be useful to reveal the predictor for graft loss earlier and implement measures based on the results. Therefore, the predicted ideal eGFR/actual eGFR was successfully developed to make the eGFRs at 1, 2, and 3 weeks more useful predictors for graft loss. This finding enabled the detection of slightly widening disparities of eGFR after KT between the graft loss and non-graft loss groups, which could not be detected using the actual eGFR after KT.

Many factors might prevent recipients from obtaining an ideal eGFR during the perioperative period, including rejection, delayed graft function, and operative complications (7, 46, 47). However, the period of 3 weeks after LDKT is early to optimize immunosuppression, treat comorbidities, and detect and treat surgical complications, including graft vascular stenosis and urinary tract obstruction. Therefore, to obtain the ideal eGFR, preventing rejection, delayed graft function, and intraoperative surgical complications, which may negatively affect graft function within 3 weeks after LDKT, might be crucial. These results are novel because long-term graft survival can be predicted using prediction models for perioperative ideal eGFRs. Accordingly, this study demonstrated the importance of obtaining an ideal eGFR during the perioperative period.

Furthermore, considering the recent advancements in artificial intelligence technology, the development of tools for predicting eGFR after KT using data from large-scale multicenter studies is expected. Therefore, this study's results, which suggest the potential utility of a predictive tool for the ideal eGFR rather than relying solely on the actual eGFR, can lead to the advancement of innovative studies in the field of KT.

The retrospective design of the study and the fact that it was conducted in a single institution to examine the impact of predicted eGFRs on graft survival were limitations of this work. Therefore, a prospective multicenter randomized study focusing on the effects of predicted ideal eGFRs/actual eGFRs on graft survival should be conducted to verify the results and elucidate the causes of failure to obtain ideal eGFRs.

In conclusion, the predicted ideal eGFRs/actual eGFRs at 1, 2, and 3 weeks after LDKT and the predicted ideal best eGFR/actual best eGFR within 3 weeks may forecast graft survival after adult LDKT. Therefore, obtaining an ideal perioperative eGFR is crucial for improving long-term graft survival.

Data availability statement

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

Ethics statement

The studies involving humans were approved by Nagoya Daini Red Cross Hospital's Institutional Review Board (Aichi, Japan; approval number: 1504). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

TH designed and acquired the data, interpreted the results, and drafted the manuscript. YH, MO, YM, AT, and TK acquired the data. KF, NG, TI, and SN interpreted the results. KU and YW approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1187777/full#supplementary-material

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ABO-incompatible living donor kidney transplantation failure due to acute blood group antibody-dependent rejection triggered by human parvovirus B19 infection: a case report and literature review

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Background: With the improvement of immunosuppressive regimens, the success rate and availability of ABO-incompatible (ABO-i) kidney transplantation (KT) have gradually increased. However, the management of immunosuppression protocols and complications associated with ABO-i KT is complex. Here, we report a clinical case of ABO-i living donor KT with allograft dysfunction caused by acute blood group antibody-dependent rejection triggered by human parvovirus B19 (B19V).

Case report: The ABO blood group of the recipient was O, and that of the donor was B. The recipient had high baseline anti-B antibody titers (IgM, 1:1024; IgG, 1:64). Before transplantation, he completed a desensitization protocol comprising plasma exchange, double-filtration plasmapheresis, and rituximab, which maintained a low blood group antibody level and resulted in successful transplantation. Two weeks after surgery, the recipient developed a B19V infection combined with acute T-cell-mediated rejection. After the anti-rejection regimen, acute rejection (AR) was successfully reversed, but B19V persisted. One week after AR stabilization, the patient experienced acute antibody-mediated rejection that was more severe and refractory, resulting in the loss of the transplanted kidney.

Conclusion: Desensitization combined with immunosuppressants can lead to overimmunosuppression and cause various infections. Infections could break the accommodation state of the patient, thereby inducing AR and resulting in the loss of the transplanted kidney.

KEYWORDS

ABO incompatibility, kidney transplantation, acute rejection, living donor, B19V infection, accommodation

1 Introduction

Kidney transplantation (KT) is the best replacement therapy for patients with end-stage kidney disease. The shortage of deceased donor kidneys and long waiting times have led to a gradual increase in the proportion of living donor transplantations. Owing to the gradual maturation of appropriate immunological preparations to remove blood group antibodies and suppress their generation, ABO-incompatible (ABO-i) KT has been performed (1). ABO-i KT has been compared with ABO-compatible KT at several centers worldwide, and several studies have shown no significant differences in patient survival or graft survival of these two procedures (2). However, other studies have shown that ABO-i transplant recipients are at higher risk for serious infections and thrombotic microangiopathy (TMA) (3–5). Moreover, the incidence of antibody-mediated rejection (AMR) during the first 3 months is 58% for ABO-i transplant recipients (6). Among these patients, acute AMR (aAMR) is the primary cause of allograft failure with solid organ transplantation (7). Despite adequate desensitization therapy, anti-ABO antibodies may rebound after ABO-i KT.

If anti-ABO antibody titers are successfully neutralized during the first 3–4 weeks, then the transplanted kidney may establish a post-transplant state of accommodation, the graft will maintain its normal function in the presence of anti-ABO antibodies and complement, and anti-ABO antibody-mediated graft injury may not occur (8–10). However, excessive immunosuppression caused by high doses of immunosuppressants targeting T and B lymphocytes during desensitization and the early post-transplantation period increase the risk of infection of ABO-i KT recipients, which may break the accommodation state, thereby largely increasing the risk of AMR and eventually leading to serious graft impairment or even loss of function (4, 11). Although successful ABO-i KT has been performed, it is important to summarize the reasons for ABO-i living donor KT (LDKT) failure caused by complex factors.

2 Case description

The LDKT recipient was a 34-year-old man (height, 174 cm; weight, 68 kg; blood group, O). The patient's primary nephropathy was chronic glomerulonephritis, and he had been on regular dialysis for >5 years. Before transplantation, donor-specific anti-human leukocyte antigen (HLA) antibodies and panel-reactive antibodies (PRA) were negative. The recipient's mother was the donor (age, 64 years; height, 157 cm; weight, 60 kg; blood group, B). Complement-dependent cytotoxicity cross-matches and flow cytometry cross-matches were negative. The recipient underwent ABO-i and HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DP, and HLA-DQ 4/12 mismatched KT. The baseline anti-B antibody titers of the recipient before transplantation were 1:1,024 (IgM) and 1:64 (IgG) (Figure 1). The donor volunteered to donate a kidney to her son and provided written informed consent. This study was approved by the ethics committees of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, and the Health Commission of Hubei Province.

The recipient was admitted to the hospital 3 weeks before surgery. We formulated and started a desensitization protocol before transplantation. First, the recipient was intravenously administered 100 mg of rituximab on day 15 before surgery. Immediately thereafter, tacrolimus (TAC) 0.1 mg/kg/day and mycophenolate mofetil (MMF) 1,500 mg/day were administered orally in two doses. Subsequently, the immunosuppressive doses were adjusted according to the TAC trough level and the area under the curve at the time of MMF. The TAC trough level before surgery fluctuated around 8 ng/mL, and the area under the curve at the time of MMF was controlled at 60 μg/h/mL, which inhibited the activation and proliferation of T and B lymphocytes and the levels of blood group antibodies. Simultaneously, three courses of plasma exchange (PE) and one course of double-filtration plasmapheresis (DFPP) were administered to remove blood group antibodies. According to the protocol, transplantation was considered when anti-B titers were less than 1:16 for 2 consecutive days. On the day of transplantation, the anti-B titers were reduced to 1:16 (IgM) and 1:2 (IgG) (Figure 1).

The donor's left kidney was subjected to warm ischemia for 1 min and promptly transplanted to the recipient. The allograft was functionally perfect after reperfusion. Perioperative immunosuppression included induction therapy with anti-human T-lymphocyte porcine immunoglobulin and methylprednisolone and triple maintenance therapy with TAC, MMF, and prednisone (intravenous porcine immunoglobulin, 500 mg, days 0–4; intravenous methylprednisolone, 500 mg, days 0–2; and oral prednisone, 50 mg, day 3). Oral prednisone was subsequently tapered by 10 mg every day to 10 mg/day for maintenance. The preoperative dosing regimens of TAC and MMF were continued. The postoperative TAC trough level was maintained at approximately 8 ng/mL. The renal function of the recipient improved postoperatively. Serum creatinine decreased from 1,125 μmol/L before surgery to 159 μmol/L on postoperative day (POD) 11. The IgG and IgM anti-B titers remained low throughout the postoperative course (1, 2). The patient was discharged after an uneventful course on POD 12.

On POD 17, the patient presented with fever and a progressive decrease in hemoglobin level to 73 g/L. Excluding other causes, the patient was readmitted to the hospital because of high suspicion of human parvovirus B19 (B19V) causing pure red cell aplasia. His serum creatinine level had increased to 514 μmol/L and was accompanied by positive B19V IgM and DNA and a sharp decrease in urine volume to 950 mL/day. The TAC trough concentration was 2.9 ng/mL; however, there was no increase in antibody titers (anti-B IgM and IgG titers of 1:8 and 1:2, respectively). Furthermore, graft perfusion had reduced, and the arterial resistance index had increased on Doppler ultrasonography. Therefore, the patient was preliminarily considered to have B19V and acute rejection (AR). To further assess the condition of the allograft, we immediately performed a graft biopsy. Pathological findings confirmed grade IA mild acute T-cell-mediated rejection (aTCMR) (Banff 2019 grade IA, i1, t1, g0, v0, ci0, ct0, cg0, cv0, ptc1, g + ptc = 1, ah0, mm0, i-IFTA0, t-IFTA0, and diffusely positive C4d3) (Figure 2A), with only a few minor peritubular

Abbreviations: ABO-i, ABO-incompatible; KT, Kidney transplantation; LDKT, Living donor kidney transplantation; B19V, Parvovirus B19; aTCMR, acute T-cell-mediated rejection; AR, acute rejection; aAMR, acute antibody-mediated rejection; TMA, Thrombotic microangiopathy; AMR, Antibody-mediated rejection; HLA, Donor-specific anti-human leukocyte antigen; PRA, Panel-reactive antibodies; TAC, Tacrolimus; MMF, Mycophenolate mofetil; PE, Plasma exchange; DFPP, Double filtration plasmapheresis; POD, Postoperative day; IVIG, Intravenous immunoglobulin.

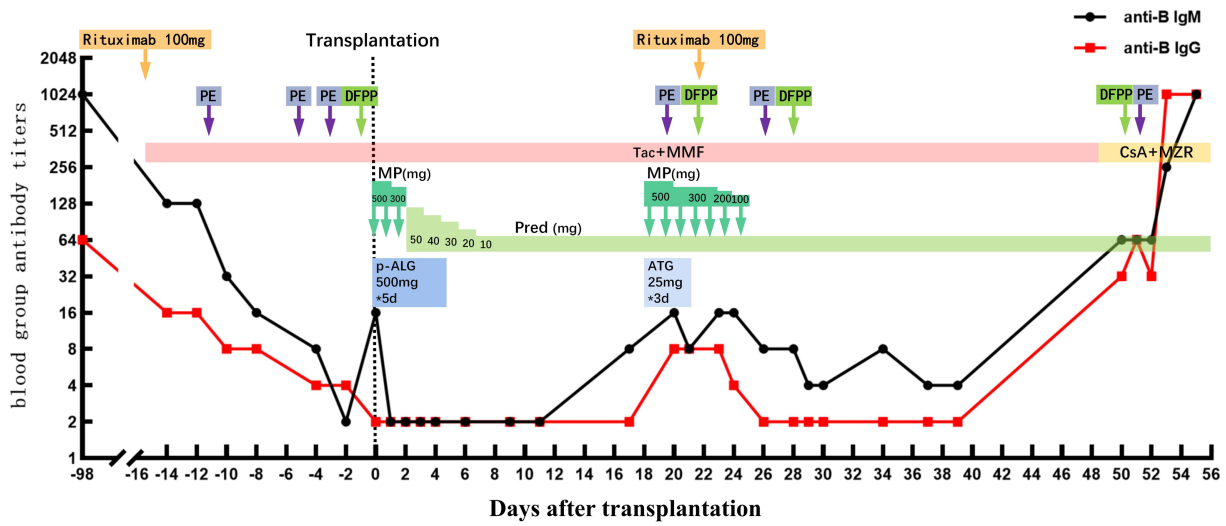

FIGURE 1
Blood group antibody titers and immunosuppressive regimen used before and after transplantation. The recipient's ABO blood group was O and the donor's blood group was B, and the recipient's baseline anti-B antibody titers were 1:1,024 (anti-B IgM) and 1:64 (anti-B IgG), respectively. The recipient completed a pre-transplant desensitization protocol, whereafter anti-B antibody titers remained at low levels (equal or less than pre-transplant level 1:2) or declined to an undetectable level. Until POD 50, the recipient's anti-B IgM and anti-B IgG had increased to 1:64 and 1:32, respectively, and then gradually increased to untreated pre-transplant levels. The pink rectangle indicates tacrolimus (Tac) and mycophenolate mofetil (MMF). The yellow rectangle indicates the change of cyclosporine (CsA) and mizoribine (MZR) on POD49. The orange arrows indicate days of using rituximab. The purple arrows indicate days of using plasma exchange (PE). The grass green arrows indicate days of using double filtration plasmapheresis (DFPP). The dark green arrows indicate days and dose of using methylprednisolone (MP). The green rectangle indicates days and dose of using prednisone (Pred), staring at 50 mg/d and then prednisone tapered by 10 mg every other day to 10 mg/d for maintenance. The dark blue and light blue boxes indicate the dose and days of using anti-human T lymphocyte porcine immunoglobulin (p-ALG) and rabbit anti-human thymocyte immunoglobulin (ATG). The black dashed line indicates the day of transplantation.

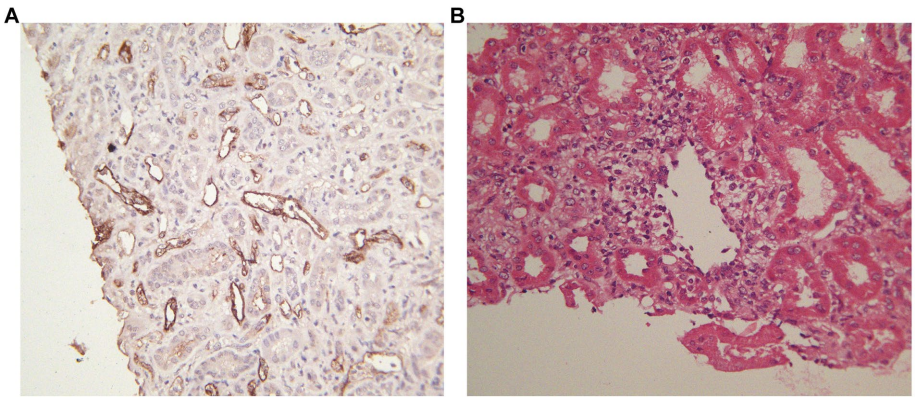

FIGURE 2
Allograft biopsy specimen obtained on POD 18. **(A)** Immunohistochemical staining: C4d was diffusely positive (×200). **(B)** One micro-vein presented with venous endotheliitis, mild renal interstitial edema, patchy and diffuse infiltration of lymphocytes in the interstitium, patchy and mild renal tubulitis, and a few peritubular capillaritis; the interstitial matrix of renal tissue did not show hyperplasia and tubular atrophy; mild water degeneration of renal tubular epithelial cells and no necrosis of renal tubular epithelial cells (H.E, ×400).

capillaritis (ptc1) in the allograft biopsy sample and no manifestation of glomerulonephritis (Figure 2B). The B19 DNA in paraffin-embedded kidney tissue was amplified by nested PCR, and the results were negative. Methylprednisolone (500 mg for 2 days, 300 mg for 3 days, 200 mg for 1 day, and 200 mg for 1 day) and rabbit anti-human thymocyte immunoglobulin pulse therapy (25 mg for 3 days) were immediately administered. The patient promptly received intravenous immunoglobulin (IVIG) at 20 g/day for 14 days. Red blood cell transfusion was simultaneously performed for pure red cell aplasia. Subsequently, hemoglobin levels gradually increased (Figure 3).

On POD 19, the anti-B IgM and IgG titers were 1:16 and 1:8, respectively, showing a rebound trend. To avoid injury caused by anti-B antibodies that trigger aAMR, the patient received 100 mg rituximab intravenously and four courses of PE or DFPP (Figure 1). The antibody titer did not increase continuously. The urine volume gradually returned to more than 2,000 mL/day, and the serum

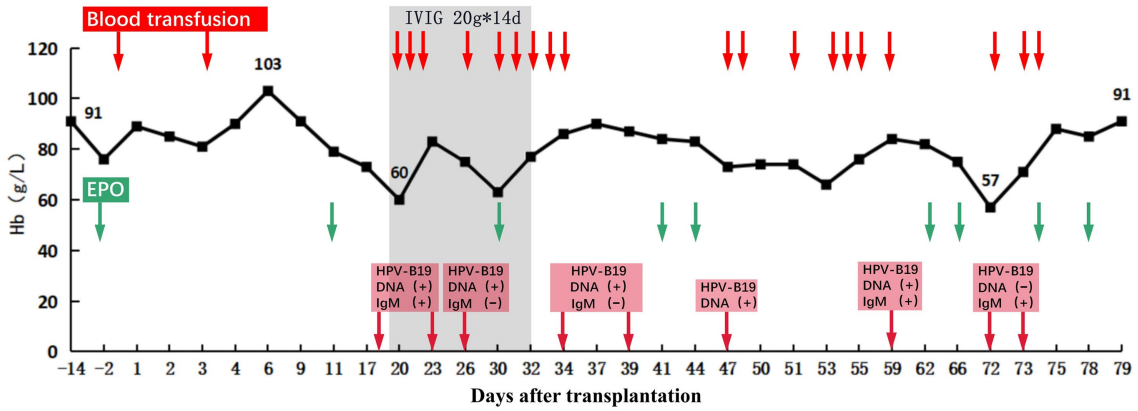

FIGURE 3
Hemoglobin levels before and after transplantation. After transplantation, the recipient's hemoglobin increased to 103 g/L at the highest level and then showed a progressive decrease in hemoglobin without obvious cause, which reached as low as 60 g/L on POD 19. After treatment, the recipient's hemoglobin (Hb) recovered and remained stable. The red arrows indicate days of using transfusion. The Green arrows indicate days of using recombinant human erythropoietin (EPO). The red squares indicate the results of HPV-B19 DNA and IgM testing, and the arrow points to the time of testing.

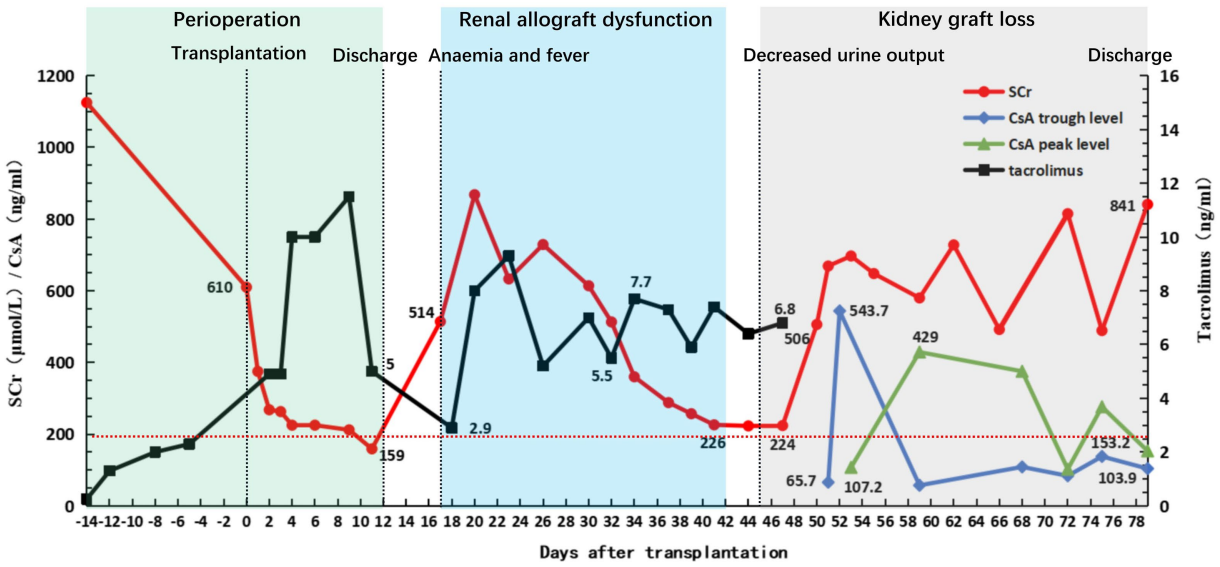

FIGURE 4
Clinical course of the patient. Graft function and fluctuation of immunosuppressant concentration before and after transplantation. The green square indicates the patient's first hospitalization and discharge with good recovery of graft function (with serum creatinine (SCr) rapidly declining to 200 μmol/L within 10 days post-transplantation). The blue square indicates that the patient was readmitted for treatment due to HPV-B19 infection and aTCMR, after which the patient improved following a series of treatments. The gray square indicates the patient's injury of graft function following a fierce anti-blood group antibody-mediated acute humoral rejection until the graft was lost and the patient was discharged again after resuming regular hemodialysis therapy.

creatinine level decreased to 226 μmol/L. The TAC trough concentration was 7.4 ng/mL (Figure 4), and the anti-B titers decreased to 1:4 (IgM) and 1:2 (IgG). Allograft perfusion was adequate, and the arterial resistance index was within the normal range, as evaluated by Doppler ultrasonography. Therefore, we believe that aTCMR was reversed after treatment.

As the patient's condition improved, we assumed that the graft function improved as well. He recovered well; however, the urine volume gradually decreased on POD 45 and the serum lactate dehydrogenase (LDH) level significantly increased to 269 U/L and the platelet level was 40 × 109/L (the preoperative serum LDH level was 126 U/L). Platelet-boosting therapy was given at this time. Repeated qualitative tests to determine B19V DNA yielded positive results. TAC was replaced with cyclosporine A as antiviral therapy on POD 48. On POD 50, the serum creatinine and serum LDH levels increased sharply to 506 mol/L and 356 U/L, the platelet level was 83×10^9/L, and anti-B titers increased to 1:64 (IgM) and 1:32 (IgG). Doppler ultrasonography revealed swelling, enhanced parenchymal echo, and sparse blood flow in the transplanted kidney; therefore, renal artery and vein embolisms were ruled out. Donor-specific anti-HLA

antibodies and PRA tests yielded negative results. This indicated that the subsequent AR was more aggressive. The patient immediately underwent two courses of PE or DFPP to remove the antibodies. We recommended another renal allograft biopsy, but the patient refused. On POD 52, Doppler ultrasonography revealed no blood supply to the renal allograft. Subsequently, the serum creatinine level increased to 697 μmol/L, and the anti-B antibody titers promptly increased (IgM, 1:1024; IgG, 1:256) (Figure 1). As the calcineurin inhibitors and lymphocyte function indicators were within reasonable ranges, we concluded that the patient had experienced an acute blood group antibody-mediated humoral rejection of the allograft and that kidney function had deteriorated drastically to an irreversible state. The patient had received salvage therapy for the transplanted kidney and returned to conventional hemodialysis.

3 Discussion

Here, we report a clinical case of renal allograft loss due to severe acute humoral rejection mediated by anti-blood group antibody after ABO-i living-related renal transplantation, probably triggered by B19V infection. Based on our current knowledge, no similar cases have been reported to date. Both inadequate and excessive immunosuppression can result in cascade reactions such as rejection and infection. The patient featured higher baseline anti-B titers (IgM 1:1024; IgG 1:64), which undoubtedly increased the frequency of preoperative PE or DFPP and the intensity of immunosuppression, and therefore, the patient lost more immunoglobulins (12). Meanwhile, high-intensity immunosuppression during desensitization therapy and in the early post-transplant period increased the risk of infection in ABO-i KT recipients. Furthermore, there has been controversy regarding the high AMR rate and early graft loss in recipients with high baseline blood group antibody titers (13). However, in any case, immunosuppressive regimens used to remove high baseline blood group antibody titers could result in a higher risk of infection (14, 15). High baseline anti-B titers in patients may contribute to the loss of the transplanted kidney. One week after transplantation, the recipient experienced decreased hemoglobin levels with no apparent trigger. Thereafter, B19V was diagnosed, implying excessive immunosuppression. Because B19V mainly manifests as pure red cell aplasia (16), the patient experienced markedly decreased hemoglobin and a significantly decreased TAC trough concentration. AR may be related to insufficient immune strength caused by the reduction and replacement of immunosuppression after B19V infection (17, 18).

Postoperative week 2 involves a high incidence of AR and is a high-risk period for ABO-i KT recipients (19). Results of the pathological biopsy performed 2 weeks after the operation in this recipient indicated mild aTCMR, with peritubular capillaritis with diffuse C4d deposits (C4d3) in the kidney allograft, which usually predict aAMR. Despite diffuse C4d peritubular capillary deposition, the transplanted kidney can establish a state of post-transplant accommodation in which the allograft maintains the normal long-term function of the host without AR injury (11, 20). Therefore, C4d staining cannot be used to diagnose AMR in ABO-i KT recipients (21, 22). Ultimately, we adopted efficacious anti-rejection therapies that successfully reversed aTCMR, and the recipient recovered from the initial renal graft function impairment.

aAMR is a major cause of graft dysfunction. It contributes to solid organ transplantation failure (7) and is mediated by preexisting and *de novo* antibodies, including major HLA, minor non-HLA, and A/B blood group antibodies. Two types of aAMR with ABO-i KT have been suggested (13). The first type of aAMR is caused by repeat sensitization to ABO antigens. If antibody production, including that of memory lymphocytes, is insufficiently inhibited, then ABO antigens induce a secondary immune response. This leads to an explosive production of antibodies, culminating in violent aAMR, which usually manifests as an increase in IgG titers accompanied by a parallel increase in IgM titers (23). When this occurs, there is no response to currently available therapies, ultimately resulting in graft loss. The other type of aAMR is attributed to primary sensitization caused by ABO antigens. This type of aAMR is associated with increased serum IgM titers. This type of aAMR progresses more slowly and is less severe. Patients with this type of aAMR respond well to treatment and have good graft survival and renal function (24). However, it is unclear which type of ABO antibody (IgM or IgG) is more clinically important in ABO-i KT. The antibody response to non-protein antigens primarily depends on IgM production. IgM is more capable of complement fixation and activation than IgG. Studies that relied on the molecular characteristics of ABO antigens and evaluated the clinical significance of anti-A/B IgM and IgG in ABOi KT suggested that anti-A/B IgM might play a critical role in AMR (25). This case appears to be consistent with a type of aAMR caused by repeat sensitization to ABO antigens, which may have been the ultimate cause of graft loss.

However, a condition known as accommodation during the post-transplantation period, which can be broadly defined as the absence of allograft injury despite the presence of anti-donor antibodies in the recipient, has been described. It is generally believed that the ABOi KT enters this stabilization period after 2 weeks. There is no blood group antibody rebound or AMR, and kidney function remains stable for a long time (11). In ABOi transplants, the rebound of A/B blood group antibodies after transplantation without any pathological manifestation of rejection is indicative of the state of accommodation, and similar phenomena with xenograft transplants have been observed (26, 27). The second occurrence of rejection in the recipient was characterized by rapid and severe acute blood group antibody-dependent rejection. We considered this to be related to the collapse of the postoperative accommodation state (10). Accommodation breakdown may be associated with high preoperative baseline antibody titers and postoperative B19V onset (28).

B19V has a tropism for renal endothelium (16) and may have direct cytopathic effects on glomerular epithelial cells or endothelial cells and glomerular deposition of immune complexes (29, 30). Kidney injury caused by B19V often results in pathological manifestations, such as focal segmental glomerulosclerosis, collapsing glomerulopathy, endocapillary proliferative glomerulonephritis, and thrombotic microangiopathy (31–35). There are no specific antiviral therapies for B19V. Reductions in the intensity of immunosuppression and IVIG constitute the cornerstone of therapeutic management of B19V after renal transplantation (36). However, both treatments may cause a rebound of blood antibody titers (37, 38). Inadequate immunosuppression may induce reactivation of T and B lymphocytes, thus inducing *de novo* blood group antibodies and resulting in titer rebound. However, there is evidence of a certain level of blood group antibodies remaining in IVIG because of manufacturing processes,

which may lead to increased blood group antibody levels in the ABO-i KT recipients after infusion (38). This recipient was treated with IVIG products from two manufacturers; therefore, we retrospectively measured the anti-B titers of the same batches of IVIG from these manufacturers. However, both had low antibody titers (Shandong Taibang Biological Products Co., Ltd.: IgM <1:2 and IgG = 1:4; Harbin Baishi Huike Biopharmaceutical Co., Ltd.: IgM < 1:2 and IgG = 1:2). Additionally, because there was no significant increase in the recipient's blood group antibody titers during IVIG infusion, the sharp rebound in anti-B titers is unlikely to have originated from IVIG infusion.

After our first successful reversal of aTCMR, we reduced the frequency of blood group antibody monitoring. The serum LDH level was more than twice that of the pre-transplantation level. The recipient also experienced a decreased platelet count. However, this was not considered during this case management. We overlooked the fact that ABO-i KT is more likely to cause TMA, which is a rare but severe complication after transplantation (37). TMA is characterized by rapidly progressive renal graft dysfunction and often has a poor prognosis. The diagnostic criteria for TMA include a postoperative lactate dehydrogenase level more than 2-fold higher than the baseline level, anemia or the need for transfusion therapy, and decreased platelet count ($<50\times10^9$/L or < 50% reduction). Additionally, viral infections and AMR are risk factors for TMA (29, 39–41). However, we did not consider the possibility of this adverse event. aAMR is a common and important cause of *de novo* TMA after transplantation, and sometimes both co-exist (42). As TMA was included in the diagnosis of aAMR and according to the Banff criteria, microvascular inflammation is also a criterion of aAMR (43). A study by Tasaki et al. revealed that all TMA cases were biopsy-proven aAMR and that TMA occurred with increasing antibody titers in these recipients whose grafts showed aAMR (3). However, we speculated that allograft loss in this recipient may have been linked to TMA because of the lack of novel transplant biopsy evidence to support this inference.

It has been hypothesized that B19V infection can lead to allograft dysfunction and acute or chronic allograft rejection through direct cytopathic effects and immune responses (32, 44–46). It is difficult to determine a causal relationship between B19V and allograft rejection or dysfunction. Nevertheless, it has been suggested that B19V targets the endothelium, which acts as an antigen-presenting cell during infection, with overexposure to major histocompatibility complex class II and the activation of acquired immunity, ultimately leading to humoral reactions, which lead to AMR and the production of donor-specific anti-HLA antibodies (29, 47, 48). Some researchers believe that endothelial cell injury caused by B19V and the subsequent sensitization of the glomerular endothelium may increase the incidence of AMR (41). Reducing the impact of the direct and indirect effects of B19V after renal transplantation is important to improve graft survival and function.

Cases of B19V after KT with allograft loss or dysfunction and rejection have been reported (30, 46, 49–51); however, literature on this aspect is limited. The first report of B19V after renal transplantation was published in 1986 (52). Zolnourian et al. (31) implicated B19V as a cause of AR and graft loss. To the best of our knowledge, there have been two cases similar to our case in China (unpublished). AMR induced renal function loss caused by persistent B19V after renal transplantation that pathologically manifested as TMA. Persistent B19V may increase the AMR incidence and is associated with a higher risk of chronic graft dysfunction (30, 53). The characteristics of the cases of B19V-triggered rejection episodes are presented in Table 1. Our case of ABOi KT involved allograft dysfunction secondary to aAMR triggered by B19V and highlights the

TABLE 1 Characteristics of the cases of B19V-triggered rejection episodes.

Study	Year	Country	Patients (n)	B19V detection	Clinical manifestation	Renal allograft pathology	Patient outcome
Barzon et al. (30)	2009	Italy	Kidney transplant patients (7)	PCR	AR (7) fever, rash, and hyporegenerative anemia (1)	AR (7) High copy numbers of B19V DNA (7) C4d-positive aAMR (1) TMA (1)	Graft survival (6) Acute graft dysfunction (1)
Zolnourian et al. (31)	2009	Northern Ireland.	Kidney transplant patients (1)	lgM, lgG, and PCR	AR (1)	Acute vascular rejection (1)	Graft failure (1)
Murer et al. (35)	2000	Italy	Kidney transplant patients (1)	lgM, lgG, and PCR	AR, fever, fatigue and arthralgia, aplastic anemia, and thrombocytopenia (1)	TMA (1) Histologic rejection (1)	Graft survival (1)
Eid et al. (49)	2006	US	Simultaneous kidney and pancreas transplant patients (1)	lgM and lgG	Chronic rejection, PRCA, and leukopenia (1)	Chronic rejection (1)	Graft failure (1)
Knysak et al. (50)	2020	Poland	Second kidney transplant patients (1)	lgM, lgG, and PCR	AR and PRCA (1)	aAMR (1) acute tubular necrosis (1) BKV infection (1)	Graft survival (1)
Ki et al. (51)	2005	Korea	Kidney transplant patients (7)	PCR	AR (7) PRCA (2)	AR (7)	Graft survival (6) Graft failure (1)
Bertazza et al. (53)	2023	Italy	Kidney transplant patients (15)	PCR	AR (15)	aAMR (8) aTCMR (7)	Graft survival (15)

AR, acute rejection; PRCA, pure red cell aplasia; TMA, thrombotic microangiopathy; aAMR, acute antibody-mediated rejection; aTCMR, acute T-cell-mediated rejection.

importance of including B1V9 in the diagnostic evaluation of graft failure after KT. This recipient was infected with B19V in the early postoperative period and aTCMR occurred at the same time. The treatments for AR and B19V infection are diametrically opposed, with the former requiring stronger immunosuppressive treatment and the latter requiring less immunosuppressive treatment. Although aTCMR was successfully reversed, the B19V infection continued, which led to the state of accommodation to break down and trigger aAMR, which resulted in the rapid deterioration of the transplanted renal function of the recipient and graft loss.

In conclusion, although ABOi KT has been widely developed, managing immunosuppressive regimens and postoperative complications is more complex than managing ABO-compatible KT. Both inadequate and excessive immunosuppression could induce rejection and infection, respectively. The treatment of infections combined with rejection and immunosuppressive therapy is challenging. The risk of graft loss increases when the accommodation status after transplantation is broken or aAMR is triggered by repeat sensitization to ABO antigens. These results should be comprehensively analyzed, and viral infections caused by excessive immunosuppression should be avoided. When blood group antibody titers rebound, it should be determined whether the recipient was in the accommodation state or whether aAMR was triggered by ABO antigens. The latter may lead to the irreversible loss of the kidney allograft. Based on this case, we believe that the postoperative hospitalization period should be appropriately extended for ABOi KT recipients to allow them to safely overcome the postoperative high-risk period. The blood group antibody titers and immune function status should be monitored for 3 months after surgery. Patients with high baseline blood group antibody titers, anti-A/B titers, and various virological parameters, especially B19V, should be closely monitored after transplantation.

Data availability statement

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

Ethics statement

The donor-recipient relationship is mother-to-son. The donor volunteered to donate a kidney for her son, and all of the donor's immediate family members signed a written informed consent form before the operation. The living-related kidney transplantation was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, and the Ethics Committee of Health Commission of Hubei Province, China. Written informed consent to participate in this study was provided by the participants. 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

SoC, W-jZ, ShC, and Z-yZ performed the surgery. L-rD wrote the manuscript. SC and X-hW revised and edited the manuscript. Y-bH performed the flow. All authors participated in patient management and data collection and contributed to the article and approved the submitted version.

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Conflict of interest

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

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1195419/full#supplementary-material

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Tacrolimus monitoring in hair samples of kidney transplant recipients

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Background: Calcineurin inhibitors, including tacrolimus, remain a cornerstone of immunosuppressive therapy after kidney transplantation. However, the therapeutic window is narrow, and nephrotoxic side effects occur with overdose, while the risk of alloimmunization and graft rejection increases with underdose. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) allows quantification of tacrolimus in biological samples from patients. This study investigates the feasibility of quantifying tacrolimus in scalp hair from kidney transplant (KT) recipients and correlates hair tacrolimus concentrations with tacrolimus dosage and blood trough levels. The aim was to provide proof-of-principle for hair tacrolimus drug monitoring in KT recipients.

Method: Single-center prospective study between September 9, 2021 and December 4, 2021, including KT recipients under tacrolimus. Minors, patients with active skin or hair diseases, and patients with scalp hair shorter than 4 cm were excluded from participation. Scalp hair was collected from the posterior vertex of patients, cut into segments, and analyzed for tacrolimus by LC-MS/MS. Patients filled out a questionnaire on hair treatments and washing habits. In parallel, tacrolimus trough levels were measured in whole blood and correlated with hair tacrolimus concentrations.

Results: In total, 39 consenting KT recipients were included, and hair samples were collected at 53 visits. Tacrolimus was detected in 98% of hair samples from patients exposed to the drug. Tacrolimus hair levels and whole blood trough levels were correlated with a beta coefficient of 0.42 (95% CI: −0.22–1.1, p = n.s.). Age and dark hair affected hair tacrolimus measurements, while different tacrolimus formulations (immediate release vs. extended release), hair washes, and permanent coloring did not. Longitudinal measurements in a subgroup of patients indicate that long-term measurement of hair tacrolimus levels is feasible.

Conclusion: Measuring tacrolimus in hair is a potentially reliable method to monitor drug exposure in KT patients. Rapid wash-in effects and consistent concentrations over time indicate that tacrolimus is incorporated into the hair matrix, allowing temporal resolution in the analysis of recent exposure and exposure history. This method provides a simple and low-risk alternative to regular blood sampling, sparing patients from frequent hospital visits through the self-collection of hair samples.

KEYWORDS

kidney transplantation, tacrolimus, C_0 level, mass-spectrometry, hair, trough levels

Introduction

Kidney transplantation (KT) is an effective treatment for advanced and end-stage kidney disease (1, 2). Although short-term outcomes have improved substantially over the last decades, long-term results are still unsatisfactory (3). The primary causes of allograft failure remain chronic antibody-mediated rejection due to relative under-immunosuppression and calcineurin inhibitor (CNI) toxicity. The latter reflects a common nephrotoxic side effect of CNI, namely, cyclosporine A (CsA) and tacrolimus (Tac) (4–6). While these agents represent a cornerstone in the treatment of solid transplant recipients, they have a narrow therapeutic range and pose a substantial toxicity risk if overdosed. In particular, the nephrotoxic effects of these drugs may lead to progressive allograft disease and premature graft failure (7). Furthermore, CNI elicits extra-renal side effects, including progressive cardio-vascular disease (8), vulnerability against infections, and risk for cancer (9), all of which contribute to increased morbidity and mortality in KT recipients (10).

In the past decades, much effort has been made to measure CNI exposure and to adjust treatment doses to pre-specified target CNI blood levels for individual patients (11, 12). Indeed, tailoring immunosuppressive therapy to each individual KT recipient is a good example of precision- and patient-centered medicine (13). Unfortunately, these efforts have not yet led to substantial breakthroughs since CNI blood levels only poorly correlate with toxicity and we cannot predict whether CNI toxicity will progress or not (14).

Drugs and metabolites can be analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) from biological matrices, including blood (15), hair (16), and nails (17). In forensic toxicology, retrospective quantification of chemicals in hair samples has gained widespread acceptance. Chemicals such as cocaine (18), ethyl glucuronide (19), and delta9-THC (20) are quantified to confirm abstinence in patients who are recovering from addiction (21, 22). Furthermore, long-term medication monitoring in hair is feasible, accurate, and predictive in specific clinical settings. For instance, tenofovir concentrations measured in hair samples can be readily used to monitor treatment adherence in HIV patients (23). However, although LC-MS/MS analysis of substances in hair is specific and sensitive, certain factors, such as hair products, hair washing routines, hair color, and artificial coloring, are known to significantly affect the results of hair analysis (20, 24, 25).

The aim of this trial was to quantify tacrolimus in the scalp hair of KT recipients and correlate concentrations with tacrolimus dosing and blood C_0 levels.

Methods

Study design and population

This study evaluates a subgroup of the Bernese transplant cohort. KT recipients on maintenance therapy with tacrolimus (Prograf®, Advagraf®, or Envarsus®) were screened and enrolled in the study during routine outpatient follow-up at the Nephrology Department of the University Hospital Insel in Bern between September 9, 2021 and December 4, 2021. Minors, patients without at least 4 cm long hair in their vertex, and patients with active skin or hair diseases were ineligible to participate. The study was approved by the Local Ethics Committee (2020-00953). All patients provided oral and written consent.

Clinical and laboratory parameters

Baseline characteristics and treatments were extracted from the electronic patient documentation. Information on hair color, care, and utilized hair treatment products was collected with a questionnaire. Tacrolimus concentration was determined 12 h after the last dose of immediate-release tacrolimus (Prograf®) and 24 h after the last dose of extended-release tacrolimus (Advagraf® or Envarsus®). The daily tacrolimus dose was recorded as a cumulative dose in mg per day. Serum creatinine was measured from plasma samples; eGFR was estimated according to the CKD-EPI equation (26) and expressed in mL/min/1.73 m^2.

Hair sampling and processing

Patients were allowed to provide hair specimens at multiple study visits. Specifically, a strand of hair with a diameter of 2–4 mm was cut at the base from the posterior scalp of participants. The end of the hair tuft adjacent to the scalp was marked. The bottom proximal 2 cm segment (S1) and the adjacent 2 cm segment (S2) of the specimens were segmented and used for further analysis. Hair specimens were cleaned, chopped into snippets, ground into a powder, and then utilized for mass spectrometry analysis. First, hair samples were cut into segments of exact length, and each segment was decontaminated with the following standard protocol for forensic hair analysis. The hair was washed once with 5 mL of deionized water and twice with 5 mL of acetone for 3 min each. After drying at room temperature, hair segments were chopped into snippets using scissors. For extraction, between 5 and 25 mg of snippets were exactly weighed into an Eppendorf vial, and the snippets were pulverized for 15 min at 30 Hz. Then, 100 µL of IS solution and 1,400 µL of methanol were added, and the samples were sonicated for 2 h at 40°C. After centrifugation for 10 min at 9,000 g, the supernatant clear solution was transferred to a vial for evaporation under a stream of nitrogen at 40°C. For injection into the LC-MS/MS system, the residue was reconstituted in 30 µL of methanol and 70 µL of 5 mM ammonium formate (pH 3) with 10% (v/v) of methanol.

Preparation of working solutions

Spiking solutions for calibrators and quality control were prepared in methanol to obtain concentrations comparable to those found in hair. As an internal standard (IS), a solution was prepared in methanol containing $13CD_4$-tacrolimus at a concentration of 800 pg/mg at a sample weight of 1 mg.

LC-MS/MS parameters

The LC–MS-MS system consisted of a Shimadzu Prominence high-performance liquid chromatography system (Shimadzu, Duisburg, Germany) and a QTrap 6500 mass spectrometer (Sciex, Darmstadt, Germany) using electrospray ionization (ESI) operating in positive mode. Separation was achieved using a Kinetex® F5 column (100 × 2.1 mm, 100 Å, 2.6 µm, Phenomenex) coupled with SecurityGuard™ ULTRA Cartridges ultra-high performance liquid chromatography (UHPLC) F5 (2.1 mm ID). A mobile phase A [water containing ammonium formate (1 mM) and formic acid (0.1%)] and a mobile phase B [acetonitrile containing ammonium formate (1 mM) and formic acid (1 mM)] were used. A post-column spray of methanol was applied with a flow rate of 0.04 mL/min to support the ionization process. The flow rate was set at 0.6 mL/min, and the gradient was programmed as follows: 0.01–1.5 min, 10% eluent B; 1.5–9 min increasing to 95% eluent B; 9–11 min, 95% eluent B; 11–11.1 min decreasing to 10% eluent B; and 11.1–12 min starting conditions (10% eluent B). The column oven was set at 40°C. The dead time (t0) was about 0.3 min (0.19-mL void volume of the column). The autosampler was operated at 15°C, and the autosampler needle was rinsed before and after aspiration of the sample using methanol. The mass spectrometry (MS) instrument was operated in the "Scheduled MRM™ Algorithm Pro" mode. Quantification was achieved by calculating the mean concentration of both transitions. MRM transitions and retention times of tacrolimus and $13CD_4$-tacrolimus (IS) are given in Supplementary Table 1. The following identification criteria were used: (1) the retention time (RT) between the analyte and the IS and (2) deviations ≤20% for the relative area ratios of the three transitions (MRM 1 to MRM 2 and MRM1 to MRM3, respectively).

Calibration curve and method validation

Three calibration concentrations (C1–C3) and a blind hair sample were prepared to establish the linearity of the calibration. Approximately 20 mg of tacrolimus-free hair was analyzed without or spiked at concentrations C1–C3. The regression was calculated using a linear model (Supplementary Table 2). The method was partially validated for the selected parameters, namely, selectivity, the lower limit of detection (LLOD), the lower limit of quantification (LLOQ), and linearity. Tacrolimus hair concentration (hC_0) was measured in picograms per sample (pg/sample) and normalized to the input weight, resulting in a hair tacrolimus concentration (pg/mg).

Longitudinal sampling

Drugs and metabolites are transported to the hair follicle via the bloodstream and permanently incorporated into the matrix. Over time and along with hair growth, the matrix moves away from the follicle and remains relatively inert in terms of component incorporation and washout. To test this notion, we analyzed hC_0 levels in the S1 segment of visit 1 (representing recent tacrolimus exposure) and the S2 segment of visit 2 (representing tacrolimus exposure 2–4 months ago). Furthermore, we compared hC_0 in S1 and S2 segments in two patients, one with recent tacrolimus withdrawal due to belatacept-conversion and one with recent tacrolimus exposure for *de novo* KT.

Statistical analysis

Results were reported as the number of participants (percentage) for categorical data and the median (interquartile range) for continuous data. To assess correlations between hC_0 and drug exposure, we employed a linear regression model with hC_0 as the dependent variable and daily dose (mg/day) as the independent parameter without (a crude model) or with potentially interfering patient-related (partial model) or cosmetic treatment-related cofactors (full model). Data were presented using histograms and xy-plots. The Pearson correlation coefficient between hC_0 and bC_0 (blood tacrolimus concentration) and the daily tacrolimus dose were calculated. A two-tailed *p*-value below 0.05 was considered statistically significant. Statistical analyses were performed using R (version 4.0.3) and R Studio (version 1.3.1093).

Results

Overall characteristics of participants and hair samples

The study cohort includes 39 KT recipients of the Bernese Transplant project. Baseline characteristics are given in Table 1. 62% of patients were female, had a median age of 53.1 years (IQR: 42.0–63.4), and had a median transplant history of 2.8 years (IQR: 0.4–6.9) at study inclusion. In total, 19% of patients suffered from glomerulonephritis as an underlying disease. A total of 24 patients (61%) were under immediate-release tacrolimus (Prograf®) and the remainder were under extended-release tacrolimus (Advagraf®, Envarsus®). In total, 74% were under low-dose prednisolone, and 83% were under antimetabolites (azathioprine, mycophenolate mofetil, or acetate). The average daily tacrolimus dose was 4.5 mg (IQR: 3–6) and the average trough (C_0) level was 6.2 ng/mL (IQR: 4.6–8.0). A total of 37 patients had been taking tacrolimus for at least 6 months prior to study entry; one patient started within 2 months before entry (recent KT); and one patient was switched to belatacept between two samplings. Characteristics of hair color and treatment are given in Table 2.

Tacrolimus trough (C_0) levels in hair and blood samples

Overall, 53 samples were collected. Eight patients participated twice, and three patients participated three times. Of the hair specimens collected, the median weight of S1 segments was 14 mg (IQR: 10–20) and S2 segments was 13 mg (IQR: 9–20). Overall, tacrolimus was detectable in 52/53 samples (98%) with a median concentration of 7.0 pg/mg (IQR: 3.5–11.0) in S1 and in 30/32 samples (93.8%) with a median concentration of 4.0 pg/mg (IQR: 2.0–6.5) in S2 (Supplementary Table 3; Supplementary Figure 1).

TABLE 1 Baseline characteristics of study population.

	Prograf $n = 24$	Advagraf $n = 11$	Envarsus $n = 4$	Overall $n = 39$	p-value
Sex (female)	15 (62%)	7 (64%)	2 (50%)	24 (62%)	>0.9
Age (years)	53.5 (42.5, 60.0)	50.2 (41.4, 60.8)	57.3 (44.0, 71.5)	53.1 (42.0, 63.4)	0.8
eGFR (mL/min/1.73 m^2)	49 (32, 62)	45 (40, 52)	63 (39, 73)	47 (32, 63)	0.9
Tac dose (mg/day)	5.0 (3.9, 6.0)	4.0 (2.5, 4.8)	4.5 (3.2, 5.0)	4.5 (3.0, 6.0)	0.2
KT history (years)	3.8 (0.4, 9.9)	2.4 (0.4, 3.5)	1.4 (0.7, 2.0)	2.8 (0.4, 6.9)	0.2
Tac C_0 (ng/mL)	7.1 (5.8, 9.2)	5.1 (4.1, 6.4)	7.0 (5.6, 8.7)	6.2 (4.6, 8.0)	0.093

Values are given as frequencies (percentages) or median values (interquartile range).
KT, kidney transplant; eGFR, estimated glomerular filtration rate; Tac C_0, tacrolimus trough levels.

TABLE 2 Characteristics of the acquired S1 samples comparing different tacrolimus formulations.

Characteristics	Prograf $N = 24$	Advagraf $N = 11$	Envarsus $N = 4$	Overall $n = 39$	p-value
Hair color					0.2
Blond	9 (38%)	6 (60%)	0 (0%)	15 (39%)	
Brown	8 (33%)	3 (30%)	1 (25%)	12 (32%)	
Black	3 (12%)	1 (10%)	1 (25%)	5 (13%)	
Gray	4 (17%)	0 (0%)	2 (50%)	6 (16%)	
Artificial coloring	6 (26%)	3 (30%)	0 (0%)	9 (24%)	0.7
Permanent structural alteration	1 (4.3%)	1 (10%)	1 (25%)	3 (8.1%)	0.2
Bleached	1 (4.2%)	0 (0%)	0 (0%)	1 (2.6%)	>0.9
Washes per week	3.0 (2.0, 7.0)	3.0 (2.1, 5.5)	3.0 (2.5, 3.5)	3.0 (2.0, 7.0)	>0.9

Correlation of tacrolimus trough levels in hair (hC_0), blood (bC_0), and daily dose

hC_0 was positively correlated with daily dose (beta coefficient 0.42 per mg tacrolimus, 95% CI: −0.22 to 1.1, $p = 0.2$) in the crude model and remained positively correlated with a similar coefficient after correction for age, sex, and drug formulation (partial model, beta 0.45, 95% CI: −0.14 to 1.0, $p = 0.13$). After additional correction for hair washes, permanent structural alteration, and dark hair color, the results remain unchanged (full model, beta 0.36, 95% CI: −0.27 to 1.0, $p = 0.3$). Tacrolimus formulation had no impact on the interaction between hC_0 and daily exposure (Table 3). Patient age negatively and dark hair positively influenced hC_0 values. hC_0 correlated with daily dose with a Pearson correlation coefficient of 0.203, while the correlation of bC_0 and dose was 0.186 (Figure 1).

Longitudinal hair tacrolimus concentrations

For five subjects in the study cohort with no change in tacrolimus medication, a hair sample was collected at visits 1 and 2, ~2 months apart. These hair samples were analyzed in segments. Assuming a hair growth rate of 1 cm/month, the proximal segment S1 of the visit 1 sample and the distal segment S2 of the later visit 2 sample represent approximately the same time period. The corresponding hC_0 values are shown in Figure 2A.

One patient with recent KT was started on tacrolimus at the time of the first visit. Both proximal S1 segments of visits 1 and 2 were compared, and tacrolimus was detected only at the second visit (Figure 2B). Conversely, one patient changed immunosuppressive treatment from tacrolimus to belatacept between the two visits. Comparison of hC_0 in the S1 segment at the two visits showed a decrease of 40% from the first measurement (Figure 2C).

Discussion

To the best of our knowledge, this is the first study assessing tacrolimus hair concentration in KT recipients and correlating results with patient-related and hair treatment-related cofactors. In the vast majority of patients, tacrolimus was detectable in the hair specimen collected from the vertex. The correlation of matrix levels (hair and blood) with daily tacrolimus exposure was rather low, yet higher in hair samples compared to blood. A correlation between hC_0 and bC_0 was not significant. The continuous deposition of tacrolimus in growing hair is supported by the analysis of patients with recent tacrolimus withdrawal and exposure. Together, these findings strongly support the assumption that tacrolimus is incorporated into the hair matrix via the bloodstream and thereafter remains detectable weeks to months after exposure, with only limited washout effects from hair washing and hair

TABLE 3 Linear regression models for hC_0 level in segment 1 with independent parameters.

	Crude model			Partial model			Full model		
	Beta	95% CI	*p*-value	Beta	95% CI	*p*-value	Beta	95% CI	*p*-value
Tacrolimus dose (mg/day)	0.42	−0.22, 1.1	0.2	0.45	−0.13, 1.0	0.13	0.35	−0.28, 0.98	0.3
Tacrolimus formulation (extended release)				0.03	−3.0, 3.0	>0.9	−1.4	−4.7, 1.8	0.4
Sex (female)				−1.3	−4.1, 1.6	0.4	−1.2	−4.8, 2.4	0.5
Age (per year)				−0.2	−0.29, −0.10	**<0.001**	−0.09	−0.22, 0.03	0.15
Washes (per week)							0.25	−0.43, 0.93	0.5
Hair color (brown/black)							3	−0.48, 6.6	0.088
Artificial coloring (yes)							2.2	−3.9, 8.2	0.5

Crude model: hC_0 in relation to daily tacrolimus dose. Partial model: hC_0 in relation to daily tacrolimus dose, tacrolimus formulation (extended vs. immediate release), sex, and patient age. Full model: Partial model and the parameters, namely, reported washes per week, hair color (dark hair vs. fair hair), and permanent treatment (yes). Beta coefficient, 95% confidence intervals (CI), and values are given. hC_0, hair tacrolimus level. Bold values are significant *p*-values (<0.05).

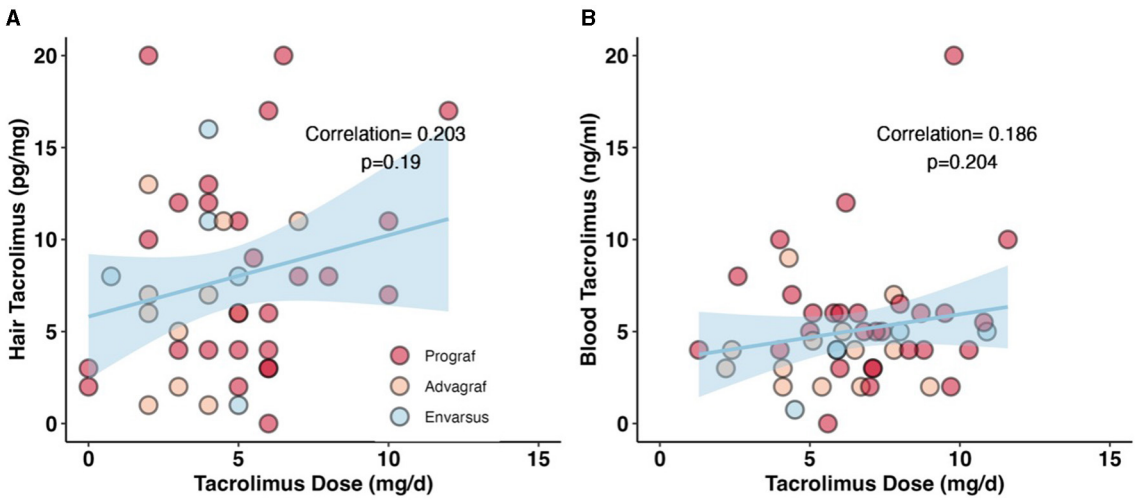

FIGURE 1
Correlation between daily dose of tacrolimus and measured drug levels in hair and blood. **(A)** Correlation between hC_0 and daily tacrolimus dose. Pearsons correlation coefficient is 0.203, $p = 0.19$. A non-significant positive correlation between the daily tacrolimus dose and the measured hC_0 levels. **(B)** Non-significant correlation between bC_0 and daily tacrolimus dose. Pearsons correlation coefficient is 0.186, $p = 0.204$. In this sample we could show a stronger correlation between hC_0 and daily dose than with bC_0.

product applications. Patient age significantly influenced results, while results were reliable and comparable among all tacrolimus formulations (Prograf®, Advagraf®, and Envarsus®).

The main differences were found between patients with different hair colors. Thus, there is a higher hC_0 in patients with darker hair (brown and black). Differing levels of metabolites depending on hair pigmentation are described in hair analyses of a variety of different drugs (27, 28). Gray hair naturally correlates with increased age; we interpret the lower hC_0 levels in older patients as a consequence of a higher fraction of gray hair.

Prednisolone has been described to induce CYP3A and/or P-glycoprotein, therefore increasing the needed tacrolimus dose to reach the target bC_0, especially after transplantation (29). In our population, the majority of patients were on low-dose prednisolone. Prednisolone maintenance therapy had no impact on Tacrolimus concentrations in hair.

This study highlights new opportunities for therapeutic drug monitoring. First, our approach enables therapeutic drug monitoring from biological samples, independent of blood collection. Hair specimens are easily accessible and may even be collected by patients themselves or their relatives. Furthermore,

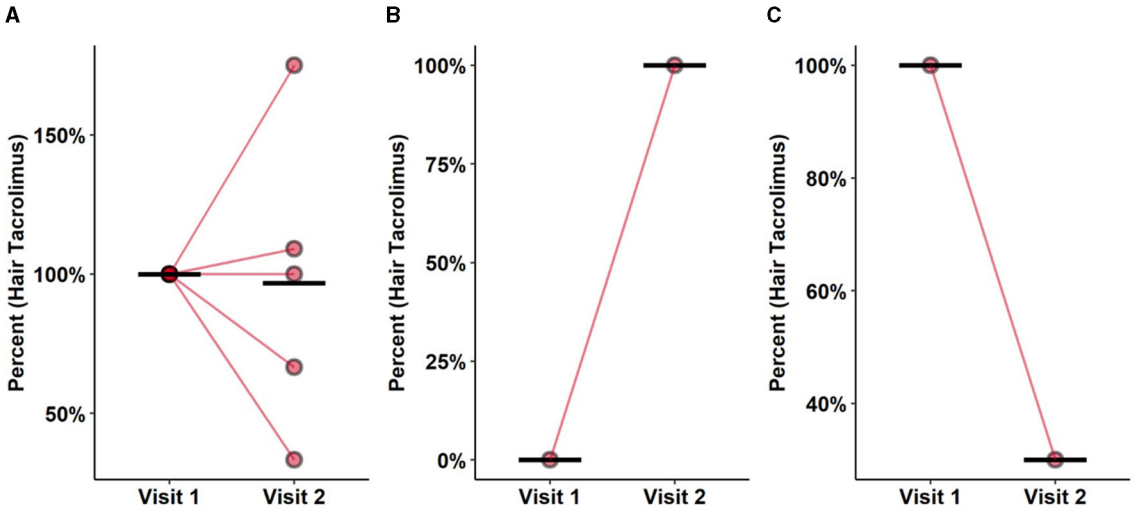

FIGURE 2
Stability over time, washin and washout. **(A)** Washout effect of hC_0 in patients with constant (±20%) bC_0 concentrations. S1 segment of the first visit was compared to S2 of the second visit, representing roughly the same time frame. Each segments represents 2 months of tacrolimus ingestion. S1 segment was cut of just above the skin therefore roughly representing the 2 months prior to analysis. Mean concentration was constant among both samples. Rather wide distribution of values between the samples indicates the presence of cofounders impacting stability of tacrolimus in hair. **(B)** Positive quantification in one patient *de novo* taking tacrolimus, comparing S1 segments of both visits showing a washin effect. **(C)** Washout effect after changing from tacrolimus to belatacept between two visits. Comparing S1 segments of both visits. Prompt washin and washout effects suggesting dose related incorporation in the hair. With measurable washin and out effects over a short period of time the possibility of temporal resolution in hair measurements is likely.

sampling is independent of healthcare facilities, does not require pre-analytical processing (centrifugation and cooling), and poses a negligible risk of transmission of infectious diseases. Since hC_0 concentrations appear to be relatively stable during the course of hair growth, this method could even be used to quantify tacrolimus exposure for weeks to months in the past.

Our study has several limitations. First, the cohort is small and comprises mainly single-time point evaluations. Second, patient- and hair treatment-associated confounders were associated with hC_0 levels. The sample size was too small and the sampling procedures too limited to test whether these confounders remain stable over time on a patient level and whether natural or hair treatment-related changes (graying of hair in aging patients, new hair products, or permanent coloring) affect longitudinal hC_0 values. Likely, further confounders have not been captured in detail, notably ethnic differences, given the predominance of Caucasian patients in this study. Although there is a wide distribution of pigmentation in hair, it is controversial if ethnicity affects hair analysis (28). Finally, tacrolimus and chronic kidney disease are known causes of alopecia (30, 31). However, not all patients were eligible for participation; notably, bald patients (predominantly elderly men) had to be excluded.

Conclusion

Tacrolimus detection in patient hair offers a reliable method to quantify drug exposure, including longitudinal measurements. Further studies are needed to determine therapeutic target levels for tacrolimus hair measurements and to quantify the effects of age, hair color, and different hair treatments on hC_0 and washout effects.

Data availability statement

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

Ethics statement

The studies involving humans were approved by Kantonale Ethikkomission Bern, Switzerland Nr. 2020-00953. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

AB: Data curation, Formal analysis, Investigation, Software, Visualization, Writing—original draft. FB: Writing—review & editing. CK: Writing—review & editing. UA: Project administration, Writing—review & editing. MB: Conceptualization, Formal analysis, Methodology, Resources, Writing—review & editing. DS: Conceptualization, Data curation, Funding acquisition, Methodology, Software, Supervision, Validation, Visualization, Writing—original draft.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1307505/full#supplementary-material

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Risk factors and current state of therapy for anemia after kidney transplantation

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Post-transplant anemia is one of the most common complications in kidney transplant recipients, severely affecting patient prognosis and quality of life, and is an independent predictor of graft kidney loss and patient mortality. However, our clinical understanding and the attention given to post-transplant anemia are currently insufficient. This paper reviews the current status, risk factors, and therapeutic progress in anemia after transplantation in kidney transplant recipients. We recommend that clinical staff pay attention to anemia and its complications in kidney transplant recipients and intervene early for anemia.

KEYWORDS

kidney transplantation, anemia after transplantation, risk factors, intervention, research progress

1 Introduction

Kidney transplantation is the most effective treatment for end-stage kidney disease (ESKD), and a successful kidney transplant can restore the patient's kidney function to almost normal levels, including endocrine functions (1, 2). Post-transplantation anemia (PTA) is a common complication after kidney transplantation, and studies have shown that the incidence of PTA is 20–50% at different stages after transplantation (3–7). Although the vast majority of cases of PTA can be corrected in the early stages following successful kidney transplantation, there are still patients who progress to anemia or secondary anemia, which can seriously affect the recipients' prognosis. PTA has been shown to reduce patient quality of life (8). Anemia has been linked with significant cardiovascular morbidity and mortality in renal transplant recipients (9, 10). Although anemia is a serious complication of transplantation, it has not attracted the attention of researchers. This paper therefore reviews the current status, risk factors, and available interventions for anemia after transplantation in kidney transplant recipients in order to prompt clinical workers to pay early attention to anemia after kidney transplantation.

2 Classification and diagnosis

Anemia describes a state in which the level of hemoglobin (Hb), the number of red blood cells, and/or the specific capacity of red blood cells within peripheral blood units is below the low normal limit (11). There is currently no exact staging or grading of the degree of anemia after transplantation (11). It is still generally graded using the normal human anemia scale now. In order to identify the effects of anemia on the post-transplant recipient and the transplanted kidney at different times, some centers have differentiated between PTA

occurring within 6 months and PTA occurring after 6 months (12), as early and late anemia, respectively.

At present, according to the World Health Organization and the American Transplant Society, anemia is diagnosed in adults living at sea level with Hb ≤130 g/L for men, Hb ≤120 g/L for women, or Hb ≤110 g/L for pregnant women (13). According to the Kidney Disease: Improving Global Outcomes (KDIGO) initiative and the European Kidney Best Practice group, anemia is defined as Hb ≤120 g/L in men and menopausal women and Hb ≤110 g/L in non-menopausal women (14, 15). The reference range for hemoglobin concentrations in the blood may vary depending on the population analyzed, age, sex, environmental conditions, and dietary habits (16).

3 Prevalence and risks of anemia in kidney transplant recipients

3.1 Prevalence of anemia in kidney transplant recipients

Anemia is one of the most common complications in patients with CKD, with both the incidence and degree of anemia gradually increasing as renal function decreases. A study has found that more than 50% of cases of CKD are combined with anemia, while the prevalence of anemia in the uremia phase reaches 90.2% (17). A retrospective study of 649 samples taken in Mexico between 2013 and 2017 found an anemia prevalence of 73.1% in patients prior to kidney transplantation (18). Similarly, another retrospective study in Turkey found that before kidney transplantation, the prevalence of anemia and severe anemia reached 86.7 and 58.8%, respectively (19). Fortunately, kidney transplantation can improve the symptoms of anemia in patients with CKD to some extent. But due to intraoperative blood loss, repeated postoperative blood tests, infection, rejection, delayed recovery of transplanted kidneys, drugs and other factors (6, 20, 21), the incidence of anemia remains high in patients after kidney transplantation. Indeed, the incidence of PTA decreased significantly as kidney function improves after transplantation. The prevalence of PTA at 1, 3, 6, and 12 months after kidney transplantation has been reported as 84.3, 39.5, 26.2, and 21.6%, respectively (22). Since then, the incidence of PTA has remained at high level and even increased. In further studies, the prevalence of post-transplant anemia after kidney transplantation ranged from 25 to 41.4% (12, 23), with a 2-year PTA prevalence of 36.6%, while the incidence of anemia at 3, 5, and 10 years after transplantation was reported to be 41.5, 35.3, and 93.2%, respectively (24).

Abbreviations: ALG, anti-lymphocytic globulin; AMR, antibody-mediated rejection; ATG, anti-thymocyte globulin; CERA, continuous erythropoietin receptor activator; CKD, chronic kidney disease; CVD, cardiovascular disease; EPO, erythropoietin; ESA, erythropoiesis stimulating agent; ESKD, end-stage kidney disease; IVIG, intravenous immunoglobulin; KDIGO, Kidney Disease: Improving Global Outcomes; MMF, mycophenolate mofetil; PTA, post-transplantation anemia.

3.2 Risks of anemia in kidney transplant recipients

3.2.1 Influence of anemia on cardiovascular function in kidney transplant recipients

Lower hemoglobin levels are associated with higher cardiovascular events (10). The annual incidence of cardiovascular disease (CVD) after kidney transplantation is 3.5–5%, which is 50-times that in the general population (25). Among the causes of death among kidney transplant recipients, CVD ranks first (40.9%) (26). Rates of cardiac death in renal transplant recipients (RTRs) are higher than in the general population, with the rate of cardiac death 10-times higher and the annual rate of fatal or non-fatal CV events 50-times that of the general population (27, 28). Anemia is an independent risk factor for clinical and echocardiographic cardiac disease, as well as mortality in end-stage renal disease patients (29). Kidney transplant recipients are considered to be a specific category of patients with CKD and are at risk for CKD-related complications (30). Long-term anemia causes hyperdynamic changes in the circulatory system and long-term overload of the heart and myocardial ischemia. This leads to anemic heart disease, as well as changes in heart rate, arrhythmias, changes in the structure of the heart, and congestive heart failure in severe cases (31). A retrospective cohort study of patients with no clinical heart disease who survived 1 year after kidney transplantation by a Canadian team showed that anemia was an important and major risk factor for left ventricular hypertrophy, 1 to 5 years after transplantation (10). However, most of these studies were observational or small intervention trials, which also focused on patients with CKD and did not take into account the poorer renal function and higher proteinuria in patients with PTA. Therefore, it is important to study whether positive treatment of anemia can improve CVD in PTA patients. However, studies have shown no benefit in correcting anemia or of high hemoglobin levels on cardiovascular disease or survival in CKD patients (32–35).

3.2.2 Influence of anemia on kidney function in kidney transplant recipients

Previous studies have shown an association between anemia and adverse transplant outcomes, including graft failure, rejection and patient survival (36). During a specific physical examination of 18,383 healthy elderly people in Tokyo, Japan, it was found that patients with Hb <12 g/dL (male) or Hb <11 g/dL (female) had a 2.215- or 2.2-fold risk, respectively, of new CKD compared with individuals with normal Hb levels (37). In addition, these patients had 2.618-times the risk of deteriorating kidney function, respectively (37). A study in 385 kidney transplant recipients showed that both persistent anemia and late-onset anemia were associated with an increased risk of graft loss (36). Furthermore, studies such as that conducted by de Andrade have shown that patients with anemia have a 3.8-fold higher risk of losing a transplanted kidney than patients without anemia (38). It's worth nothing that each increase in the degree of anemia increases the risk of graft loss by 2.77-times (hazard ratio [HR], 2.77; 95% confidence interval [CI], 1.50–5.13) (22). Studies such as that conducted by Jones have shown that patients with anemia have a 5.25-fold risk of transplant kidney failure compared with non-anemic patients (39). PTA also increases the incidence of post-transplant rejection, which is 1.8-times greater than in non-anemic patients (40). Previous studies have suggested that residual renal function is the most important

predictor of PTA and that impaired renal function in renal transplant recipients is proportional to the severity of anemia (41). However, the role of correcting anemia on graft kidney function is not clear. A meta-analysis showed no difference between the ESA and no ESA groups (42). Similarly, in CKD patients, no nephroprotective effect of anemia correction has been observed (32). In contrast, the prospective study by Tsujita et al. showed that correcting anemia to target levels (12.5–13.5 g/dL) slowed the time to deterioration of renal function (43). These studies suggest that the occurrence of PTA after kidney transplantation is detrimental to the recipient's transplant kidney function. We need to pay attention to the occurrence of PTA in the recipient at an early stage. The effectiveness and safety of anemia correction in improving graft outcomes in renal transplant patients remains to be further validated.

3.2.3 Influence of anemia on survival and quality of life in kidney transplant recipients

Previous studies have demonstrated that anemia is associated with increased mortality and morbidity in patients with various diseases. For example, anemia is associated with shortened survival in patients with lung and cervical cancers (44), while severe anemia (Hb <11 g/dL) is consistently associated with high mortality (HR, 4.36; 95% CI, 3.04–6.27) (5). The development of anemia after kidney transplantation has a very significant impact on the recipient's survival and is usually indirectly caused by the effect of anemia on other functions. However, due to the observational design of studies, causality cannot be affirmed. A retrospective study of 4,217 kidney transplant recipients in France found that all-cause mortality was as high as 6.8% in PTA recipients and 4.55% in the no-PTA group (23). The main reasons for this may be related to the type of the patient's primary disease, the deterioration of the transplanted kidney function and the long-term use of immunosuppressive drugs. Anemia can also seriously affect the quality of life of kidney transplant recipients. For example, patients with anemia have been shown to experience chronic fatigue, decreased activity endurance, and cognitive decline, as well as increased length of hospital stay and costs associated with anemia (8, 45). Treatment of anemia may have some benefits on the quality of life of patients after kidney transplantation, but whether it will reduce the complications and mortality of patient needs further study.

4 Risk factors for the occurrence of PTA

4.1 Causes of anemia before kidney transplantation

Patients with ESKD often have varying degrees of anemia before surgery. The main reason for this is bone marrow suppression due to decreased erythropoietin (EPO) secreted by the renal interstitial cells and the accumulation of uremic toxins in the blood that inhibit the activity of EPO (46), resulting in decreased erythrocyte production. Additionally, the blood loss caused by hemodialysis or the abnormal iron metabolism caused by blood loss and microvascular inflammation can also lead to the occurrence of anemia (47). Patients with ESKD often use daily diet to control the production of toxins, and may also suffer from a loss of appetite because of the disease, while patients with end-stage kidney disease tend to have uremia toxins exceeding the level that causes catabolism, which may lead to maladaptation of nutrients and a lack of folic acid, vitamin B12, iron, and other hematopoietic substrate. This eventually triggers anemia (11). Anemia may occur in the context of bone metastases in kidney cancer, lupus nephropathy, and hemolytic uremic syndrome, as well as multiple myeloma and diabetic nephropathy (48–50). A retrospective analysis of 410 patients (Hb <10 g/dL) in South Korea found that those with 25-hydroxyvitamin D < 10 ng/dL before kidney transplantation had a higher risk of developing anemia than those with 25-hydroxyvitamin D ≥ 10 ng/dL, while vitamin D deficiency may also be a risk factor for anemia in patients with ESKD (21). A deficiency of levonidin has also been shown to be associated with anemia (51). The degree of anemia in pre-transplant patients affects the development of anemia in post-transplant patients to varying degrees (52). Therefore, the correction of anemia of patient needs to be considered before kidney transplantation and receive early post-transplant work-up.

4.2 Early causes of anemia after transplantation

Early anemia after kidney transplantation is often attributed to iron deficiency, blood loss, immunosuppression, and viral infections. Inadequate iron storage during transplantation, blood loss during surgery, increased iron utilization for compensatory production of red blood cells due to blood loss, and malnutrition can all contribute to iron deficiency (53). Moreover, frequent blood tests in the early postoperative period resulting in frequent small blood loss in patients can exacerbate the incidence and extent of anemia (54). Immunosuppressive drugs are usually used after kidney transplantation to prevent the occurrence of graft and receptor rejection; however, immunosuppression causes the patient's entire immune system to be suppressed, inhibiting the bone marrow hematopoietic system and leading to the possibility of anemia. A study in Israel evaluating the incidence of anemia in pediatric patients with kidney transplantation and CKD showed that renal function recovered and glomerular filtration rates improved after kidney transplantation, but recipients had a higher incidence of anemia, possibly due to immunosuppressive therapy and EPO resistance (47). In a large cohort study enrolling 864 adult subjects, the prevalence of severe anemia immediately after kidney transplantation was 62.7%, significantly associated with anti-thymocyte globulin (ATG) /anti-lymphocytic globulin (ALG) administration (55). Simultaneously, the use of immunosuppressants decreases the body's resistance to infection, resulting in various infections (56); cytomegalovirus (57, 58), and parvovirus B19 (59) typically occur early after transplantation, with a very low proportion and count of reticulocytes (60). Infection with parvovirus B19 must be strongly suspected when refractory and severe anemia with reticulocytopenia develops after transplantation (61). Studies have also shown that avoiding the use of steroids in the first 6 months after kidney transplantation also increases the incidence of PTA (62). Acute kidney injury and nutritional deficiencies are also important influencing factors for early PTA (5). Therefore, it is important to monitor the iron stores and immunosuppression status of patients in the early post-transplant period and make timely adjustments to avoid the development of PTA.

4.3 Cause for late PTA

The onset of late PTA is associated with impaired kidney graft function and the development of renal insufficiency (63). Delayed graft function, impaired kidney graft function, and acute rejection are risk factors for PTA (62). Serum creatinine and glomerular filtration rates have been shown to affect Hb in patients (20). For example, when eGFR >60 mL/min/1.73 m^2, 18.7% of kidney transplant recipients experienced anemia, compared with only 2.4% of the general population. Further, when eGFR was 30–60 mL/min/1.73 m^2, 34.8% of recipients developed anemia after kidney transplantation and 3.8% of the general population suffered from anemia (64). Therefore, renal transplant recipients are more susceptible to the influences of inadequate glomerular filtration rate, which leads to the development of PTA. We need to pay early attention to recipients with impaired kidney graft function and delayed graft function. In kidney transplant patients, kidney graft function deteriorates over time due to various factors. A multicenter study in France confirmed that deterioration of kidney graft function is a significant risk factor for PTA, and that renal transplant recipients have increased proteinuria early in the deterioration of kidney function (23). An increase in urine protein has been shown to be significantly associated with anemia (65). In addition, multiple studies have reported that a longer transplant time is an important influencing factor PTA (9, 23, 66).

4.4 Other causes of anemia after transplantation

Sex is a contributing factor in PTA, and previous studies have suggested a higher incidence of PTA in female kidney transplant recipients (19, 67), which may be associated with menstrual blood loss and resulting iron deficiency in women. Pre-menopausal women recipients may need more attention. In terms of age, pediatric transplant recipients may be more likely to develop PTA than adults (68), and in a multicenter study in Argentina, Hb levels were significantly correlated with age, as lower Hb levels in children (19, 69). At the same time, donor age has been suggested to be a risk factor for PTA (70). This may be because the donor kidney in older donors may have poorer endocrine function. At the same time, studies have pointed out that the non-use of EPO before transplantation is also a major predictor of PTA (66). There are other causes, such as a meta-analysis of 29,061 kidney transplant recipients that showed a significantly increased risk of anemia in patients receiving renin-angiotensin system (RAS) (71).

5 Status of interventions for anemia in kidney transplant recipients

Anemia is a syndrome, not a disease. Therefore, the cause must always be investigated, and treatment must be primarily for causal disorders (72). Based on published evidence, comprehensive anemia testing approximately 3 months after transplantation allows for prompt correction of anemia and treatment and improved prognosis (73). However, there are no specific recommendations for PTA treatment in kidney transplant recipients in KDIGO guidelines, and the current treatment of anemia after kidney transplantation is mainly based on CKD rational for the use of anemia-associated erythropoiesis stimulating agent (ESA), iron therapy, hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHI), and other (74, 75) (Table 1).

5.1 Erythrocyte-producing stimulating hormone (ESA)

According to the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative, hemoglobin (Hb) levels below 11 g/dL are currently proposed treatment targets for anemia (87). KDIGO guidelines recommend initiating ESA therapy in patients with CKD only when Hb concentrations <10.0 g/dL and target Hb levels of 11.5 g/dL (14). Multiple evidence-based medical data show that ESA treatment significantly improves postoperative quality of life and reduces the need for blood transfusions (88). Recombinant human erythropoietin (rhEPO) is the first generation of ESA applied to the clinic, rhEPO is a short-acting preparation, 2 to 3 per week sub-administration; Daepotin α (DPO) belongs to the second generation of ESA. The mechanism of improving anemia is the same as that of rhEPO, but it is more biologically active and only needs to be administered once every 1 to 2 weeks; Persistent Erythropoietin Receptor Activator (CERA) is a third-generation ESA with advantages such as long half-life and low frequency of administration, but studies have confirmed its long-term efficacy and safety are inferior to other ESA (89). ESA is effective in correcting anemia and maintaining hemoglobin concentrations in the target range in most patients with CKD (90). Many post-transplant patients are anemic but only few receive adequate anemia treatment. Even transplant recipients with severe anemia received epoetin in only 17.8% of cases (12). Study showed that target hemoglobin level of 11.5 to 13.5 g/dL improves the vitality and mental health domains in quality of life Short Form (SF) - 36 scores and not associated with adverse changes in cardiovascular outcomes or increased cardiovascular morbidity or thrombotic events. However, treatment did not reduce the rate of decline in graft function (76). The main reason for this may be that some physicians believe that even without treatment, most patients will have symptoms of anemia improving on their own in the short term after surgery. Other studies have shown no effect of ESA on kidney function (91). It has also been reported that the treatment of EPO can cause complications such as hypertension and stroke, resulting in a poor prognosis for patients (92, 93). The laboratory evaluation of anemia including iron status and other substrates and replacement should be performed prior to treatment with ESA with a view to rational use of ESA for desired efficacy. The specific efficacy and influence of the use of ESA requires further study in the future.

5.2 Iron therapy

Iron is the basic ingredient for the synthesis of Hb, and studies have shown that early anemia after kidney transplantation is not due to the incomplete recovery of kidney function, but rather iron deficiency (94). Therefore, kidney transplant recipients with anemia after transplantation should routinely undergo measurement of serum ferritin, transferrin saturation, and other indicators, supplemented by serum hypersensitivity C-reactive protein and other deficit indicators if judged necessary. In addition, the causes of the iron deficiency

TABLE 1 Status of interventions for anemia in kidney transplant recipients.

Treatment	Reference	Interventions	Outcomes	Adverse effects
Erythrocyte-producing stimulating hormone	Pile et al. (76)	Epoetin beta: a starting dose of 50 U/kg once per week	Improvements in quality of life	NA
	Choukroun et al. (77)	Epoetin-β: start with a low dose weekly, dose escalation,	Less progress to ESKD; longer graft survival; lower cardiovascular events; improvements in quality of life	More return to dialysis and more death in low Hb-target group
	Heinze et al. (78)	Erythropoietin	High risk of mortality	Higher doses of erythropoietin with a higher incidence of cardiovascular, malignant, infection-related deaths
	Sánchez-Fructuoso et al. (79)	Erythropoietin receptor activator continuity	Better target Hb levels	Hypertension
	Budde et al. (80)	Epoetin α/βat: every 2–4 weeks for once; Continuous erythropoietin receptor activator: once-monthly	Stable Hb levels; good tolerability	Hemolytic anemia; pancytopenia; thrombocytopenia; angina pectoris; unstable angina; deep vein thrombosis; hypertension; injection site pain
	Bloom et al. (81)	Darbepoetin α: every 2 weeks for 24 weeks	Improved Hb levels; better HRQOL	Urinary tract infection; acute renal failure; nausea
Iron therapy	Iorember et al. (82)	Parenteral iron therapy: a single infusion of 1–2 mg/kg	Lower prevalence of early- and late-onset anemia; lower requirement for either ESA rescue or blood transfusion	NA
	Mudge et al. (83)	IV iron polymaltose: 500 mg single dose Oral ferrous sulfate: 210 mg elemental iron daily, continuously	Fewer gastrointestinal side-effects; fewer blood transfusions;	NA
	Rozen-Zvi et al. (84)	IV iron supplementation	Associated with increased Hb levels; lower rete of decline of eGFR	Chest pain; palpitations; weakness; nausea; dyspnea
Hypoxia-inducible prolyl hydroxylase inhibitor	Li et al. (54)	Roxadustat: orally three times a week	Improve Hb levels; good safety performance; stable renal function; no rejection	Symptoms of fatigue
Blood transfusion therapy	Ferrandiz et al. (85)	Blood transfusion	Higher incidence of DSAs	Rejection reaction
	Khedjat et al. (86)	Blood transfusion	No association with DSA, rejection graft loss; no influence with long-term outcomes	NA

HRQOL, health-related quality of life; IV, intravenous; DSAs, donor-specific antibodies; NA, not available; Hb, hemoglobin; ESKD, end-stage kidney disease.

should be investigated, and timely iron treatment administered according to the needs of patients, iron supplements before or after the kidney transplant for prevention might also be considered.

Iron therapy is typically divided into two categories: oral iron and intravenous iron. Commonly used forms of oral iron include ferrous sulfate, ferrous gluconate, and the newly emerged iron citrate, heme iron polypeptide, for example. The impact of oral iron on iron metabolism is close to the physiological state (95). However, the main disadvantages of oral iron are gastrointestinal adverse effects and the slow absorption of oral iron, which is not conducive to maximum iron utilization and rapid supplementation for patients in urgent need of iron supplementation. Intravenous iron agents include low-molecular weight dextran iron, iron sucrose, iron isomalt sucrose and iron carboxymaltose. The effectiveness and safety of intravenous iron in correcting renal anemia have been confirmed by a of evidence-based medical guidelines (96). However, the irregular application of intravenous iron can cause iron overload and damage to vital organs such as the liver and heart (97). Iron carboxymaltose, commonly used in intravenous iron preparations, was found to induce severe fibroblast growth factor 23-induced hypophosphatemia (98). Hence, we need to be vigilant about the amount of intravenous iron we use and avoid overloading. Gafter-Gvili et al. recommend that the use of an appropriate combination of erythropoietin stimulators and iron agents may be more beneficial in maintaining hemoglobin targeting at 12.5–13 g/dL in kidney transplant recipients (73). The Kidney Disease: Improving Global Outcomes (KDIGO) guidelines for the care of kidney transplant recipients recommend that anemia in kidney transplant patients should be monitored and treated in the same way as patients with CKD (99),

while, CKD populations have indicated that an increased risk of stroke and venous thromboembolism when ESA therapy is used to target high Hb levels (32, 35, 100). CAPRIT (77) as the only clinical randomized cntrolled study evaluating the therapeutic target of PTA, divided Hb into normal group (130–150 g/L) and partially corrected group (105–115 g/L). The preservation of renal function and cardiovascular events in the normal group were better than those in the partially corrected group. Therefore, it is suggested that the therapeutic target of PTA is higher than that of other CKD patients.

5.3 Hypoxia-inducible prolyl hydroxylase inhibitor (HIF-PHI)

The safety of ESA in the treatment of anemia has been questioned after an increased risk of death, cardiovascular events, and stroke was observed in the ESA intervention trial (35). Therefore, compared with external supplementation of EPO, stimulating the production of endogenous EPO by other drugs might have higher safety and applicability. HIF-PHI is a novel oral small molecule drug class that stimulates endogenous EPO production and improves iron utilization (101). Four HIF-PHI agents, roxadustat, daprodustat, vadadustat and molidustat, have been investigated in clinical trials. Roxadustat was the first HIF-PHI to enter a Phase 3 clinical trial, and was recently approved for the oral treatment of anemia in China (102). Roxadustat has been shown to correct anemia and maintain hemoglobin levels in the presence of low ferritin saturation and a gradual decline in ferritin levels (103). Roxadustat has also been shown to significantly increase hemoglobin levels and has shown good safety, renal stability, and no rejection in patients with PTA (54). HIF-PHIs were not inferior to ESAs in correcting anemia when using the Hb increase from baseline to the evaluation period as the primary endpoint in most trials (104, 105). However, its high price limits its large application by most patients, while several side effects of roxadustat need to be noted, including gastrointestinal diseases, nasopharyngitis, and back pain (102). A recent meta-analysis showed a 31% higher risk of thrombosis events versus ESAs (105). At present, daprodustat and vadadustat are also approved for listing in Japan, but their effects and safety need to be verified by further clinical studies. In the absence of conclusive data on the reduction of cardiovascular risk with the use of HIF-PHI, their use to correct hemoglobin levels in transplant recipients should be treated with more caution. It is noted that no randomized controlled trial has been designed for kidney transplant recipients so far, given their metabolization by CYP enzymes, possible drug interactions in kidney transplant recipients should also be carefully evaluated.

5.4 Anti-infective and antiviral therapy

Kidney transplant recipients require long-term immunosuppressants after surgery, which primarily work by suppressing the body's immune system, making patients more susceptible to bacterial and viral infection (106). Anemia due to chronic infection is often improved with anti-infective therapy. To prevent opportunistic infection in kidney transplant recipients, anti-infective and antiviral prophylaxis is recommended after kidney transplantation. In patients with microvirus B19 infection, intravenous immunoglobulin (IVIG) is required in addition to reduced exposure to immunosuppressive drugs, and high doses of IVIG do not aggravate anemia in patients (61, 107).

5.5 Adaptation of immunosuppressants

Many immunosuppressive drugs used after transplantation have potential myelosuppressive effects, and immunosuppressants such as tacrolimus and mycophenolate mofetil (MMF) have been reported to be a cause of PTA after kidney transplantation (108). Anemia has been reported to be more common in patients taking MMF and sirolimus (12). Some studies have shown no relationship between anemia and immunosuppression, which may be due to the already poorer function of the transplanted kidney in such patients (30). Current regimens for adjusting immunosuppressive therapy include switching to low-intensity immunosuppressants or reducing the dose of immunosuppressants (109). However, it is important to note that in the process of reducing immunosuppression intensity, the risk of rejection may be increased.

5.6 Blood transfusion

In non-emergency situations, blood transfusion therapy is generally not recommended for kidney transplant patients. Study has shown that donor-specific antibodies and antibody-mediated rejection (AMR) occurs in patients undergoing transfusion therapy after kidney transplantation (85). The probability of AMR is significantly higher than in non-transfusion patients. In addition, this might interfere with the patients' opportunity to be re-transplanted. Therefore, a strategy of blood transfusion in the clinic should always be treated with caution. However, in cases where it is necessary, we still do not hesitate to carry out antibody clearance in conjunction with blood transfusions.

6 Discussion

PTA is a common complication after kidney transplantation and yet, despite its high prevalence, low treatment rate, and serious consequences, the condition has not currently attracted sufficient attention. Due to differences in the definition of anemia, ethnicity, follow-up time, and intervention factors, for example, the reported incidence of PTA varies greatly among research centers. Therefore, we need to explore and reach consensus on the assessment criteria applicable to anemia in renal transplant patients in the future.

The main risk factors associated with the development of PTA include transplanted kidney function, polypharmacy, and infection. Risk factors can either aggravate PTA or worsen the disease caused by PTA. There are no national or international detailed systematic reviews or guidelines for the treatment of PTA. There is also insufficient guidance for the diagnosis and treatment of PTA in kidney transplant recipients, while the target level of Hb after PTA treatment also remains controversial. Optimal treatment of PTA may be ambivalent, depending on the underlying cause; for example, infection caused by PTA requires reduced or even discontinued immunosuppressant therapy, while PTA caused by kidney rejection requires immunosuppression to be strengthened. The short-term effects of PTA on kidney transplant patients are unclear and reversible, but their long-term negative effects are known.

This paper reviews the current epidemiology status, risk factors and available interventions for PTA in patients with kidney transplantation. We hope that clinicians will pay attention to PTA after renal transplantation and that systematic guidelines for the prevention and management of PTA after renal transplantation will be available in the near future.

Author contributions

YT and JG drafted the manuscript, undertook the systematic literature search. ZW and JL are conceived and designed the study. JZ and TQ obtained funding and supervised the study. TQ, JL, and JZ had important intellectual input. All authors have approved the final version of manuscript before submission.

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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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Dynamics of torque teno virus load in kidney transplant recipients with indication biopsy and therapeutic modifications of immunosuppression

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Following kidney transplantation, lifelong immunosuppressive therapy is essential to prevent graft rejection. On the downside, immunosuppression increases the risk of severe infections, a major cause of death among kidney transplant recipients (KTRs). To improve post-transplant outcomes, adequate immunosuppressive therapy is therefore a challenging but vital aspect of clinical practice. Torque teno virus load (TTVL) was shown to reflect immune competence in KTRs, with low TTVL linked to an elevated risk for rejections and high TTVL associated with infections in the first year post-transplantation. Yet, little is known about the dynamics of TTVL after the first year following transplantation and how TTVL changes with respect to short-term modifications in immunosuppressive therapy. Therefore, we quantified TTVL in 106 KTRs with 108 clinically indicated biopsies, including 65 biopsies performed >12 months post-transplantation, and correlated TTVL to histopathology. In addition, TTVL was quantified at 7, 30, and 90 days post-biopsy to evaluate how TTVL was affected by changes in immunosuppression resulting from interventions based on histopathological reporting. TTVL was highest in patients biopsied between 1 and 12 months post-transplantation (N = 23, median 2.98×10^7 c/mL) compared with those biopsied within 30 days (N = 20, median 7.35×10^3 c/mL) and > 1 year post-transplantation (N = 65, median 1.41×10^4 c/mL; $p < 0.001$ for both). Patients with BK virus-associated nephropathy (BKVAN) had significantly higher TTVL than patients with rejection ($p < 0.01$) or other pathologies ($p < 0.001$). When converted from mycophenolic acid to a mTOR inhibitor following the diagnosis of BKVAN, TTVL decreased significantly between biopsy and 30 and 90 days post-biopsy ($p < 0.01$ for both). In KTR with high-dose corticosteroid pulse therapy for rejection, TTVL increased significantly between biopsy and 30 and 90 days post-biopsy ($p < 0.05$ and $p < 0.01$, respectively). Of note, no significant changes were seen in TTVL within 7 days of changes in immunosuppressive therapy. Additionally, TTVL varied considerably with time since transplantation

and among individuals, with a significant influence of age and BMI on TTVL ($p < 0.05$ for all). In conclusion, our findings indicate that TTVL reflects changes in immunosuppressive therapy, even in the later stages of post-transplantation. To guide immunosuppressive therapy based on TTVL, one should consider inter- and intraindividual variations, as well as potential confounding factors.

KEYWORDS

kidney transplantation, immune monitoring, immunosuppression, torque teno virus, precision medicine

1 Introduction

Lifelong immunosuppressive maintenance therapy is mandatory following kidney transplantation to prevent graft rejection and minimize risks for allograft failure (1, 2). However, immunosuppression increases the risk for severe infectious complications, which represent the leading non-cardiovascular cause of death among kidney transplant recipients (KTRs) (3–5). Therefore, optimal dosing of immunosuppressive drugs and balancing the risks of rejection and infection is important to improve outcomes after kidney transplantation.

Currently, monitoring of immunosuppression is mainly based on measuring calcineurin inhibitor trough levels, but acute rejection can occur even if trough levels are within the target range (6). Previously, Vasudev et al. introduced a semi-quantitative immunosuppression (IS) scale to assess the immunosuppressive burden in KTRs (7), which has been adopted and validated by various groups in different immunocompromised cohorts (8–11). However, as the IS scale is calculated using simply the dosages of immunosuppressive medication, it fails to address the high inter- and intrapatient variability in the dosage required to reach certain trough levels of immunosuppressive agents such as tacrolimus (12, 13). Thus, new surrogate parameters that measure a patient's individual immunosuppressive burden are urgently needed to monitor immunocompetence.

Recently, monitoring torque teno virus (TTV) load within the first year post-transplantation has emerged as a promising approach to identify KTRs at risk of rejection or infection (14–17). Previous studies showed that TTV is profoundly influenced by the initiation of immunosuppressive therapy but largely evades the impact of antiviral drug therapy, such as cytomegalovirus prophylaxis administered after transplantation (18, 19). Additionally, a correlation has been observed between the intensity of immunosuppression and TTV load, indicating a potential link between TTV load and the likelihood of immunosuppression-related complications, including infections and rejections (14, 20). Recent data suggest that TTV loads may even help in monitoring short-term changes in immunosuppressive therapy, although there is still no thorough understanding of viral load kinetics due to changes in immunosuppression (21–23). To assess the possibility of guiding immunosuppression based on TTV loads in KTRs in the first year after transplantation, the multicentric, randomized, and controlled phase II TTVguideIT trial was initiated, with results expected in 2024 (24).

With limited data on the dynamics of TTV loads in KTRs transplanted >12 months ago, this study seeks to explore potential associations between TTV loads and various graft-associated pathologies, especially in KTRs beyond the first year post-transplantation. Additionally, this study aims to investigate changes in TTV loads upon modifications in immunosuppression in a well-characterized cohort of KTRs with indication biopsy at different time points post-transplantation.

2 Materials and methods

2.1 Study design

A total of 108 KTRs with indication biopsy at the Department of Nephrology, Heidelberg University Hospital, were enrolled in this prospective single-center study to evaluate new biomarkers in post-transplant care (DRKS00023604). Serum was obtained on the day of biopsy (T_0), as well as 7 (T_1), 30 (T_2), and 90 days (T_3) post-biopsy (Figure 1A), and TTV loads were quantified as a *post-hoc* analysis.

As immunosuppressive medication is reduced following initial transplantation and immunosuppressive burden consecutively declines with time since transplantation, KTRs were classified into four groups based on the timing of the biopsy in relation to initial transplantation (<30 days, $N = 20$; 1–12 months, $N = 23$; 1–5 years, $N = 30$; and > 5 years post-transplantation, $N = 35$) to compare TTV loads at the time of biopsy (T_0) between these four groups. Biopsies were only performed in KTRs with no clinically apparent concomitant infection. Histopathology was assessed by two board-examined pathologists according to the BANFF 2018 reference guide (25), as reported previously (26).

Clinical management following histopathological reporting included corticosteroid pulse therapy in 31 patients with suspected rejection. Among the 13 KTRs with biopsy-proven BK virus-associated nephropathy (BKVAN), immunosuppression was switched from a calcineurin inhibitor (CNI)-mycophenolic acid (MPA) to a CNI-mTOR-based regimen including lower CNI target ranges. In six of these patients, MPA had already been reduced by 50% before biopsy due to the prior detection of BK viremia. Additionally, in six patients with suspected CNI toxicity ($ah \geq 1$), immunosuppression with CNI was switched to belatacept. One of these patients further received corticosteroid pulse therapy for concomitant borderline lesions.

The study was approved by the ethics committee of the University of Heidelberg and conducted in accordance with the Declaration of Helsinki. All transplant procedures were performed in accordance with the Declaration of Istanbul. Written informed consent was

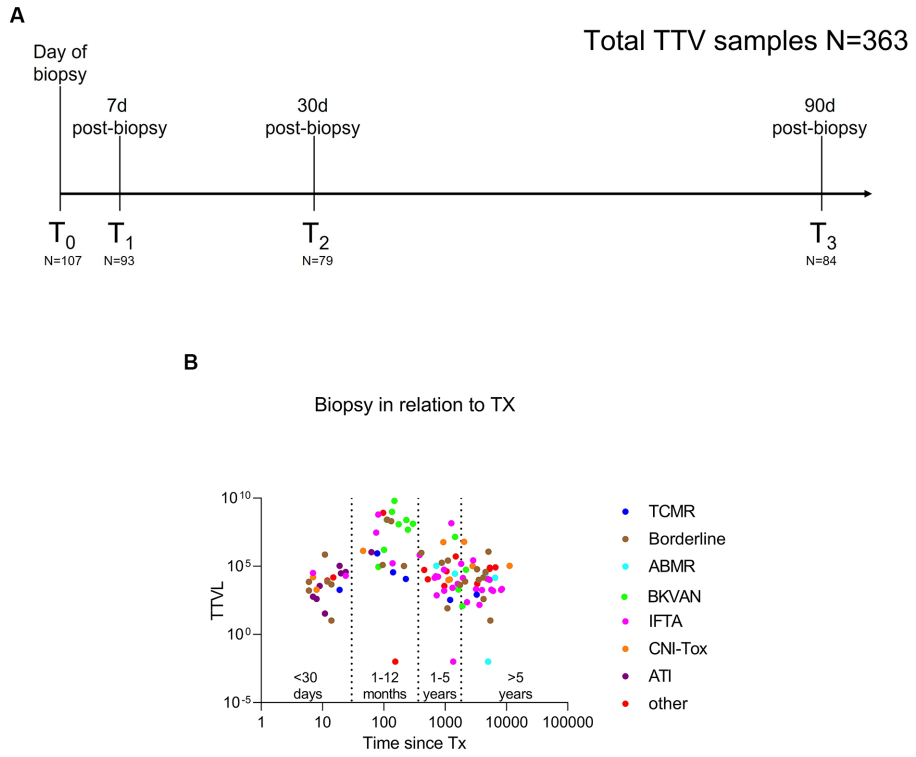

FIGURE 1
Study design to assess the dynamics of torque teno virus load in kidney transplant recipients with indication biopsy and therapeutic modifications of immunosuppression. **(A)** Torque teno virus load was quantified on the day of biopsy (T_0, $N = 107$) and 7 days (T_1, $N = 93$), 30 days (T_2, $N = 79$) and 90 days (T_3, $N = 84$) post-biopsy. In total, 363 patient samples were analyzed. **(B)** Torque teno virus load at biopsy (T_0) in relation to timing of the biopsy relative to transplantation. Specifically, 20 KTRs received a biopsy within 30 days of transplantation, 23 KTRs received a biopsy between 1 and 12 months post-transplantation, and 65 KTRs underwent a biopsy >1 year post-transplantation. Different colors represent the different histopathological findings. 36/108 (33%) of indication biopsies were classified as rejection, including seven patients with ABMR (turquoise), six with TCMR (blue), and 23 with borderline changes (brown). BKVAN (green) was diagnosed in 13 KTRs. A total of 29 biopsies were graded as IFTA (pink), while 9 KTR showed signs of CNI toxicity (orange) and 8 presented ATI (purple). Other (red) diagnoses included eight patients with normal/unspecific histopathology, four KTR with recurrent disease, and one KTR with infect-related graft deterioration. ABMR, antibody-mediated rejection; ATI, acute tubular injury; BKVAN, BK virus-associated nephropathy; CNI, calcineurin inhibitor; IFTA, interstitial fibrosis and tubular atrophy; KTRs, kidney transplant recipients; TCMR, T cell-mediated rejection; TTVL, torque teno virus load; Tx, transplantation.

obtained from all study participants. The main goals of this analysis were to: (i) assess differences in TTV loads between different graft-associated pathologies, particularly in KTRs transplanted >12 months ago; and (ii) analyze the effects of modifications in immunosuppressive therapy on TTV loads.

2.2 Quantification of torque teno virus load

TTV quantification was performed using the TTV R-Gene® assay (BioMérieux, Marcy-l'Etoile, France), a real-time polymerase chain reaction (PCR) assay targeting the TTV 5′ untranslated region (27). The assay has a dynamic range of 250 to 10^9 copies/mL, with a limit of detection at 250 copies/mL. The assay was developed for quantifying TTV in plasma and whole blood samples, for which it is validated and widely used (28). We recently demonstrated that TTV load as quantified by the TTV R-Gene® assay did not differ significantly whether quantified in serum or plasma and observed a very strong and highly statistically significant correlation between serum and plasma TTV load, underscoring the interchangeability of serum and plasma for TTV quantification (21).

TTV DNA was extracted from serum samples using the QIAsymphony SP platform (QIAGEN, Venlo, the Netherlands), and PCR was conducted on a Light Cycler® 480 Instrument II (Roche Diagnostics, Rotkreuz, Switzerland) according to the manufacturer's instructions. Viral load was determined using a standard curve, and specimens with undetectable viral load were assigned a value of 0.01 copies/mL for analysis purposes, as previously done by Fernández-Ruiz et al. (20).

2.3 Immunosuppression scale

To assess the overall burden of immunosuppression and to correlate results to TTV loads, an empiric, semi-quantitative score as previously published by Vasudev et al. was used (7). One score unit was assigned to each of the following doses of immunosuppressive drugs: tacrolimus 2 mg, cyclosporine 100 mg, mycophenolate mofetil 500 mg, azathioprine 100 mg, sirolimus 2 mg, and prednisolone 5 mg. For methylprednisolone and mycophenolate sodium, we assigned one unit to the equivalent doses of 4 mg and 360 mg, respectively. Additionally, we assigned one unit for 1.5 mg of everolimus, as previously done by Baumann et al. (29).

2.4 Statistical analysis

Quantitative data are presented as median with interquartile range (IQR). Due to TTV loads not conforming to normality, only non-parametric statistical analyses were performed. The Mann–Whitney *U* test or the Kruskal–Wallis test was used to compare continuous variables. To analyze repeated measures between pairs, the Wilcoxon matched-pairs rank test was applied. To correlate TTV load and IS score, medication, or BKV load, Spearman's rho was calculated. The area under the ROC curve (AUC) was calculated to evaluate the performance of the TTV load to discriminate rejection from no rejection or BKVAN from no BKVAN. To identify possible confounders to TTV load, multiple linear regression was performed. For this analysis, TTV loads were $\log_{10}$-transformed beforehand. Statistical analysis was performed using GraphPad Prism version 9.5.1 (GraphPad Software, San Diego, CA, United States), and statistical significance was assumed at a p-value <0.05.

3 Results

3.1 Study cohort

Indication biopsy was performed at a median (IQR) of 2.6 (0.3–7.8) years post-transplantation, with 43/108 (40%) KTRs receiving a biopsy within the first year of transplantation. Specifically, 20 KTRs received a biopsy within 30 days of transplantation, 23 KTRs received a biopsy between 1 and 12 months post-transplantation, and 65 KTRs underwent a biopsy >1 year post-transplantation.

Histopathology revealed biopsy-proven rejection in 36 KTRs, including 7 KTRs with antibody-mediated rejection (ABMR), 6 KTRs with T-cell–mediated rejection (TCMR), and 23 KTRs with borderline changes. Patients with borderline changes were analyzed within the rejection group as all biopsies were performed on clinical indication. BKVAN (SV40+) was histopathologically proven in 13 KTRs. The other 59 KTRs were grouped as "No Rejection/BKVAN", including 29 KTRs with interstitial fibrosis and tubular atrophy (IFTA), eight with acute tubular injury (ATI), nine with CNI toxicity, and 13 with other changes.

Figure 1B illustrates TTV loads in KTRs at the time of biopsy, considering the biopsy's timing relative to time since transplantation, and color-coding representing the different histopathological diagnoses. Characteristics of the study cohort are presented in Table 1. Mean (±SD) age at biopsy was 49 (±14) years, 35 (32%) of the participants were female. Comorbidities and underlying renal pathologies for 108 KTRs with indication biopsy are shown in Supplementary Table S1.

3.2 Torque teno virus prevalence and virus load kinetics according to timing of the biopsy

TTV was detectable in 107 (99%) of the study patients. Virus DNA was detectable in every sample in 104 patients, while three patients had one sample with a viral load below the threshold of detection. In total, 361 serum samples and 2 plasma samples were analyzed, with a mean of 3 samples analyzed per patient. TTV was quantifiable in 98.9% (359 of 363) of all samples with a median (IQR) viral load of 5.28×10^4 c/mL (5.59×10^3 c/mL–1.03×10^6 c/mL). TTV load varied markedly between patients, ranging from 10.3 c/mL to 7.44×10^9 c/mL.

TABLE 1 Characteristics of the study cohort.

Variable	All	Rejection	BKVAN	Other
Number of samples, *N*	108	36	13	59
Female, *N* (%)	35 (32)	12 (33)	4 (31)	19 (32)
Age at enrollment, mean ± SD	49 ± 14	46 ± 15	51 ± 11	51 ± 15
Donor type Deceased Donor, *N* (%) Living Donor, *N* (%)	 70 (65) 38 (35)	 16 (44) 20 (56)	 10 (77) 3 (23)	 44 (75) 15 (25)
HLA class 1 mismatches, mean ± SD	1.7 ± 1.2	1.9 ± 1.2	1.6 ± 1.2	1.6 ± 1.1
HLA class 2 mismatches, mean ± SD	0.8 ± 0.7	0.9 ± 0.7	0.8 ± 0.7	0.7 ± 0.6
Months post-transplant at time of biopsy, mean ± SD	60 ± 76	65 ± 73	22 ± 27	65 ± 84
DSA MFI > 500, *N* (%)	30 (29) [+]	14 (39) [++]	2 (15)	14 (24) [+++]
DSA MFI > 1,000, *N* (%)	21 (20) [+]	9 (25) [++]	2 (15)	10 (17) [+++]
S-Creatinine [mg/dl], mean ± SD	3.0 ± 2.3	2.9 ± 1.7	2.6 ± 1.2	3.1 ± 2.8
eGFR [ml/min/1.73 m^2], mean ± SD	30.5 ± 16.3	30.0 ± 14.5	33.0 ± 19.5	30.4 ± 17.0
Proteinuria [g/molCr], mean ± SD	140.7 ± 217.2	209.6 ± 291.9	74.9 ± 115.4	114.6 ± 168.2
TTVL [copies/mL], median (IQR)	1.9×10^4 (2.2×10^3–2.7×10^5)	1.4×10^4 (1.9×10^3–1.2×10^5)	4.9×10^7 (7.3×10^4–2.5×10^8)	1.5×10^4 (2.2×10^3–1.1×10^5)
BKV load [IU/mL], median (IQR)			2.4×10^5 (5.1×10^4–6.9×10^5)	

BKV, BK virus; BKVAN, BK virus-associated nephropathy; DSA, donor-specific antibodies; eGFR, estimated glomerular filtration rate; SD, standard deviation; TTVL, torque teno virus load.
[+]Not possible to determine DSA in five patients with missing data. [++]not possible to determine DSA in two patients with missing data.
[+++]Not possible to determine DSA in three patients with missing data.

In patients who underwent indication biopsy less than 30 days post-transplantation, the median (IQR) TTV load was 7.35×10^3 c/mL (1.71×10^3 c/mL–2.78×10^4 c/mL). TTV load was with a median (IQR) of 2.98×10^7 c/mL (1.24×10^5 c/mL–2.58×10^8 c/mL) significantly higher in patients that received a biopsy in between 1 and 12 months post-transplantation ($p < 0.001$). Subsequently, with a reduction in immunosuppression, TTV load was significantly lower in KTR with indication biopsies within 1 and 5 years (median 1.72×10^4 c/mL, IQR 2.68×10^3 c/mL–2.66×10^5 c/mL, $p = 0.001$) and > 5 years post-transplantation (median 1.12×10^4 c/mL, IQR 1.75×10^3 c/mL–6.77×10^4 c/mL; $p < 0.001$) compared with those with biopsies between 1 and 12 months post-transplantation (Figure 2A).

A multiple linear regression analysis revealed a significant influence of age, BMI, and time since transplantation on TTV loads ($p < 0.05$ for all; Supplementary Table S2).

3.3 Torque teno virus loads in kidney transplant recipients with different graft-associated pathologies

Patients with BKVAN had, with a median (IQR) of 4.85×10^7 c/mL (7.34×10^5 c/mL–2.54×10^8 c/mL), significantly higher TTV loads at the time of biopsy than patients with histopathological signs of rejection (median 1.44×10^4 c/mL, IQR 1.87×10^3 c/mL–1.24×10^5 c/mL) or other pathologies (median 1.51×10^4 c/mL, IQR 2.17×10^3 c/mL–1.06×10^5 c/mL) ($p < 0.01$ and $p < 0.001$, respectively; Figure 2B). A total of two KTRs with rejection had a TTV load higher than the median TTV load for KTRs with BKVAN; these patients either had concurrent histoplasmosis or BK viremia at the time of biopsy. Additionally, three patients with no rejection or BKVAN showed higher TTV loads than the median TTV load for BKVAN. Of these three, two patients underwent indication biopsy within 3 to 4 months post-transplantation, a period where TTV loads in KTRs typically reach their peak levels, while the third patient was diagnosed with Pneumocystis jirovecii pneumonia shortly after biopsy.

Considering all 108 KTRs with indication biopsy, the AUC to differentiate BKVAN from no BKVAN was at 0.79 (95% CI 0.63–0.96), and the AUC to discriminate rejection from no rejection was at 0.58 (95%CI 0.46–0.69; Figure 3A). When only including patients that received a biopsy within 1 year after transplantation (N = 43), the AUC to discriminate BKVAN from no BKVAN and rejection from no rejection increased to 0.88 (95% CI 0.78–0.99) and 0.62 (95% CI 0.44–0.79), respectively (Figure 3B). Figure 3C displays the ROC curves for KTRs that received a biopsy more than 1 year post-transplantation with an AUC of 0.50 (95% CI 0.15–0.86) to discriminate BKVAN from no BKVAN and an AUC of 0.53 (95% CI 0.38–0.69) to discriminate rejection from no rejection.

3.4 Influence of changes in immunosuppressive therapy on torque teno virus load

When converted from mycophenolic acid (MPA) to the mTOR inhibitor following diagnosis for BKVAN ($N = 13$), TTV loads decreased significantly in these patients from a median (IQR) of 4.85×10^7 c/mL (7.34×10^5 c/mL–2.54×10^8 c/mL) to a median (IQR) of 3.52×10^6 c/mL (7.48×10^3 c/mL–2.53×10^7 c/mL) 30 days post-biopsy (T_2; $p < 0.01$) and a median (IQR) of 1.32×10^5 c/mL (8.33×10^3 c/mL–2.73×10^5 c/mL) 90 days post-biopsy (T_3; $p < 0.01$; Figure 4A).

On the other side, in KTR who received high-dose corticosteroid pulse therapy as anti-rejection therapy ($N = 31$), a significant increase in TTV loads was observed between biopsy (T_0) (median 1.17×10^4 c/

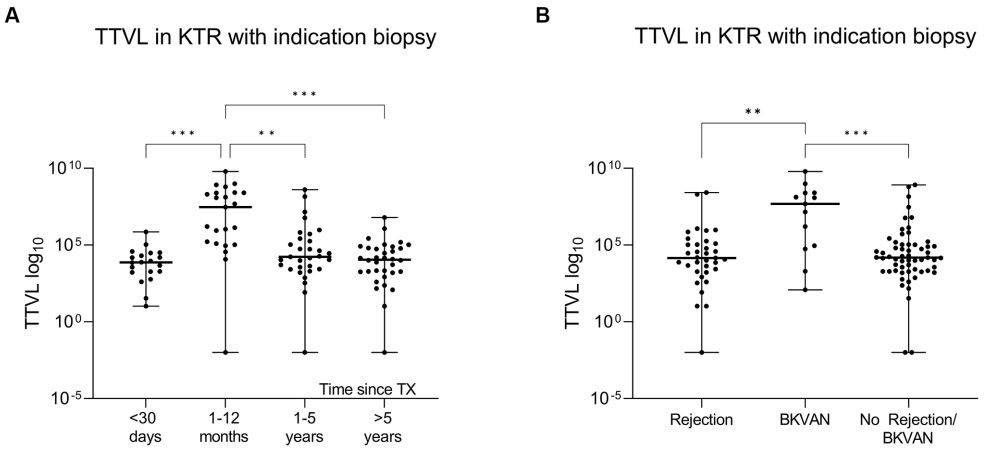

FIGURE 2
Differences in torque teno virus load based on time since transplantation and histopathology. **(A)** To compare TTVL at the time of biopsy (T_0) in relation to time since transplantation, KTRs were categorized into four groups based on the timing of the biopsy relative to initial transplantation (<30 days, 1–12 months, 1–5 years and > 5 years post-transplantation). The x-axis displays the respective group, and the virus loads are shown on the y-axis. The scatter dot plots present the distribution of data with a horizontal line representing the median. The lower and upper edges display the minimum and maximum range, respectively. Individual values are shown as dots. **(B)** TTVL at time of biopsy (T_0) in KTRs with rejection, BKVAN or other pathology. KTRs with BKVAN have significantly higher loads. The x-axis displays the respective group, and the virus loads are shown on the y-axis. The scatter dot plots present the distribution of data with a horizontal representing the median. The lower and upper edges display the minimum and maximum range, respectively. Individual values are shown as dots. BKVAN, BK virus-associated nephropathy; KTRs, kidney transplant recipients; TTVL, torque teno virus load; TX, transplantation; ***$p < 0.001$; **$p < 0.01$.

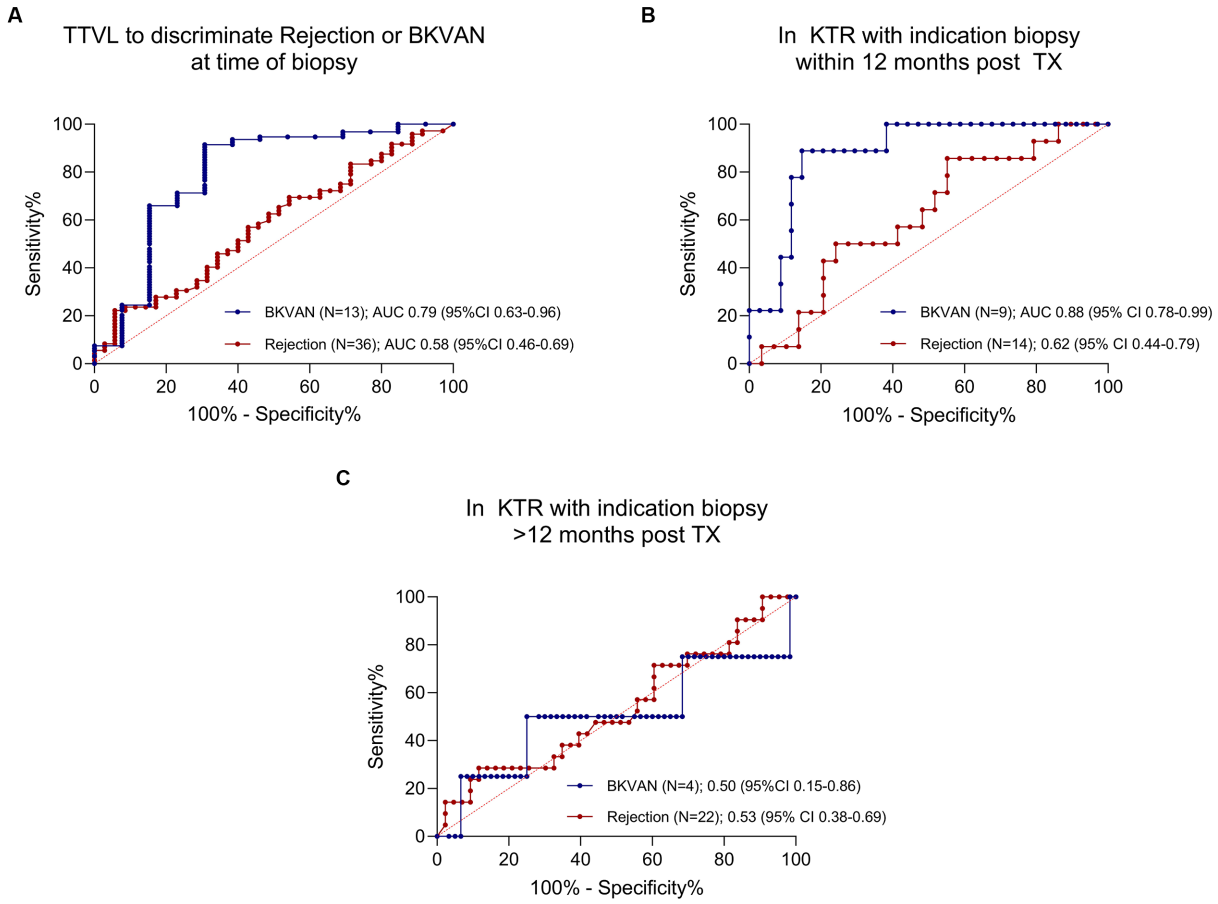

FIGURE 3
ROC curves for torque teno virus load to discriminate rejection and polyomavirus nephropathy from other diagnoses. **(A)** ROC curve for TTVL to discriminate rejection from no rejection and BKVAN from no BKVAN in all patients ($N = 108$). AUC for BKVAN = 0.79 (95% CI 0.63–0.96); AUC for rejection = 0.58 (95% CI 0.46–0.69). **(B)** ROC curve for TTVL to discriminate rejection from no rejection and BKVAN from no BKVAN in patients receiving a biopsy within the first year of transplantation ($N = 43$). AUC for BKVAN = 0.88 (95% CI 0.78–0.99); AUC for rejection = 0.62 (95% CI 0.44–0.79). **(C)** ROC curve for TTVL to discriminate rejection from no rejection and BKVAN from no BKVAN in KTRs receiving a biopsy after the first year of transplantation ($N = 65$). AUC for BKVAN = 0.50 (95% CI 0.15–0.86); AUC for rejection = 0.53 (95% CI 0.38–0.69). 100%-specificity % is displayed on the *x*-axis and sensitivity on the y-axis. The ROC curve to discriminate rejection is plotted in red whereas the ROC curve for BKVAN is plotted in blue. AUC, area under the curve; BKVAN, BK virus-associated nephropathy; CI, confidence interval; ROC, receiver operating characteristics; TTVL, torque teno virus load; TX, transplantation.

mL, IQR 1.66×10^3 c/mL–1.03×10^5 c/mL) to 30 days (T_2) (median 7.53×10^4 c/mL, IQR 1.14×10^4 c/mL–1.34×10^6 c/mL) and 90 days (T_3) (median 1.83×10^5 c/mL, IQR 1.89×10^4 c/mL–3.72×10^7 c/mL) post-biopsy ($p < 0.05$ and $p < 0.01$, respectively; Figure 4B).

Patients whose immunosuppressive therapy was converted from calcineurin inhibitors (CNI) to belatacept ($N = 6$) showed an increase in TTV loads as well, albeit less significant, with TTV loads surging from a median (IQR) of 3.78×10^4 c/mL (7.36×10^3 c/mL–1.15×10^6 c/mL) at time of biopsy (T_0) to a median (IQR) of 1.32×10^7 c/mL (2.14×10^6 c/mL–2.25×10^7 c/mL) 90 days post-biopsy (T_3; $p < 0.05$; Figure 4C).

3.5 Correlation of torque teno virus load to immunosuppressive medication, histopathological lesion score, and BK viremia

There was no significant correlation between TTV load and time since transplantation or IS scale when considering all KTR, irrespective of the timing of the biopsy ($r = -0.17$ and $r = 0.03$, respectively; Table 2). When only including KTR with an indication biopsy at least 30 days post-transplantation whose viral loads had already increased to higher levels post-transplantation (N = 84), TTV load correlated significantly and strongly with time since transplantation and moderately with IS scale ($r = -0.53$, $p < 0.001$ and $r = 0.28$, $p < 0.01$, respectively; Table 2). When examining the correlation between single immunosuppressants to TTV load, only an immunosuppressive regimen including everolimus showed a significant, negative correlation to TTV load ($r = -0.20$, $p < 0.05$; Table 3).

When analyzing the correlation between TTV loads at the time of biopsy and histopathological BANFF lesion scores, TTV load correlated weakly to BANFF lesion score for inflammation (i; $r = 0.23$, $p < 0.05$) and moderately to polyomavirus-associated interstitial nephritis score (PVI, $r = 0.35$, $p < 0.001$), while there was no significant correlation to other BANFF lesion scores (Supplementary Table S3).

In KTR with BKVAN, there was a moderate correlation between BKV loads and TTV loads, with higher BK viremia being

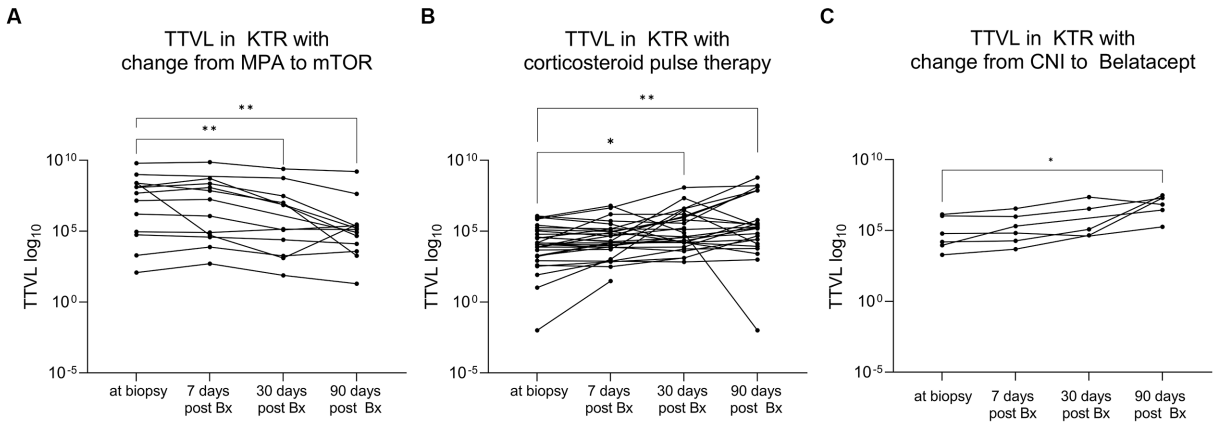

FIGURE 4
Dynamic changes in torque teno virus load following changes in immunosuppression. **(A)** KTRs with conversion from MPA to mTOR inhibitor following diagnosis for BKVAN (N = 13) showed a significant decrease in viral load. The *x*-axis displays the different time points of TTV sampling while TTVL is presented on the *y*-axis. The lines connect different samples of the same patient. **(B)** KTRs who received high-dose corticosteroid pulse therapy to treat rejection (N = 31) showed a significant increase in viral load. The *x*-axis displays the different time points while TTVL is presented on the y-axis. The lines connect different samples of the same patient. **(C)** KTRs who converted from CNI to belatacept (*N* = 6) showed a significant increase in TTVL. The x-axis displays the different time points while TTVL is presented on the y-axis. The lines connect different samples of the same patient. BKVAN, BK virus-associated nephropathy; Bx, biopsy; CNI, calcineurin inhibitor; KTRs, kidney transplant recipients; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin; TTV, torque teno virus; TTVL, torque teno virus load; $^{**}p < 0.01$; $^{*}p < 0.05$.

TABLE 2 **Correlation between torque teno virus load, immunosuppression scale, and time since transplantation.**

Variable	All KTR (*N* = 103) [+]		KTR with biopsy>30days post-Tx (*N*=84) [++]	
IS Scale: median (IQR)	6.0 (4.0–9.5)		5.0 (4.0–7.0)	
Correlation	**Spearman's rho (95% CI)**	***p* value**	**Spearman's rho (95% CI)**	***P* value**
TTVL/Time since Tx	-0.17 (-0.36–0.03)	0.09	-0.53 (-0.67– -0.35)	<0.001 (***)
IS Scale/TTVL	0.03 (-0.17–0.23)	0.76	0.28 (0.06–0.47)	0.01 (**)
IS Scale/Time since Tx	-0.74 (-0.82– -0.64)	<0.001 (***)	-0.55 (-0.68– -0.37)	<0.001 (***)

CI, confidence interval; IS, immunosuppression; KTR, kidney transplant recipients; TTVL, torque teno virus load; Tx, transplantation.
[+]five patients whose immunosuppressive regimen at biopsy included belatacept were excluded from the analysis [++] four patients whose immunosuppressive regimen at biopsy included belatacept were excluded from the analysis.

TABLE 3 **Immunosuppressive medication and correlation to torque teno virus loads.**

Immunosuppressive regimen: *N* (%)	All	Spearman's rho (95% CI)	*P*-value
Tacrolimus	82 (76)	0.17 (-0.02–0.36)	0.07
Cyclosporine	19 (18)	-0.09 (-0.28–0.11)	0.35
Mycophenolic acid	91 (84)	0.03 (-0.17–0.22)	0.78
Azathioprine	3 (3)	-0.08 (-0.27–0.11)	0.40
Everolimus	8 (7)	-0.20 (-0.38–0.00)	0.04 (*)
Sirolimus	1 (1)	-0.09 (-0.28–0.11)	0.38
Belatacept	5 (5)	0.00 (-0.20–0.19)	0.97
Corticosteroid	103 (95)	0.13 (-0.07–0.32)	0.18

CI, confidence interval; $^{*}P < 0.05$.

associated with higher TTV load ($r = 0.36$, $p < 0.05$). Following conversion from MPA to mTOR inhibitor (N = 13), a notable reduction in BKV loads was observed when comparing levels at 30 and 90 days post-biopsy to BKV loads 7 days post-biopsy ($p = 0.01$ for both, Supplementary Figure S1). Of thirteen, three KTR (23%) demonstrated an increase in BK viremia, despite a concurrent decrease in TTV loads. When comparing BKV and TTV loads at time of biopsy to those documented 90 days post-biopsy, there was no significant correlation between the delta viral loads ($p = 0.57$).

4 Discussion

Our results validate the potential of quantifying TTV loads to monitor immunocompetence in KTR within the first year post-transplantation. However, it appears to be challenging to correctly identify patients with borderline lesions suspicious for TCMR even within the first year post-transplantation, as well as differentiating between KTR with BKVAN or rejection and those without beyond 12 months post-transplantation, solely based on TTV loads. On another note, we show that TTV loads mirror adjustments in immunosuppressive therapy, albeit TTV loads do not seem to be affected immediately (within 7 days) following corticosteroid pulse therapy or switching immunosuppression to an mTOR-based regimen.

Our data demonstrate that KTR with BKVAN had significantly higher TTV loads compared with KTR with rejection or other pathologies, which was also reflected by a moderate correlation between TTV loads to histopathological SV40 positivity. In addition, higher BKV loads were associated with higher TTV loads in KTR with BK viremia, as previously shown by Solis et al. (30). Higher TTV loads may reflect a higher immunosuppressive burden, possibly fueling the replication of BK virus and the potential development of BKVAN. While a general association between TTV loads and BK viremia in the first 3 months post-transplantation may not be evident (31), possibly due to other confounding factors such as induction therapy, our findings suggest that higher TTV loads could help in identifying patients with risk for BKVAN, especially in the first year post-transplantation.

On the other hand, KTRs with active rejection had significantly lower TTV loads compared with KTRs with BKVAN; however, no significant differences were seen between KTRs with histopathologically diagnosed rejection and those with histopathology other than rejection or BKVAN. This could be attributed to the substantial number of patients with borderline lesions ($N = 23$) among those with rejection ($N = 36$). Despite the ongoing controversy regarding the pathological relevance and the need for treatment of borderline lesions, previous studies have associated these lesions with adverse outcomes such as late rejection, functional impairment, donor-specific antibody formation, and allograft failure (32), which would advocate for the necessity to timely detect KTRs with those lesions.

Jaksch et al. proposed an optimal TTV load range of 4.6 $\log_{10}$ c/mL to 6.6 $\log_{10}$ c/mL for KTR within 3 months and 1 year post-transplantation that strikes a balance between risks for rejection and infection (33). In our study cohort, there were 17 KTRs within this time frame: of the eight patients, seven (88%) with BKVAN were above the proposed upper cut-off indicating an elevated risk for infection ($p < 0.05$). Applying the lower threshold to identify patients at risk for rejection however only correctly diagnosed two patients with active TCMR, while all four patients with borderline changes had TTV loads above the threshold and would have been missed ($p = 0.51$). Admittedly, the small sample size ($N = 17$) to whom the cut-offs were applicable in our cohort limits a definitive conclusion; however, the proposed cut-offs by Jaksch et al. appear to be promising indicators for infection but seem to fail to early identify patients with borderline changes suspicious for TCMR. Corresponding to that, the AUC of 0.62 to discriminate rejection from no rejection in KTRs that were biopsied within 1 year post-transplantation in our study cohort was rather weak and not conclusive.

The results to correctly identify rejection or BKVAN beyond 12 months post-transplantation were rather disappointing. This may be attributed to the fact that we noted significant variations in TTV loads depending on the timing of the indication biopsy relative to transplantation, even beyond the first year post-transplantation, which is consistent with previous research (34, 35). Furthermore, we revealed a significant association between higher TTV loads and age, which has been demonstrated before for healthy adults (36, 37) as well as for KTRs (20, 34), and may be explained by immunosenescence and the accompanying higher susceptibility to pathogens in the elderly population (38). Moreover, a higher BMI was linked to elevated TTV loads, suggesting impaired immune functionality in obese patients compared with non-obese individuals (39).

Of note, TTV loads reflected changes in immunosuppressive therapy within our study. KTRs switching to mTOR inhibitors following diagnosis for BKVAN displayed a notable decrease in TTV loads, consistent with the negative correlation we found between mTOR inhibitor intake and TTV loads. Our findings align with results obtained by Schiemann et al., revealing significantly reduced TTV levels in KTRs under mTOR inhibitor-based maintenance therapy (34). This effect may reflect the antiviral properties of mTOR inhibitors but possibly also the generally reduced efficacy compared with CNIs (40). Contrarily, patients receiving high-dose corticosteroid pulse therapy for rejection subsequently developed significantly higher TTV loads. This aligns with de Vlaminck et al. who reported that higher prednisone doses early after transplantation result in an increased presence of TTV (18). Our results add that TTV loads could additionally serve as an indicator of the higher immunosuppressive burden in KTRs receiving anti-rejection therapy beyond the first year following transplantation. Furthermore, our data support the hypothesis that changes in TTV load reflect modifications in immunosuppression, as previously shown for short-time cessation of mycophenolate in KTRs (21, 22). Interestingly, the decline in TTV loads observed in our patients with BKVAN transitioning from MPA to an mTOR-based regimen did not consistently align with a reduction in BK viremia, preventing the utilization of TTV loads as a means to monitor treatment response in individuals with BK viremia. Additionally, further investigation is needed to determine the extent and pace of changes in TTV loads as a response to modifications in CNI dosages, as well as the potential utility of TTV in guiding CNI dosage adjustments (24).

It seems evident that simple tools such as the immunosuppression scale proposed by Vasudev et al. in 2005 to evaluate the degree of immunosuppression in KTRs with BKVAN (7) seem insufficient to monitor both, infections and rejections post-transplantation. Within our cohort, TTV loads correlated more strongly to time since transplantation than to the IS scale, emphasizing that a mere scoring system for various immunosuppressants does not sufficiently capture the true extent of immunosuppression in a patient. Our data suggest that TTV loads are more accurate than the IS scale or drug trough levels in reflecting the immunosuppressive burden in immunocompromised patients and may prove particularly useful for monitoring complications related to over- or under immunosuppression in older and obese patients.

In general, our study has some limitations. First, the single-center design compromises external generalization. Second, the small sample size of some sub-analyses may limit their validity. All proposed correlations should therefore merely be considered as hypothesis-generating, emphasizing the need for further studies. Additionally, as no re-biopsies were performed within this study cohort, it was not possible to establish a correlation between TTV loads and potential histopathological resolution of injury in patients experiencing rejections or BKVAN.

In conclusion, we were able to reproduce previous findings that TTV loads are highest in patients within the first year after transplantation and gradually become lower in patients transplanted long ago, corresponding to a reduction in immunosuppression following transplantation. To guide immunosuppressive therapy based on TTV loads, one should consider inter- and intraindividual variations, as well as confounding factors such as age, BMI, and, most

importantly, time since transplantation, even beyond the first year post-transplantation. Another potential use case of monitoring TTV loads could be to follow up on changes in immunosuppressive therapy, although viral replication does not appear to be immediately impacted following corticosteroid pulse therapy or the transition to an mTOR-based immunosuppressive regimen.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Ethikkommission der Medizinischen Fakultät Heidelberg. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MRe: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. CMo: Funding acquisition, Resources, Supervision, Validation, Writing – review & editing. CS: Validation, Writing – review & editing. MRu: Data curation, Writing – review & editing. CB: Investigation, Methodology, Supervision, Writing – review & editing. JK: Investigation, Methodology, Supervision, Writing – review & editing. CMa: Data curation, Writing – review & editing. FK: Data curation, Writing – review & editing. CN: Data curation, Writing – review & editing. JB: Data curation, Writing – review & editing. MZ: Data curation, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. RB: Methodology, Project administration, Resources, Supervision, Writing – review & editing. PS: Data curation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. LB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft.

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Conflict of interest

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

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Supplementary material

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Frequency and impact on renal transplant outcomes of urinary tract infections due to extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella species*

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Background: *Enterobacterales* are often responsible for urinary tract infection (UTI) in kidney transplant recipients. Among these, *Escherichia coli* or *Klebsiella species* producing extended-spectrum beta-lactamase (ESBL) are emerging. However, there are only scarce data on frequency and impact of ESBL-UTI on transplant outcomes.

Methods: We investigated frequency and impact of first-year UTI events with ESBL *Escherichia coli* and/or *Klebsiella species* in a prospective multicenter cohort consisting of 1,482 kidney transplants performed between 2012 and 2017, focusing only on 389 kidney transplants having at least one UTI with *Escherichia coli* and/or *Klebsiella species*. The cohort had a median follow-up of four years.

Results: In total, 139/825 (17%) first-year UTI events in 69/389 (18%) transplant recipients were caused by ESBL-producing strains. Both UTI phenotypes and proportion among all UTI events over time were not different compared with UTI caused by non-ESBL-producing strains. However, hospitalizations in UTI with ESBL-producing strains were more often observed (39% versus 26%, $p = 0.04$). Transplant recipients with first-year UTI events with an ESBL-producing strain had more frequently recurrent UTI (33% versus 18%, $p = 0.02$) but there was no significant difference in one-year kidney function as well as longer-term graft and patient survival between patients with and without ESBL-UTI.

Conclusion: First-year UTI events with ESBL-producing *Escherichia coli* and/or *Klebsiella species* are associated with a higher need for hospitalization but do neither impact allograft function nor allograft and patient survival.

KEYWORDS

kidney transplantation, urinary tract infection, Enterobacterales, *E. coli*, *Klebsiella*, ESBL – extended-spectrum beta-lactamase, graft survival

1 Introduction

Infections are still an important cause of morbidity and mortality following kidney transplantation (1–3). Since the most intense immunosuppression is applied during the first year post-transplant, the incidence of infections is highest during this period (4). Urinary tract infection (UTI) comprises the most frequently observed type of infection (5, 6). As causative pathogens, *Enterobacterales* play a major role and are responsible for UTI in 50 to 80% of cases, mostly caused by *Escherichia (E.) coli* and *Klebsiella spp*. (7, 8). While susceptible strains can be treated by commonly available antibiotics, the increasing percentage of infections by *Enterobacterales* producing extended-spectrum beta-lactamase (ESBL) often requires treatment with carbapenems that need to be applied intravenously and are more expensive compared with most standard antibiotics. In addition, infections with ESBL-producing strains have been associated with a higher clinical and economic burden of disease, longer duration of hospitalization as well as increased mortality (9, 10).

Kidney transplant recipients might be particularly prone to develop UTI with ESBL-producing *Enterobacterales* due to the antimicrobial escape pressure provoked by the use of antibiotic prophylaxis as well as early empiric treatment in case of suspected infection. In addition, UTI with ESBL-producing strains may affect the outcome of transplantation. Only few single-center studies have so far investigated UTI by ESBL-producing *Enterobacterales* in kidney transplant recipients in more detail. In a cohort of kidney transplant recipients from Paris (France), Pilmis et al. described an 11% prevalence of bacteriuria with ESBL-producing strains and about 50% of patients developed an UTI (11). In another larger study from Spain, Bodro et al. found a higher proportion of UTI caused by ESBL-producing *Enterobacterales* among transplant recipients with recurrent episodes of UTI versus non-recurrent UTI (12). Brakemeier et al. reported a lower patient survival but similar death-censored allograft survival in patients with ESBL-UTI compared with a control group (13). Notably, patients of all cohorts were transplanted about or even more than 10 years ago and were, as expected, frequently on cyclosporine and mTOR inhibitors for maintenance immunosuppression, which does not represent the current standard of immunosuppression. Only the study of Brakemeier et al. did investigate the role of UTI with ESBL-producing *Enterobacterales* with respect to graft and patient survival. Therefore, the aim of this study was to describe the frequency as well as the impact of first-year ESBL-UTI on transplant outcomes in a large nationwide contemporary cohort of kidney transplant recipients.

Abbreviations: CFU, colony forming units; DSA, donor-specific HLA antibodies; eGFR, estimated glomerular filtration rate; ESBL, extended-spectrum beta-lactamase; *E. coli*, *Escherichia coli*; UTI, urinary tract infection; *spp.*, *species*; STCS, Swiss Transplant Cohort Study.

2 Materials and methods

2.1 Data source

The Swiss Transplant Cohort Study (STCS) is a multicenter, observational and long-term follow-up cohort project recruiting solid organ transplant recipients at all six Swiss transplant centers since 2008. Design, methodology and details on the cohort of the STCS have been previously published (14, 15). This study (project number FUP168) was nested within the STCS and separately approved by the ethics committee of Northwestern and Central Switzerland (www.eknz.ch; project ID 2021-00360). Detailed patient- and transplant-specific data, including infectious disease episodes, are prospectively collected in the STCS. In addition, information on ESBL-production of the causative pathogen has been captured since 2012.

2.2 Study cohort

Between January 2012 and December 2017, 1799 kidney transplantations were performed in Switzerland. For this study, 317 (18%) transplantations were excluded for the following reasons: no STCS consent ($n = 151$), multiorgan transplants ($n = 96$), pediatric recipients ($n = 60$), missing pre-transplant donor-specific HLA antibody assignment ($n = 8$), no complete first-year follow-up ($n = 2$). This resulted in a cohort consisting of 1,482 transplants in adults eligible for study inclusion (Figure 1). In order to be able to comparatively analyze the impact of first-year ESBL-UTI, we subsequently focused only on transplants experiencing at least one first-year UTI event with *E. coli* and/or *Klebsiella spp*. This decision was made because 96% of all infections caused by ESBL-producing strains belonged to these two bacterial species. The final study population consisted of 389 kidney transplants (Figure 1). The median age of the cohort was 56 years (47–64 years) and 58% were women.

2.3 Definitions

For this study, the same classification of UTI events as in a previous study of our group was used (7). Briefly, all UTI events were classified by an infectious disease specialist and/or nephrologist based

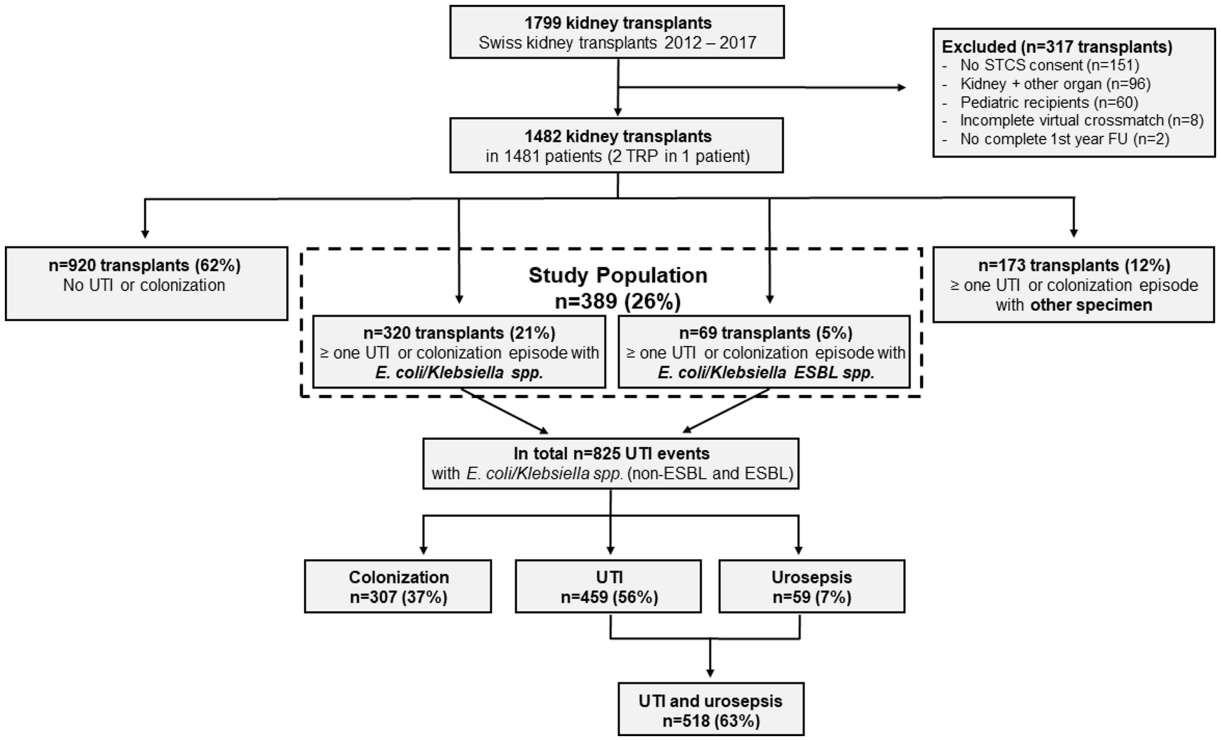

FIGURE 1
Study flowchart. ESBL, extended-spectrum beta-lactamase; *E. coli, Escherichia coli*; FU, follow-up; STCS, Swiss Transplant Cohort Study; TRP, transplantation; UTI, urinary tract infection.

on microbiological cultures, urine analyses, and recorded clinical symptoms as follows:

a) Urinary colonization (equivalent to 'asymptomatic bacteriuria/UTI') was defined as the presence of bacteria in the urine with $\geq 10^5$ colony forming units (CFU)/ml in the absence of local and systemic signs or symptoms of infection.
b) UTI was defined as the presence of bacteria in the urine with $\geq 10^5$ CFU/mL in the presence of local and/or systemic signs or symptoms of infection. No distinction between lower UTI (i.e., cystitis) and upper UTI (i.e., pyelonephritis) was recorded in the STCS database.
c) Urosepsis was defined as the detection of the same pathogen in urine and blood cultures in the presence of local and/or systemic symptoms of infection.

Recurrent UTI were defined as ≥ three UTI events within the first year. At all six transplant centers, urine cultures were taken in case of leucocyturia and/or symptoms referring to an UTI. Additionally, at one center, urine cultures were taken at each consultation during the first 6 months after transplantation.

2.4 Treatment of UTI

At all transplant centers, UTI were consistently treated. Colonizations were only treated in 2/6 centers early after transplantation (for the first 6 months after transplantation and as long as the double J-stent was *in situ*, respectively). At all centers, patients with recurrent UTI underwent thorough clinical work-up for underlying gynecological or urogenital pathologies.

2.5 Catheter policy and infection prophylaxis

At all six kidney transplant centers, the allograft recipients received a Foley catheter after transplantation, which was removed between postoperative days 4 and 7. A double J-stent was inserted during transplantation as a standard procedure in 5/6 transplant centers, which was removed between two and eight weeks after transplantation. At all centers, patients received trimethoprim-sulfamethoxazole as pneumocystis prophylaxis for 6 months after transplantation. Additionally, at one transplant center, the patients received antibiotic prophylaxis with either amoxicillin/clavulanic acid or ciprofloxacin until the double J-stent was removed.

2.6 Diagnosis of rejection

Transplant biopsies were performed at any time in case of suspected rejection or unexplained graft dysfunction. Only one of the six Swiss transplant centers performed protocol biopsies at month 3 and month 6 on a regular basis. Biopsy-proven rejection episodes were graded according to the Banff 2017 classification, excluding the 'borderline changes' category.

2.7 Outcomes

Data of the study population were analyzed on the patient level as well as on the UTI level. On the UTI level, we investigated the incidence of infections with *E. coli and/or Klebsiella spp*., the proportion of UTI with ESBL-producing strains as well as the frequency of treatment and the risk for hospitalization due to UTI. On the patient level, the investigated outcomes were graft function (i.e., estimated glomerular filtration rate [eGFR] according to the Chronic Kidney Disease Epidemiology Collaboration equation) at one-year post-transplant, occurrence of rejection as well as short- and long-term death-censored allograft and patient survival.

2.8 Statistical analysis

JMP Pro version 16 software (SAS Institute Inc., Cary, NC, United States) was used for statistical analysis. Data were visualized by GraphPad Prism version 10 (GraphPad Software, San Diego, CA, United States). Categorical data are presented as counts and/or percentages and were analyzed by chi-square test or Fisher's exact test as appropriate. Continuous data are shown as median and interquartile ranges (IQR) and compared by Wilcoxon rank sum tests. For all tests, a (two-tailed) value of $p < 0.05$ was considered to indicate statistical significance. Survival curves were generated by the Kaplan–Meier method, and the groups compared using the log-rank test.

3 Results

3.1 Baseline characteristics of patient groups

On the patient level, 389 kidney transplant recipients experienced UTI events with *E. coli* and/or *Klebsiella spp*. Of these, 18% (69/389) had a least one UTI event with an ESBL-producing strain within the first year post-transplant (Figure 1). The baseline characteristics of the cohort grouped by the ESBL status are detailed in Table 1. Transplant recipients in both groups were in median 56 years old. In both the non-ESBL and the ESBL group, female sex was more common (60% versus 51%, respectively) but there were no significant differences between the groups ($p = 0.16$). There was also no difference with respect to the underlying renal diseases, with a special focus on those that may pose patients at higher risk for UTI events. In this contemporary cohort, both groups were mostly (89% versus 87%, respectively) treated with a maintenance immunosuppression consisting of tacrolimus, mycophenolate and steroids and 29% in both groups received an induction therapy with a T cell-depleting agent.

TABLE 1 Baseline characteristics of patients with *Escherichia coli* and/or *Klebsiella spp*. UTI in the first year post-transplant grouped according to ESBL status.

Parameter	No ESBL ($n = 320$)	ESBL ($n = 69$)	p-value
Recipient age	56 (47–63)	56 (46–65)	0.81
Female sex	192 (60%)	35 (51%)	0.16
Recipient renal disease			0.56
- ADPKD	75 (23%)	21 (30%)	
- Diabetic Nephropathy	22 (7%)	6 (9%)	
- Reflux/Pyelonephritis	29 (9%)	5 (7%)	
- Other	194 (61%)	37 (54%)	
RRT prior to transplantation			0.46
- HD	228 (71%)	54 (78%)	
- PD	37 (12%)	5 (7%)	
- None	54 (17%)	10 (15%)	
Donor age	54 (43–63)	55 (47–64)	0.61
Deceased donor	200 (63%)	47 (68%)	0.38
Cold ischemia time [h]	7.2 (1.8–10.4)	8.6 (2.0–11.9)	0.12
CMV constellation			0.80
- High risk	54 (17%)	13 (19%)	
- Intermediate risk	204 (64%)	44 (64%)	
- Low risk	58 (18%)	12 (17%)	
- Unknown	4 (1%)	0	
Pre-transplant HLA-DSA	71 (22%)	12 (17%)	0.38
AB0 incompatible	22 (7%)	4 (6%)	0.75
A/B/DRB1 mismatches	4 (3–5)	4 (3–5)	0.43
A/B/DRB1-5/DQB1 mismatches ($n = 355$)	5 (4–7)	5 (4–7)	0.30
Induction therapy			0.93
- ATG/Thymoglobulin	94 (29%)	20 (29%)	
- Basiliximab	223 (70%)	48 (70%)	
- None	3 (1%)	1 (1%)	
Maintenance immunosuppression			0.84
- FK/MPA/Pred	286 (89%)	60 (87%)	
- CyA/MPA/Pred	27 (9%)	7 (10%)	
- Other	7 (2%)	2 (3%)	

ADPKD, autosomal polycystic kidney disease; RRT, renal replacement therapy; HD, Hemodialysis; PD, Peritoneal dialysis; CMV, cytomegalovirus; HLA-DSA, donor-specific HLA antibodies; ATG, anti-T cell globulin; Tac, tacrolimus; MPA, mycophenolic acid; Pred, prednisone; CyA, cyclosporine.

3.2 Major one-year outcomes according to ESBL status

We compared major one-year outcomes among transplant recipients according to their grouped ESBL status (Table 2). Overall, graft loss and patients' death were rare events in both groups and no statistically significant differences were observed (2.2% versus 2.9%, $p = 0.72$). Graft function at one year did not differ among the two groups (Table 2, Figure 2). Furthermore, we did not observe differences with respect to the occurrence of rejection. However, transplant recipients with at least one first-year UTI event with an ESBL-producing strain experienced more frequently colonization episodes ($p = 0.0004$). In addition, there was a significantly higher proportion of recurrent UTI in the ESBL group (18.4% versus 33.3%, respectively). Notably, the number of severe UTI, namely urosepsis episodes, was not statistically significant different among the two groups ($p = 0.83$).

Within the ESBL group, infections with an ESBL-producing strain were in 34 (34/69; 49%) transplant recipients the first recorded UTI event. Among the other 35 patients, a majority (26/35; 74%) had previously received an antibiotic therapy for an antecedent non-ESBL

TABLE 2 First-year outcomes in patients with *Escherichia coli* and/*or Klebsiella spp*. UTI in the first year post-transplant grouped according to ESBL status

Parameter	No ESBL (*n* = 320)	ESBL (*n* = 69)	*p*-value
Graft loss or death	7 (2.2%)	2 (2.9%)	0.72
Death	5 (1.6%)	1 (1.5%)	0.95
Graft loss	2 (0.6%)	1 (1.5%)	0.48
eGFR [ml/min]	49 (38–65)	50 (37–67)	0.98
Number of transplant biopsies			
- None	137 (42.8%)	29 (42.0%)	0.98
- One	100 (31.3%)	21 (30.4%)	
- Two	58 (18.1%)	14 (20.3%)	
- More than two	25 (7.8%)	5 (7.3%)	
Number of rejections			
- None	275 (85.9%)	58 (84.1%)	0.16
- One	39 (12.2%)	7 (10.1%)	
- Two or more	6 (1.9%)	4 (5.8%)	
Number of colonization episodes	1 (0–2)	2 (1–3)	**0.0004**
Number of UTI			
- None	86 (26.9%)	19 (27.6%)	**0.02**
- One	117 (36.6%)	15 (21.7%)	
- Two	58 (18.1%)	12 (17.4%)	
- More than two	59 (18.4%)	23 (33.3%)	
UTI phenotype			
- Only colonization	86 (26.9%)	19 (27.6%)	**0.013**
- Occasional UTI (1–2 UTI)	175 (54.7%)	27 (39.1%)	
- Recurrent UTI (≥3 UTI)	59 (18.4%)	23 (33.3%)	
Any Urosepsis episodes			
- None	282 (88.2%)	58 (84.1%)	0.83
- One	32 (10%)	9 (13.1%)	
- Two	3 (0.9%)	1 (1.4%)	
- More than two	3 (0.9%)	1 (1.4%)	

eGFR, estimated glomerular filtration rate; UTI, urinary tract infection.
P-values indicating significant results are shown in bold.

UTI event. These non-ESBL UTI events were mostly (21/26; 81%) previous UTI or urosepsis episodes and in only 5 patients (5/26; 19%) previously treated colonization (data not shown).

3.3 Impact of ESBL-UTI on longer-term patient and graft survival and evolution of graft function

Then, we focused on the impact of UTI with ESBL-producing strains on the longer-term patient and allograft survival (Figure 3). Patients were followed for a median of 4.0 years (2.1–5.1 years). Both death-censored allograft survival and patient survival were not different among the two groups ($p = 0.63$ and $p = 0.67$, respectively). This finding did not change when we excluded patients who had only colonization episodes (n = 84; 28% and n = 18; 28%, respectively) but no UTI. Notably, the outcome of both groups was similar when compared to transplant recipients without any first-year UTI event (Supplementary Figure 1).

Beside patient and death-censored graft survival, we also investigated the evolution of graft function on the longer term. As expected, there was no statistically significant difference between the two groups at three and five years post-transplant (Figure 2).

3.4 Incidence of infections with *Escherichia coli* and/or *Klebsiella spp*. on the UTI level

In a next step, we focused on details of the infections on the UTI level. In the cohort consisting of 389 kidney transplants, 1,133 UTI events occurred in total. Of these, 825/1133 (73%) were caused by *E. coli* and/or *Klebsiella spp*. (Figure 1).

Most of these UTI events could be exclusively attributed to *E. coli* and/or *Klebsiella spp*. (719/825; 87%, Figure 4A). In about 5% of cases, both pathogens were found at the same time. If concomitant bacteria were present, these were mostly *Enterococcus spp*. (67/106; 63%, data not shown). As expected, the frequency of UTI events with *E. coli* was higher than with *Klebsiella spp*. (Figure 4A).

Regarding the clinical phenotypes observed among all UTI events caused by *E. coli* and/or *Klebsiella spp*. (non-ESBL and ESBL), 37% (307/825) were colonization and 56% (459/825) UTI episodes (Figure 1). Urosepsis episodes were considerably less frequent (7%, 59/825;).

Overall, ESBL-producing strains were detected in 139/825 (17%) of all UTI events caused by *E. coli* and/or *Klebsiella spp*. (Figure 4B). The distribution of *E. coli* and/or *Klebsiella spp*. among UTI events as compared with non-ESBL-producing strains was very similar (Figure 4B). We did not observe a difference in the proportion of UTI with ESBL-producing strains with respect to the clinical phenotype (62/307; 20% of colonization, 67/459; 15% of UTI, 10/59; 17% of urosepsis episodes, respectively, $p = 0.13$). In addition, the proportion of UTI events with an ESBL-producing strain remained rather stable over time (Figure 5).

3.5 Frequency of treatment and risk for hospitalization

Compared with UTI and urosepsis episodes that were almost always treated with antibiotics (100% in urosepsis and 99.4% in UTI), colonization was only treated in 66/307 episodes (21%). Moreover, when comparing colonization episodes with and without ESBL-producing strains, treatment frequency was very similar (53/245; 21.6% without and 13/62; 21.0% with ESBL, $p = 0.91$).

Within the dataset, information on the need for infection-related hospitalization was available in 97% of UTI events. Hospitalization was required in 97% of urosepsis and 28% of UTI events. In cases of colonization, hospitalization only rarely occurred (<3%). While urosepsis almost always prompted hospitalization regardless of the ESBL status (100 and 95.9%, respectively), there was a significantly higher proportion of hospitalizations in UTI with ESBL-producing strains (39% versus 26%, $p = 0.04$).

4 Discussion

In this nationwide multicenter study, we investigated the impact of UTI due to ESBL-producing *E. coli* and/or *Klebsiella spp*. on renal

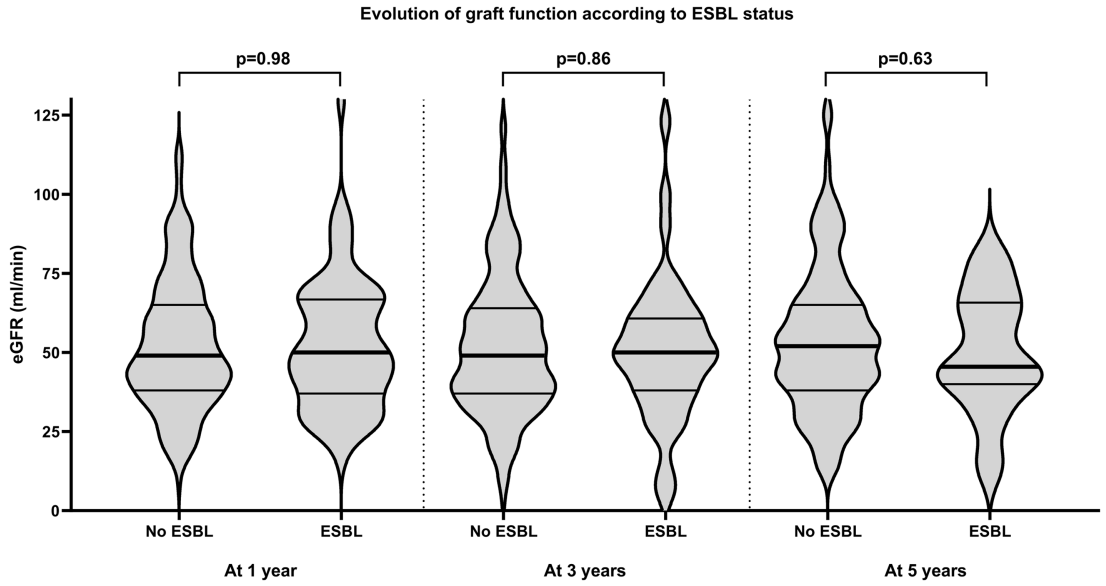

FIGURE 2
Evolution of graft function in patients with *Escherichia coli* and/*or Klebsiella spp*. UTI in the first year post-transplant grouped according to ESBL status, shown by violin plots (the lines represent the median (bold) as well as the interquartile ranges). eGFR, estimated glomerular filtration rate; ESBL, extended-spectrum beta-lactamase.

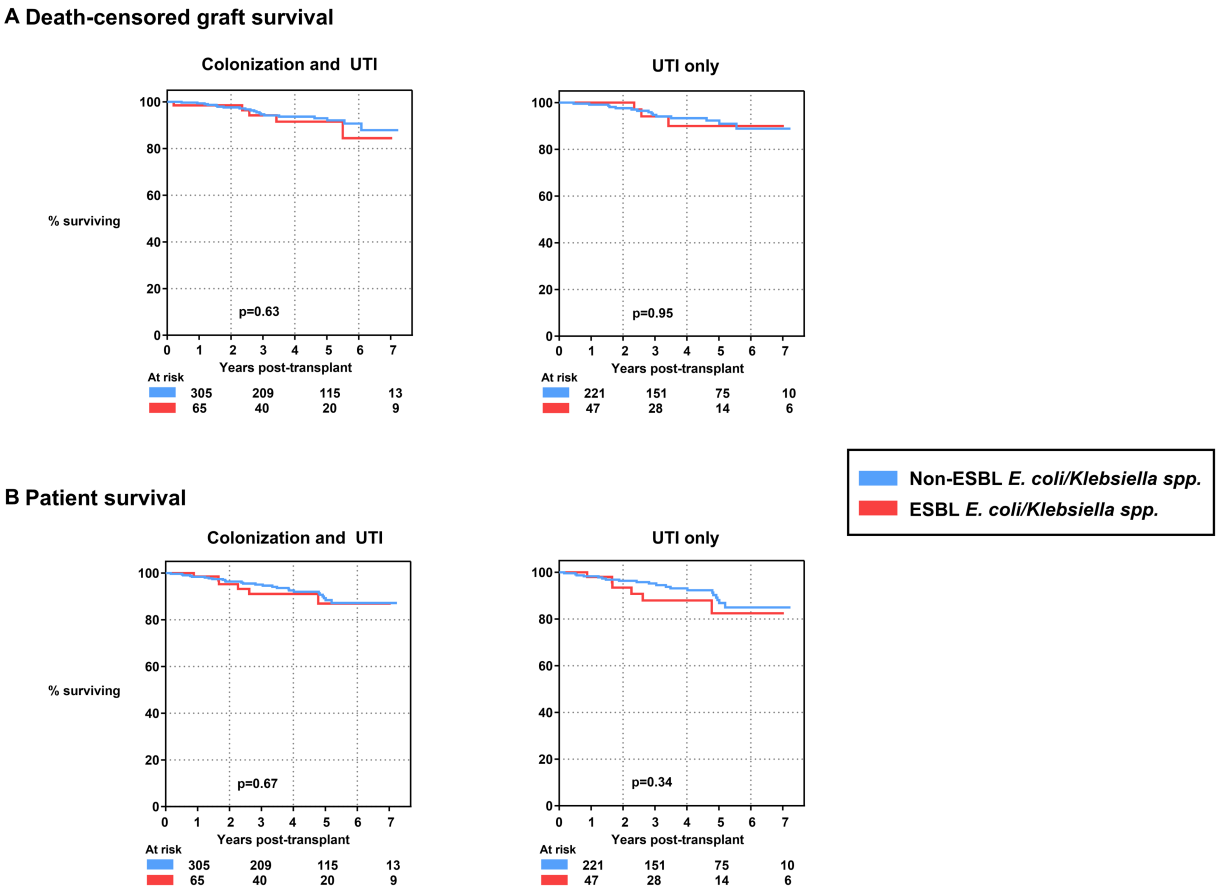

FIGURE 3
(A) Death-censored graft survival of kidney transplants experiencing first-year UTI events with and without ESBL-producing *E. coli* and/or *Klebsiella spp*., shown for colonization and UTI events (left) as well as UTI only (right). **(B)** Patient survival of kidney transplants experiencing first-year UTI events with and without ESBL-producing *E. coli* and/or *Klebsiella spp*., shown for colonization and UTI events (left) as well as UTI only (right). ESBL, extended-spectrum beta-lactamase; *E. coli, Escherichia coli*; UTI, urinary tract infection.

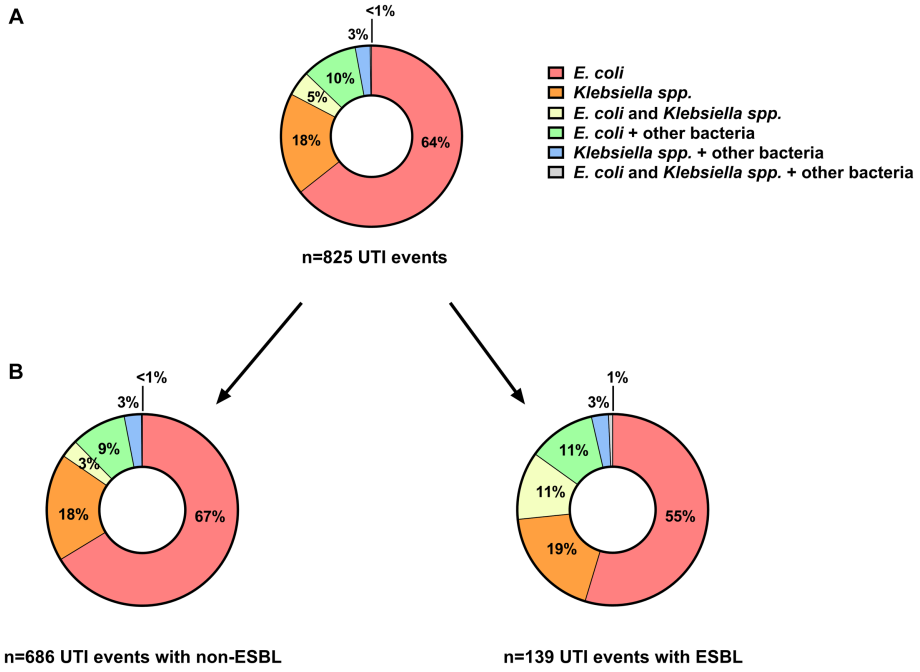

FIGURE 4
Distribution of *E. coli* and/or *Klebsiella spp.* in **(A)** all UTI events (n = 825) as well as separately shown in **(B)** UTI events without (n = 686) and with (n = 139) ESBL-producing strains. ESBL, extended-spectrum beta-lactamase; *E. coli, Escherichia coli*; UTI, urinary tract infection.

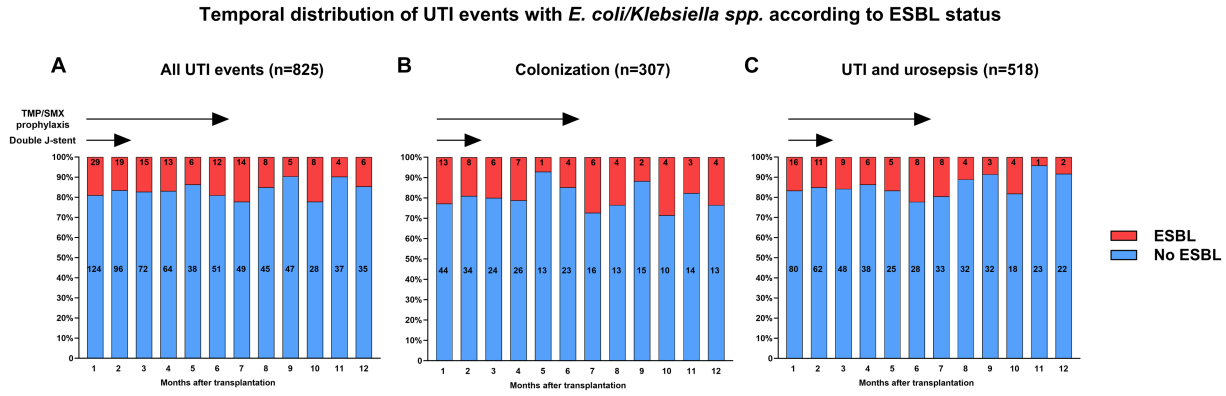

FIGURE 5
Temporal distribution of UTI events with *E. coli/Klebsiella spp.* grouped by the presence and absence of an ESBL-producing strain for **(A)** all UTI events as well as separately shown for **(B)** colonization and **(C)** UTI and urosepsis episodes. ESBL, extended-spectrum beta-lactamase; *E. coli, Escherichia coli*; TMP/SMP, trimethoprim-sulfamethoxazole; UTI, urinary tract infection.

transplant outcomes. The key observation of this study is that occurrence of UTI with ESBL-producing strains did not affect allograft and patient survival. Furthermore, there was no difference in allograft function between patients with and without at least one UTI event with an ESBL-producing strain.

The results of this study suggest a limited clinical and predominantly epidemiological significance of ESBL-producing *Enterobacterales*. In this regard, our results are in part contradictory compared with results of previous studies pointing toward a lower patient survival, a higher case fatality rate as well as a higher virulence of infections caused by such resistant bacterial strains (13, 16, 17). Some reasons might explain these discrepancies. First, we hypothesize that the clinical overall awareness for ESBL-producing bacteria increased over the last 5–10 years, which might have influenced management of patients in case of a lacking clinical response following initial empiric therapy. Secondly, antibiotic resistance profiles are nowadays usually available within 24 to maximum 72 h, facilitating a timely adaptation of antibiotics. Third, the percentage of severe UTI events, namely urosepsis episodes, caused by ESBL-producing strains was rather low (in total 10 events) in our cohort. Therefore, we cannot exclude that a delay of appropriate treatment is more detrimental in this particular subgroup. However, the overall rather low frequency of urosepsis episodes of 7% in our cohort generally suggests that current post-transplant surveillance and instruction of patients is often able

to prevent development of such severe infections (i.e., urosepsis), which might mitigate a potential risk conferred by ESBL production in this regard.

Consistent with the study of Bodro et al. we found that transplant recipients in the ESBL group had a higher proportion of recurrent UTI (12). In addition, there was a significantly higher proportion of hospitalizations required in UTI with ESBL-producing strains. These results underline the economic burden of disease (10, 18). It seems likely that, given the limited availability of outpatient parenteral antibiotic application services, intravenous application was often the main reason for hospitalization. Since this might have provoked a tendency toward a shorter treatment, it could also explain more recurrent UTI in the ESBL group. Facing lacking options for oral treatment in infections with ESBL-producing bacteria in many cases, improvement of management options by expansion of outpatient services for intravenous antibiotic administration as well as better antibiotic counseling in terms of optimal treatment duration are needed.

In this study, we observed that 18% of transplant recipients developing UTI with *E. coli* and/or *Klebsiella spp*. within the first year post-transplant experience at least one infection with an ESBL-producing strain. With respect to the whole cohort consisting of 1,482 transplants, this corresponds to an absolute frequency of 5%. The detected frequency is consistent with results of other studies reporting an overall prevalence of about 5% among kidney transplant recipients and a proportion of 20% among *Enterobacterales* in Europe (11, 19). Neither classical risk factors for UTI, such as female sex and the type of underlying renal disease (e.g., ADPKD, Diabetes, reflux nephropathy), nor the intensity of immunosuppression, such as an induction therapy with a T cell-depleting agent, were associated with the occurrence of UTI with an ESBL-producing strain. In addition, there was a rather stable monthly proportion of 10 to 20% of ESBL-UTI over time, making an influence of the antibiotic prophylaxis with trimethoprim-sulfamethoxazole for pneumocystis prevention as well as the DJ catheter *in situ* rather unlikely. Notably, we observed that the infection with an ESBL-producing strain was the first recorded UTI event in about 50% of transplant recipients ultimately developing such an UTI, suggesting that pre-existing unrecognized colonization or acquisition in the community might be underestimated risk factors. While pre-existing colonization could potentially be counterbalanced by the use of perioperative prophylaxis with carbapenems, future studies should focus on further delineating the mechanism of infection as well as the prevention of selection pressure conferred by antecedent antibiotic therapies (20, 21). In this regard, it is important to further study the wide range of virulence factors, especially since it was recently nicely shown that there is no regular pattern for ESBL production with respect to for instance type 3 fimbriae comprising an important superficial virulence factor (22, 23).

One particular strength of our study lies in the detailed analysis of a large and unselected multicenter cohort with a follow-up of a median of 4 years. Notably, our cohort is one of the largest focusing on UTI caused by ESBL-producing *Enterobacterales* in kidney transplant recipients. Furthermore, all patients studied were treated with contemporary immunosuppression. Another strength is the strict separation of the different UTI phenotypes (i.e., colonization, UTI and urosepsis) in our cohort.

However, our study is also subject to limitations. First, we had no information on the type and the lengths of antibiotics used for treatment of UTI. Therefore, treatment failure, especially in UTI with ESBL-producing *E. coli* and/or *Klebsiella spp*., cannot be ruled out and might have influenced the results with respect to the frequency of infections. Second, our analysis focuses only on UTI events occurring within the first year post-transplant. This decision was made based on the completeness of data as well as the fact that the first year is the period with the highest incidence of infections in general. Third, we focused only on UTI and can therefore not exclude that patients developed infections of other organs or colonization with ESBL-producing strains at other locations. However, an impact of other severe infections seems unlikely in light of the similar patient survival of both groups. Fourth, we had no information that allowed us to distinguish between upper and lower urinary tract infections. Additionally, we could not analyze complications of UTI such as the abscess development or obstruction. Lastly, the results of this study might have been influenced by local epidemiological factors and clinical practice, limiting its general validity in other countries.

In conclusion, overall 5% of all patients and about 20% of patients with UTI caused by *E. coli* and/or *Klebsiella spp*. develop at least one first-year UTI with an ESBL-producing strain. These infections are associated with a higher need for hospitalization but do not impact allograft function as well as allograft and patient survival.

Data availability statement

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

Ethics statement

The studies involving humans were approved by Ethics Committee of Northwestern and Central Switzerland (www.eknz.ch; project ID 2021-00360). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JB: Formal analysis, Investigation, Writing – original draft. MD: Writing – review & editing. DS: Writing – review & editing. LW: Writing – review & editing. DG: Writing – review & editing. OM: Writing – review & editing. FH: Writing – review & editing. DN: Writing – review & editing. AS: Writing – review & editing. KB: Writing – review & editing. TM: Writing – review & editing. TS: Writing – review & editing. NK: Writing – review & editing. SS: Conceptualization, Writing – review & editing. CW: Conceptualization, Formal analysis, Investigation, Writing – original draft.

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Conflict of interest

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1329778/full#supplementary-material

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Collapsing glomerulopathy is likely a major contributing factor for worse allograft survival in patients receiving kidney transplants from black donors

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Although a few registry-based studies have shown associations between receiving kidney allografts from Black donors and shorter allograft survival, detailed, large, single-center studies accounting for common confounding factors are lacking. Furthermore, pathologic alterations underlying this potential disparity have not been systematically studied. We performed a retrospective clinical-pathological study of kidney transplant recipients who received kidney allografts from either Black ($n = 407$) or White ($n = 1{,}494$) donors at Columbia University Irving Medical Center from 2005 to 2018, with median follow-up of 4.5 years post-transplantation. Black donor race was independently associated with allograft failure (adjusted HR = 1.34, $p = 0.02$) and recipients of kidney allografts from Black donors had a higher incidence of collapsing glomerulopathy [7.4% vs. 1.9%, OR = 4.17, $p < 0.001$]. When causes of allograft failure were examined, only allograft failure following development of collapsing glomerulopathy was more frequent in recipients of allografts from Black donors [15% vs. 5%, OR = 3.16, $p = 0.004$]. Notably, when patients who developed collapsing glomerulopathy were excluded from analysis, receiving kidney allografts from Black donors was not independently associated with allograft failure (adjusted HR = 1.24, $p = 0.10$). These findings revealed that, compared with recipients of kidney allografts from White donors, recipients of kidneys from Black donors have modestly shorter allograft survival and a higher probability of developing collapsing glomerulopathy, which negatively impacts allograft outcome. Identification of collapsing glomerulopathy risk factors may help decrease this complication and improve allograft survival, which optimally may reduce racial disparities post-transplantation.

KEYWORDS

kidney transplantation, kidney pathology, collapsing glomerulopathy, racial disparities, allograft outcomes

Introduction

While kidney transplantation is the optimal treatment for kidney failure, apparent racial and ethnic disparities persist after transplantation. Several registry-based studies have shown that kidneys from deceased Black donors exhibit shorter allograft survival compared to kidneys transplanted from White donors (1, 2). This has led to the inclusion of Black donor race in the calculation of the kidney donor profile index (KDPI) used to evaluate deceased donor kidney quality in the United States, and, thus, has contributed to an increased likelihood of kidney non-procurement and non-utilization of organs from deceased Black donors (3).

Post-transplant collapsing glomerulopathy (CG) is an infrequent complication of the kidney allograft that has detrimental effects on allograft survival (4). Whereas a few studies have suggested an association between CG and receiving kidney allografts from Black donors, especially those harboring Apolipoprotein L1 gene (*APOL1*) high-risk genotypes (4–6), systematic comparisons of the incidence and prognosis of post-transplant CG between different donor races/ethnicities are currently lacking.

We hypothesized that the association between Black donor race and inferior allograft survival is mediated by the development of CG in a minority of transplants. To address this issue, we performed the largest single-center clinical-pathologic study to date in which we aimed to confirm the disparity in outcomes between recipients of kidney allografts from Black vs. White donors, after adjusting for major confounding factors, and to investigate the histopathologic changes that may explain the observed disparities.

Materials and methods

With approval of the Institutional Review Board at Columbia University, we conducted a retrospective study of kidney transplant patients who received kidney allografts from self-identified Black and White donors at the Columbia University Irving Medical Center (CUIMC) between January 1, 2005, and December 31, 2018. Briefly, all patients who underwent kidney transplantation at CUIMC were identified, and only patients who received allografts from donors identified as Black or non-Hispanic White were included.

Forty-six patients received more than one kidney allograft (including 41 recipients of kidneys from White donors and 5 recipients of kidneys from Black donors). For statistical purposes, these subjects were included once for each corresponding transplant. Our final cohort included 1901 allografts, combining 407 transplants from Black donors and 1,494 from White donors.

In our center, the majority of patients are maintained on tacrolimus and mycophenolate sodium, without maintenance corticosteroids. Clinical parameters were extracted from medical records. These included recipient and donor demographics (age, sex, and race), cause of native kidney failure, history of previous kidney transplant, induction immunosuppression therapy, donor-recipient human leukocyte antigen (HLA) mismatch (0–6 based on A, B, and DR antigens), and the presence of pre-transplant circulating donor-specific antibodies [DSA, defined as mean fluorescence intensity >1,000 as assessed by Luminex single-antigen bead assay (One Lambda Inc., Canoga Park, CA)]. To provide a measurable proxy of patients' socioeconomic status, median household income according to the ZIP code of each recipient's residence was used (7). We used the median ZIP code household income data from the American Community Survey 2014 5-Year Estimates Subject Tables, which included the 5-year interval (2010–2014) covering the middle of the study period.

Follow-up

Patients were censored at the end of the follow-up period (December 31, 2019). Death-censored allograft failure (determined as re-initiation of maintenance kidney replacement therapy or re-transplantation) was considered the primary outcome, while the development of CG (Figure 1) was considered a secondary outcome. As explained previously (4), CG was defined using the Columbia classification of focal segmental glomerulosclerosis (FSGS) as ≥1 glomerulus with segmental or global wrinkling and retraction of the glomerular basement membranes, accompanied by hypertrophy and hyperplasia of overlying glomerular epithelial cells (8), and the diagnosis was confirmed by two pathologists.

In patients who did not develop CG, we aimed to assess progression of other histologic changes over time, depending on the findings extracted from pathology reports of the post-reperfusion biopsy and last available allograft biopsy performed beyond 1 year after transplantation and before the end of the follow-up period. At CUIMC, post-reperfusion biopsies are routinely performed, while subsequent kidney allograft biopsies are performed for clinical indications (elevation of serum creatinine or proteinuria), or per-protocol, mainly in patients with pre-transplant DSA or a positive pre-transplant flow cytometry crossmatch (at 1 week, 2 weeks, 1 month, 3 months, 6 months, 1 year, 2 years, 3 years, and 5 years after transplantation). All biopsies were processed for light microscopy using the standard procedure employed in processing of kidney biopsy specimens, including staining with hematoxylin and eosin, periodic acid-Schiff, Masson trichrome, and Jones methenamine silver.

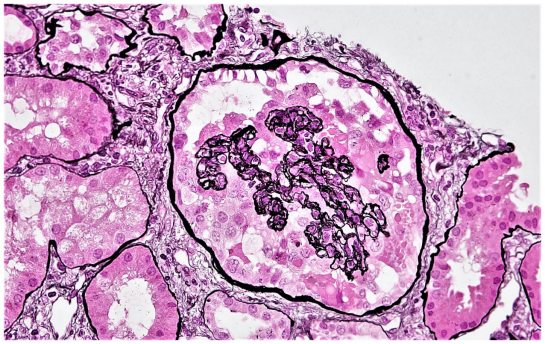

FIGURE 1
Representative photomicrograph showing collapsing glomerulopathy in a kidney allograft. A glomerulus displaying features of collapsing glomerulopathy in a kidney allograft biopsy. This glomerulus displays global collapse of the glomerular tuft, wrinkled and retracted glomerular basement membranes, and hypertrophy and hyperplasia of the overlying glomerular epithelial cells (Jones methenamine silver stain). Original magnification ×400.

Assessed histologic parameters included the number of glomeruli present, number and percentage of glomeruli displaying each of global glomerulosclerosis (GGS) and non-collapsing FSGS, percentage of interstitial fibrosis/tubular atrophy (IFTA), and histologic scores for arteriosclerosis (0–3) and arteriolar hyalinosis (0–3), as assessed using Banff criteria (9).

The rate of progression of IFTA over time in the same subjects was calculated using the following equation: [ΔIFTA = (%IFTA in last allograft biopsy – %IFTA in post-reperfusion biopsy) / number of months post-transplantation between these two biopsies]. Analogous equations were used to determine the rates of progression of GGS, FSGS, arteriosclerosis, and arteriolar hyalinosis over time, substituting the % of IFTA with each of % of GGS, % of FSGS, arteriosclerosis score, and arteriolar hyalinosis score, respectively.

If any required data were missing from the pathology reports, a review of the original biopsy slides was undertaken by two pathologists (LDF and IB) to obtain the missing data. If a specific histologic parameter was unable to be evaluated due to sampling limitations, the corresponding data point was labeled as missing.

Statistical analysis

Statistical analysis was performed using Prism version 9 (GraphPad Inc., San Diego, CA) and SPSS Statistics version 27 (IBM, Armonk, NY). Continuous data were presented as median and interquartile range (IQR; 25th and 75th percentile), and compared using the Mann–Whitney test, while categorical variables were compared using Fisher's exact test. Kaplan–Meier methodology and log-rank test were utilized to assess allograft survival, while Cox proportional hazards models were constructed to account for confounders. Variables with $p < 0.10$ on univariate analyses and variables that were different between patients receiving kidney allografts from Black vs. White donors were included in the multivariable analyses. Individuals with missing information for a tested predictor were excluded from the corresponding univariable time-to-event analysis, and those with missing data in one or more predictors in the multivariable analyses were also excluded from the latter analyses. p values <0.05 with two-sided hypothesis testing were considered statistically significant.

Results

Demographic, clinical, and pathologic features

Between January 2005 and December 2018, 1901 kidney allografts were transplanted from either self-identified Black ($n = 407$, 21%) or White ($n = 1{,}494$, 79%) donors at CUIMC. Overall, kidney allograft recipients had a median age of 53 years, 38% were female, 22% self-identified as Black, 15% had undergone prior kidney transplantation, and 52% received kidney allografts from deceased donors (Table 1). The donors had an average age at donation of 46 years and 49% were female. The median number of HLA mismatches was 4, and 19% of the kidney allograft recipients had pre-transplant DSA.

The most commonly reported etiology of native kidney failure among allograft recipients was diabetes mellitus (24%), followed by glomerulonephritis (20%), and hypertension (17%). The majority of recipients (82%) received depleting induction therapy, including 67% who received thymoglobulin (Table 1).

Recipients of kidney allografts from Black donors ($n = 407$) were more likely to be Black than those receiving allografts from White donors (53% vs. 14%, $p < 0.001$; Table 1). Additionally, recipients of kidney allografts from Black donors were more likely to be younger (median age: 51 vs. 54 years, $p = 0.03$) and female (44% vs. 37%, $p = 0.006$), and to have lower household income ($p < 0.001$). Recipients of kidney allografts from Black donors were also more likely to have developed native kidney failure attributed to FSGS (14% vs. 7%, $p < 0.001$) and to receive kidneys from deceased donors (61% vs. 50%, $p = 0.001$) that were younger (median age: 42 vs. 46, $p < 0.001$), and HLA-mismatched (97% vs. 93%, $p < 0.001$; Table 1).

Follow-up

Kidney allograft recipients were followed for a median of 4.5 years post-transplantation (IQRs: 2.2, 7.3 years). Of these, 388 (20%) experienced graft failure during follow-up. Notably, allograft survival was shorter for recipients of Black donor kidneys than for recipients of White donor kidneys (HR = 1.47, $p = 0.0008$; Figure 2). To study the independent effect of receiving a kidney allograft from a Black donor on allograft survival, we performed a multivariate analysis that included all variables with $p < 0.1$ on univariate analyses, in addition to variables that were different between patients who received kidney allografts from Black vs. White donors. The latter analysis revealed that receiving a kidney from a Black donor was still associated with a significant, albeit modest, increased risk of allograft failure (aHR = 1.34, $p = 0.02$; Table 2). Other independent predictors included receiving a kidney from a deceased donor (aHR = 2.01, $p < 0.001$), the presence of pre-transplant DSA (aHR = 1.42, $p = 0.008$), and receiving a kidney from an older donor (aHR = 1.01 per year, $p = 0.03$; Table 2).

When the secondary outcome of CG was assessed, CG was more frequent in kidney allografts procured from Black donors [30/407 (7.4%) recipients of allografts from Black donors vs. 28/1494 (1.9%) recipients of allografts from White donors, OR = 4.17, $p < 0.001$; Figure 3]. This increased frequency of CG in kidneys from Black donors was observed in recipients of kidneys from both living donors [9/157 (6%) Black donors vs. 9/750 (1%) White donors, OR = 5.0, $p = 0.001$] and deceased donors [21/250 (8%) Black donors vs. 19/744 (3%) White donors, OR = 3.5, $p < 0.001$]. When the potential relationship between Black recipient race and CG was examined among patients who received kidneys from Black donors, no difference in the frequency of Black recipients was found between those who developed CG [15/30 (50%)] and those who did not [200/377 (53%), $p = 0.85$].

Lastly, evaluation of the rate of change of histologic parameters over time for patients who did not develop CG revealed no statistically significant differences in any of the measured parameters when comparing recipients of kidney allografts from Black and White donors (Figure 4). Only a trend toward a lower rate of progression of GGS in recipients of kidneys from Black donors was observed (ΔGGS = 0.23 for Black donors vs. 0.27 for White donors, $p = 0.05$; Supplementary Table S1).

TABLE 1 Demographics and clinical characteristics of recipients of kidney allografts from Black vs. White donors.

	Total ($n = 1901$)	Recipients of kidneys from Black donors ($n = 407$)	Recipients of kidneys from White donors ($n = 1,494$)	p value (Black vs. White donors)
Recipient age (median, years)	53 (41, 62)	51 (39, 61)	54 (42, 62)	**0.03**
Recipient female sex	728/1901 (38%)	180/407 (44%)	548/1494 (37%)	**0.006**
Recipient Black race	418/1901 (22%)	215/407 (53%)	203/1494 (14%)	**<0.001**
Donor age[1] (median, years)	46 (34, 54)	42 (29, 52)	46 (35, 55)	**<0.001**
Donor female sex	937/1901 (49%)	199/407 (49%)	738/1494 (49%)	0.87
Deceased donor	994/1901 (52%)	250/407 (61%)	744/1494 (50%)	**0.001**
# of HLA mismatches[2]	4 (3, 5)	4 (3, 5)	4 (3, 5)	**0.001**
0 Mismatch	117/1900 (6%)	11/407 (3%)	106/1493 (7%)	**<0.001**
1 Mismatch	42/1900 (2%)	7/407 (2%)	35/1493 (2%)	0.57
2 Mismatch	173/1900 (9%)	30/407 (7%)	143/1493 (10%)	0.21
3 Mismatch	312/1900 (16%)	71/407 (18%)	241/1493 (16%)	0.57
4 Mismatch	429/1900 (23%)	87/407 (21%)	342/1493 (23%)	0.55
5 Mismatch	552/1900 (29%)	130/407 (32%)	422/1493 (28%)	0.16
6 Mismatch	275/1900 (15%)	71/407 (17%)	204/1493 (14%)	0.06
Pre-transplant DSA[3]	353/1900 (19%)	86/407 (21%)	267/1493 (18%)	0.15
Etiology of native kidney failure				
Diabetes mellitus	459/1901 (24%)	102/407 (25%)	357/1494 (24%)	0.65
Glomerulonephritis	382/1901 (20%)	75/407 (18%)	307/1494 (21%)	0.36
Hypertension	323/1901 (17%)	79/407 (19%)	244/1494 (16%)	0.16
Cystic changes	193/1901 (10%)	26/407 (6%)	167/1494 (11%)	**0.004**
FSGS	156/1901 (8%)	56/407 (14%)	100/1494 (7%)	**<0.001**
Obstruction/reflux	54/1901 (3%)	12/407 (3%)	42/1494 (3%)	0.87
Others	299/1901 (16%)	51/407 (13%)	248/1494 (16%)	0.05
Unknown	35/1901 (2%)	6/407 (2%)	29/1494 (2%)	0.68
Previous Transplantation[4]	292/1899 (15%)	59/407 (15%)	233/1492 (16%)	0.64
Induction therapy				
Depletion therapy	1567/1901 (82%)	338/407 (83%)	1229/1494 (82%)	0.77
Thymoglobulin	1286/1901 (67%)	279/407 (69%)	1007/1494 (67%)	0.68
Alemtuzumab	281/1901 (15%)	59/407 (14%)	222/1494 (15%)	0.94
Non-depletion	333/1901 (18%)	69/407 (17%)	264/1494 (18%)	0.77
IL2R Inhibitor	323/1901 (17%)	66/407 (16%)	257/1494 (17%)	0.71
No induction	11/1901 (1%)	3/407 (1%)	8/1494 (1%)	0.71
Median ZIP code household income[5]	71,494 (4,7,050, 9,6,454)	59,653 (42,587, 88,293)	74,784 (51,961, 98,532)	**<0.001**

DSA, donor-specific antibodies; FSGS, focal segmental glomerulosclerosis; HLA, human leukocyte antigen; IL2R, interleukin-2 receptor.
[1]Donor age: data on donor age were unavailable for 6 kidney donors (including 3 Black donors and 3 White donors).
[2]HLA-mismatch: data on number of HLA mismatches were unavailable for 1 kidney donor-recipient pair (with a White donor).
[3]Pre-transplant DSA: data regarding presence of pre-transplant DSA were unavailable for 1 kidney donor-recipient pair (from a White donor).
[4]Previous transplantation: data regarding previous renal transplantation were unavailable for 2 kidney recipients (both receiving kidneys from White donors).
[5]Median ZIP code household income: data regarding household income were unavailable for 21 kidney recipients (including 4 Black donors and 17 White donors).

CG and allograft failure

When causes of allograft failure in recipients of kidney allografts from Black and White donors were compared, only allograft failure following the development of CG was more common in recipients of allografts from Black donors [$n = 15$ (15%) Black donors vs. $n = 15$, (5%) White donors; OR = 3.16, $p = 0.004$; Figure 5], while other causes were similar (Supplementary Table S2).

To explore the association of receiving kidneys from Black donors with allograft survival in patients who did not develop CG, time-to-event analyses were repeated after excluding the 58 allografts that developed CG during follow-up (30 from Black donors and 28

from White donors; Table 3). Notably, receiving a kidney from a Black donor was no longer associated with allograft failure (aHR = 1.24, $p = 0.10$), while receiving a kidney from a deceased donor (aHR = 2.02, $p < 0.001$) and presence of pre-transplant DSA (aHR = 1.45, $p = 0.007$) remained independently associated with allograft failure (Table 3).

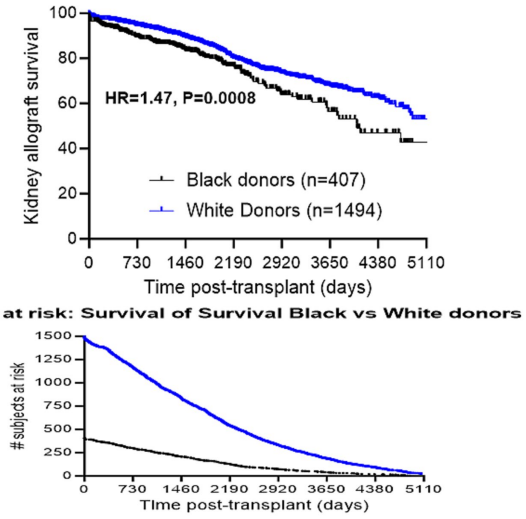

FIGURE 2
Comparison of allograft survival in recipients of kidney allografts from Black vs. White donors.

Discussion

Most, although not all, studies have demonstrated shorter allograft longevity for recipients of kidney transplants from Black donors. Using data from the United Network for Organ Sharing (UNOS), Hariharan et al. found inferior allograft survival in recipients of kidney allografts procured from older deceased Black donors (1), while Callender et al. have expanded the above observations, and demonstrated that kidney allografts obtained from either deceased or living Black donors had shorter allograft survival (2). Using data from Veterans Affairs and US Renal Data System information, Taber et al. have also shown that recipients of kidney allografts from Black donors experience substantially reduced allograft survival (10). However, when restricting the evaluation to recipients of living donor transplants from the UNOS database, Isaacs et al. found that the effect of donor race was less demonstrable (11). Similarly, using UK Transplant Registry Data, Pisavadia et al. did not find a significant difference in allograft survival in recipients of kidney allografts from living or deceased Black donors when compared to those who received kidneys from White donors (12). Nevertheless, it should be noted that the aforementioned reports were registry-based studies performed on data from national kidney transplant tracking programs, where it is not possible to adjust for important confounders such as pre-transplant DSA, given the absence of these data, nor is it possible to study histopathologic changes in the allografts.

A single-center study found that Black donor race was associated with increased risk of allograft failure on multivariate analysis (HR = 1.56, $p = 0.047$) (13). However, that particular study was relatively small (including 118 kidneys from Black donors and 845

TABLE 2 Univariable and multivariable analyses of death-censored allograft survival.

Variables	Univariable ($n = 1901$)			Multivariable ($n = 1872$), N events = 383	
	N events	HR (95% CI)	*p* value	aHR (95% CI)	*p* value
Recipient age at transplant (per each year)	388	1.00 (0.99–1.00)	0.49	0.99 (0.99–1.00)	0.12
Recipient female gender	388	0.94 (0.77–1.16)	0.58	0.88 (0.71–1.08)	0.22
Recipient Black race	388	1.38 (1.11–1.73)	**0.004**	1.08 (0.85–1.38)	0.54
Donor age at transplant (per each year)[1]	387	1.00 (0.99–1.01)	0.66	1.01 (1.00–1.02)	**0.03**
Donor female sex	388	0.99 (0.81–1.21)	0.92		
Donor Black race	388	1.47 (1.17–1.85)	**0.001**	1.34 (1.05–1.71)	**0.02**
Allograft from Deceased donor	388	2.10 (1.69–2.60)	**<0.001**	2.01 (1.57–2.57)	**<0.001**
Previous transplant[2]	388	1.47 (1.16–1.88)	**0.002**	1.28 (0.97–1.68)	0.08
Pre-transplant DSA[3]	388	1.62 (1.29–2.04)	**<0.001**	1.42 (1.10–1.84)	**0.008**
# HLA mismatches (per antigen: 0–6)[4]	388	1.12 (1.05–1.20)	**0.001**	1.03 (0.96–1.11)	0.46
Induction with Depleting therapy	388	1.18 (0.90–1.54)	0.23		
Native kidney failure due to DM	388	1.05 (0.83–1.32)	0.71		
Native kidney failure due to FSGS	388	1.47 (1.07–2.02)	**0.02**	1.29 (0.93–1.80)	0.13
Median ZIP code household income (per each $10,000/year)[5]	383	0.96 (0.93–0.99)	**0.007**	1.00 (0.96–1.03)	0.84

DM, diabetes mellitus; DSA, donor-specific antibody; FSGS, focal segmental glomerulosclerosis.
[1]Donor age: data on donor age were unavailable for 6 kidney donors (including 3 Black donors and 3 White donors).
[2]Previous transplantation: data regarding previous renal transplantation were unavailable for 2 kidney recipients (both receiving kidneys from White donors).
[3]Pre-transplant DSA: data regarding presence of pre-transplant DSA were unavailable for 1 kidney recipient (from White donor).
[4]HLA-mismatch: data on number of HLA mismatches were unavailable for 1 kidney donor-recipient pair (with a White donor).
[5]Median ZIP code household income: data regarding household income were unavailable for 21 kidney recipients (including 4 Black donors and 17 White donors).

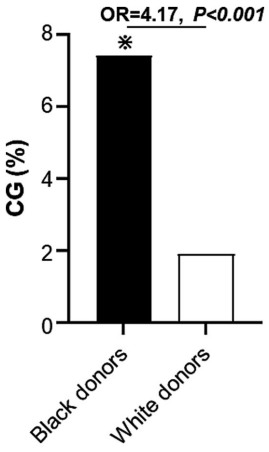

FIGURE 3
Incidence of collapsing glomerulopathy during follow-up period in recipients of kidney allografts from Black vs. White donors. CG, collapsing glomerulopathy.

kidneys from White donors) and the investigators did not adjust for major immunologic confounders, including HLA-mismatch and the presence of pre-transplant DSA (13).

The KDPI is a tool that was developed in 2009 by Rao et al. with the intent of predicting the risk of allograft failure based on clinical and demographic characteristics of an eligible deceased donor (14). Ten donor characteristics, including Black vs. non-Black donor race, are used to calculate the KDPI (14). Unfortunately, the apparent negative impact of Black donor race on allograft survival and its inclusion in KDPI calculation have led to a higher discard rate for kidneys from Black donors (3), despite the fact that the pathophysiology behind such observations are still uncertain.

To understand the mechanisms underlying the observed racial disparities in kidney allograft survival, one must examine the histopathologic changes that occur in the kidney allograft. Development of CG in the kidney allograft is an infrequently encountered complication, but it is characterized by a dismal prognosis (4). While prior studies have suggested an association between CG and receiving kidney allografts from Black donors (4–6),

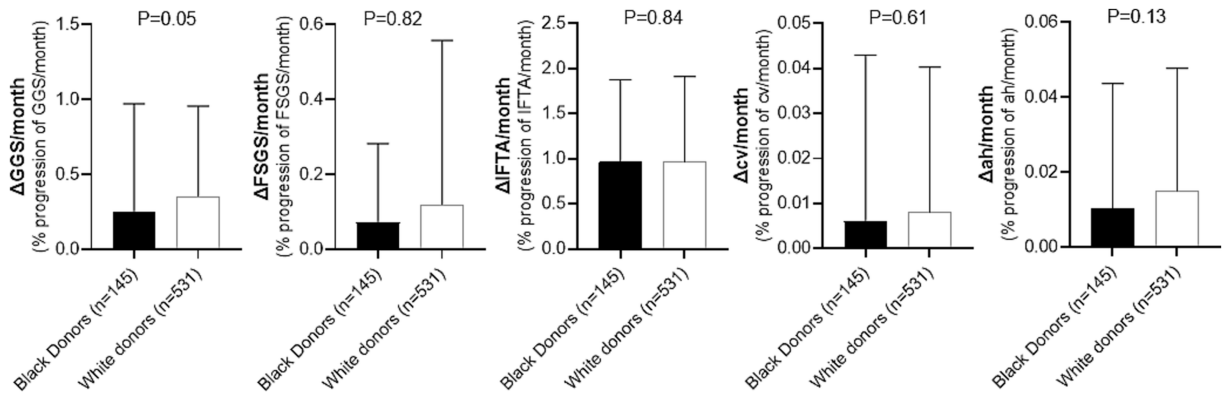

FIGURE 4
Comparison of histologic changes over time during follow-up period in recipients of kidney allografts from Black vs. White donors who did not develop CG. GGS, global glomerulosclerosis; FSGS, segmental glomerulosclerosis; IFTA, interstitial fibrosis/tubular atrophy; cv, arterial fibrointimal sclerosis; ah, arteriolar hyalinosis. Detailed comparison is presented in Supplementary Table S1.

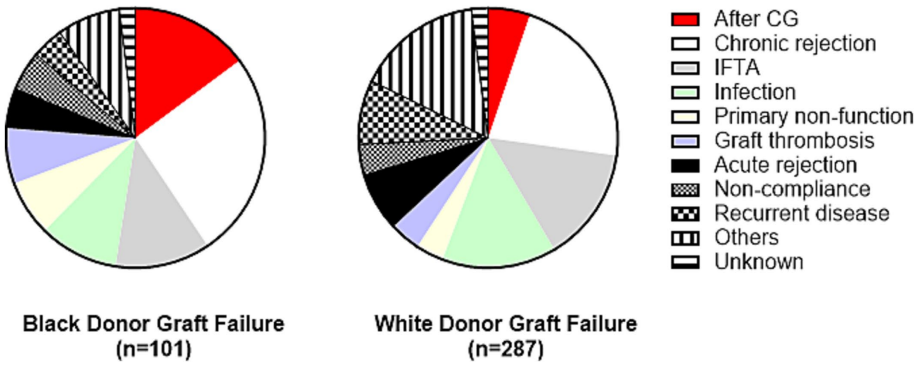

FIGURE 5
Causes of allograft failure in recipients of kidney allografts from Black vs. White donors. Only allograft failure after CG was significantly different between these two subgroups [OR = 3.16, $p = 0.004$]. Detailed comparison is presented in Supplementary Table S2. CG, collapsing glomerulopathy; IFTA, interstitial fibrosis/tubular atrophy.

TABLE 3 Univariable and multivariable analyses of death-censored allograft survival after excluding patients who developed CG.

Variables	Univariable (n = 1843)			Multivariable (n = 1814), N events = 353	
	N events	HR (95% CI)	p value	aHR (95% CI)	p value
Recipient age at transplant (per each year)	358	1.00 (0.99–1.01)	0.59	0.99 (0.99–1.00)	0.16
Recipient female gender	358	0.94 (0.76–1.16)	0.54	0.87 (0.70–1.08)	0.20
Recipient Black race	358	1.37 (1.09–1.73)	**0.008**	1.08 (0.84–1.39)	0.56
Donor age at transplant (per each year)[1]	357	1.00 (0.99–1.01)	0.64	1.01 (1.00–1.02)	0.05
Donor female sex	358	0.99 (0.80–1.22)	0.91		
Donor Black race	358	1.37 (1.07–1.74)	**0.01**	1.24 (0.96–1.61)	0.10
Allograft from Deceased donor	358	2.13 (1.70–2.66)	**<0.001**	2.02 (1.56–2.60)	**<0.001**
Previous transplant[2]	358	1.52 (1.18–1.96)	**0.001**	1.30 (0.98–1.73)	0.07
Pre-transplant DSA[3]	358	1.66 (1.31–2.10)	**<0.001**	1.45 (1.11–1.89)	**0.007**
# HLA mismatches (per antigen: 0–6)[4]	358	1.13 (1.05–1.21)	**0.001**	1.04 (0.96–1.12)	0.37
Induction with Depleting therapy	358	1.21 (0.91–1.60)	0.19		
Native kidney failure due to DM	358	1.05 (0.83–1.35)	0.68		
Native kidney failure due to FSGS	358	1.47 (1.06–2.07)	**0.02**	1.30 (0.92–1.83)	0.13
Median ZIP code household income (per each $10,000/year)[5]	353	0.96 (0.93–0.99)	**0.01**	1.00 (0.96–1.03)	0.81

DM, diabetes mellitus; DSA, donor-specific antibody; FSGS, focal segmental glomerulosclerosis.
[1]Donor age: data on donor age were unavailable for 6 kidney donors (including 3 Black donors and 3 White donors).
[2]Previous transplantation: data regarding previous renal transplantation were unavailable for 2 kidney recipients (both receiving kidneys from White donors).
[3]Pre-transplant DSA: data regarding presence of pre-transplant DSA were unavailable for 1 kidney recipient (from White donor).
[4]HLA-mismatch: data on number of HLA mismatches were unavailable for 1 kidney donor-recipient pair (with a White donor).
[5]Median ZIP code household income: data regarding household income were unavailable for 21 kidney recipients (including 4 Black donors and 17 White donors).
Significant P values are in bold.

none of these studies have compared the prevalence of CG between recipients of allograft kidneys from Black vs. White donors. Similarly, while accelerated vascular sclerosis and development of FSGS in native kidneys are more frequently observed in Black patients (15–18), a systematic assessment of the progression of such chronic changes in transplant recipients, stratified based on their donor races/ethnicities, is lacking.

Our study, which is the largest single-center investigation to date, and the first to assess histologic alterations in this patient population, has shown that, compared to recipients of kidneys from White donors, recipients of kidneys from Black donors incur only a modestly (19) increased risk of allograft loss (aHR = 1.34, $p = 0.02$), after adjusting for relevant risk factors, including recipients' and donors' demographics, kidney source, HLA-mismatches, history of previous transplantation, pre-transplant DSA, cause of native kidney failure, and socioeconomic status. Notably, recipients of kidney allografts from Black donors were more likely to be Black than those who received kidneys from White donors. The reasons for this are likely multifactorial, and include consanguinity (e.g., living-related donation), and attempts to match donor-recipient pairs with regard to blood group and HLA antigens, which are differentially distributed across different races/ethnicities (20, 21). Nonetheless, recipient race did not show an independent association with allograft survival after adjusting for other variables, including donor race.

With regard to histologic changes and outcome, the development of CG was more frequent in recipients of allograft kidneys taken from Black donors. Furthermore, when we examined the etiologies of allograft loss between kidneys from Black vs. White donors, the development of CG appeared to account for the majority of this observed disparity. In fact, patients who did not develop CG during follow-up showed similar rate of progression of other histopathologic parameters over time (including glomerulosclerosis, IFTA, and vascular sclerosis) in recipients of kidney allografts from Black and White donors. Moreover, receiving kidney allografts from Black donors lost its independent association with allograft failure after excluding patients who did not develop CG. Together, these findings support that CG is the main driver behind the worse prognosis observed in patients receiving kidneys from Black donors when compared to patients receiving kidneys from White donors.

Importantly, a few prior studies have shown that a relatively high proportion of patients who develop posttransplant CG have received allografts from donors with high-risk *APOL1* genotypes, which are increased in population with recent African ancestry (22). Moreover, a multicenter study demonstrated that the presence of high-risk *APOL1* genotypes was more appropriate indicator of risk of allograft failure than donor Black race (23). Therefore, it is plausible that the increased risk of allograft loss observed in kidneys procured from Black donors might be mediated by CG that develop following a second hit in patients, who received kidneys from donors with susceptible genetic background. Hence, precise identification of risk factors and molecular signals predisposing for the development of CG may increase utilization of kidney allografts from Black donors by identifying the minority of donors at the highest risk for CG. This might help eliminating a portion of the currently observed racial disparity in allograft survival, especially now that drugs are being developed to mitigate the effects of *APOL1* kidney-risk variants (24).

Some limitations of this report include the retrospective nature of the study and relying on self-defined race/ethnicity rather than genetic

ancestry. The fact that this is a single-center study may also introduce recruitment bias. Furthermore, it is worth noting that data on donors' race/ethnicity may also be difficult to obtain in some countries. Nevertheless, to our knowledge, this report is the first large study to compare the histologic changes in recipients of kidney allografts from Black vs. White donors with a special focus on CG. Another major strength of this report is the ability to account for different confounding factors.

Overall, our data show that recipients of allografts from Black kidney donors demonstrate modestly shorter allograft survival than recipients of White donor kidneys, and that this difference is driven by the development of CG in a minority of the allografts taken from Black donors, which negatively impacts allograft survival.

Data availability statement

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

Ethics statement

The studies involving humans were approved by IRB Columbia University Irving Medical center. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because retrospective pathologic study where contacting the patients may not be possible.

Author contributions

LD: Formal analysis, Writing – original draft, Data curation, Conceptualization. ED: Data curation, Writing – review & editing. GS: Writing – review & editing, Data curation. ST: Writing – review & editing, Data curation. DS: Writing – review & editing, Data curation. LR: Investigation, Writing – review & editing. VD'A: Conceptualization, Writing – review & editing. E-RV: Data curation, Writing – review & editing. SH: Formal analysis, Investigation, Writing – review & editing, Data curation. IB: Supervision, Formal analysis, Conceptualization, Writing – review & editing.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1369225/full#supplementary-material

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Different profiles of acute graft pyelonephritis among kidney recipients from standard or elderly donors

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Background: Acute graft pyelonephritis (AGPN) is a relatively common complication in kidney transplants (KTs); however, the effects on allograft function, diagnostic criteria, and risk factors are not well established.

Methods: Retrospective analysis of all consecutive adult KTs was performed between 01 January 2011 and 31 December 2018 (follow-up ended on 31 December 2019) to examine the association between the diagnosis of AGPN (confirmed with magnetic resonance imaging [MRI]) during the first post-transplantation year and graft outcomes.

Results: Among the 939 consecutive KTs (≈50% with donors ≥60 years), we identified 130 MRI-confirmed AGPN episodes, with a documented association with recurrent and multidrug-resistant bacterial urinary tract infections (UTIs) ($p < 0.005$). Ureteral stenosis was the only risk factor associated with AGPN (OR 2.9 [95% CI, 1.6 to 5.2]). KTs with AGPN had a decreased allograft function at the first year (ΔeGFR 6 mL/min/1.73 m^2 [−2–15] in non-AGPN vs. −0.2 [−6.5–8.5] in AGPN, $p < 0.001$), with similar and negative profiles in KTs from standard or elderly donors. However, only KTs with AGPN and a donor <60 years showed reduced death-censored graft survival ($p = 0.015$); most of this subgroup received anti-thymocyte globulin (ATG) induction (40.4% vs. 17.7%), and their MRI presented either a multifocal AGPN pattern (73.9% vs. 56.7%) or abscedation (28.3% vs. 11.7%). No difference was noted in death-censored graft survival between early (<3 months post-KT) or late (3–12 months) AGPN, solitary/recurrent forms, or types of multidrug-resistant pathogens. Linear regression confirmed the independent role of multifocal pattern, abscedation, ATG induction, and donor age on the eGFR at the first year.

Conclusion: AGPN, influenced by multifocal presentation, ATG induction, donor age, and abscedation, affects kidney function and significantly impacts allograft survival in KTs with donors <60 years.

KEYWORDS

acute pyelonephritis, kidney transplantation, urinary tract infections, multidrug resistant pathogens, ureteral stenosis

1 Introduction

Infectious complications remain a significant cause of morbidity and mortality in solid organ transplant (SOT) patients (1). Among them, urinary tract infections (UTIs) were common in all SOTs but had the highest incidence in kidney transplanted (KT) patients (2). UTIs may evolve with graft involvement, causing acute graft pyelonephritis (AGPN).

Although AGPN occurs in a significant percentage of KTs worldwide, some concerns have emerged about the definition of AGPN and its potential role in allograft dysfunction (3, 4).

For example, diagnostic criteria for AGPN included only suggestive clinical symptoms and typical laboratory findings without radiological confirmation (5), and differences between early or late occurrences after transplant are a matter of debate (6, 7).

Based on the microbiological viewpoint, Gram-negative bacilli account for more than 70% of UTIs in KTs (8–11). Additionally, many AGPN episodes are caused by multidrug resistant (MDR) pathogens (12–14) with potentially life-threatening complications (30% vs. 10% of mortality in cases of carbapenem resistance) (15) and a higher recurrence risk (13).

Surgical complications after KT were associated with AGPN but results were mixed and thus inconclusive (16); some authors suggested that there is greater AGPN incidence among patients who experienced ureteral stenosis (UrS) (17).

It can be affirmed that all these characteristics, especially the impact on graft function, may occur and evolve differently in elderly or extended criteria donors (ECDs), but there is limited case evidence reported in the literature.

Identifying phenotypes and determinants for AGPN may be particularly important for KTs, where inappropriate antibiotic therapy may pose crucial problems with immunosuppressive medication and cause MDR pathogen selection (13, 14).

Our study aimed to retrospectively analyze our cohort of consecutive KTs with many elderly donors, evaluating the clinical and microbiological characteristics of all AGPN episodes and considering the impact of AGPN on allograft function and survival.

2 Methods

2.1 Study patients and ethical statement

We performed a retrospective observational study of all consecutive adult recipients who received a KT at Turin University Renal Transplant Center "A. Vercellone" from January 2011 to December 2018. The local Ethical Committee approved this study (*Comitato Etico Interaziendale A.O.U. Città Della Salute e Della Scienza di Torino - A.O. Ordine Mauriziano - A.S.L. Città di Torino*, resolution number 1449/2019 on 11 August 2019). This study was conducted according to the principles of the Helsinki and Istanbul Declarations. All participants provided written informed consent about the use of their data/information for this retrospective analysis. Follow-up was terminated on 31 December 2019.

2.2 Exposure

According to the *American Society of Transplantation Infectious Diseases Community of Practice* indications (5), AGPN was clinically suspected when suggestive clinical symptoms (i.e., fever with flank/allograft pain and/or symptoms of lower UTI including frequency, urgency, dysuria, and/or suprapubic pain) and typical laboratory findings (i.e., urinalysis showing leukocyte counts >10 per mm^3 or > 10^4 colony-forming units of bacteria per milliliter of urine; leukocytosis either with or without bacteria isolated from blood cultures) appeared. Additionally, each clinically suspected episode was further investigated with magnetic resonance imaging (MRI) within 24 h of initial symptoms for radiological confirmation/exclusion [detailed protocol is described in Faletti et al. (18)].

AGPN episodes that required hospitalization in different centers were considered and collected in case of available MRI confirmation. All of the AGPN episodes were evaluated by three authors (RT, GC, and AM) through a retrospective review of hospital records. AGPN episodes were then classified according to radiological characteristics (multifocal vs. unifocal and abscessed vs. non-abscessed) and time after transplantation [early (<3 months after KT) vs. late (3–12 months)].

2.3 Posttransplant management and data collection

All patients were initially managed by the Renal Transplant Center (Hub center) and received induction therapy (steroids and basiliximab/anti-thymocyte globulin [ATG] according to donor type and immune risk) and maintenance immunosuppression mainly composed of tacrolimus (10–15 ng/mL for the first 3 months and 6–8 ng/mL thereafter), mycophenolate mofetil/mycophenolic acid, and/or steroids (progressively tapered to 5 mg/day). The urological anastomosis was usually performed with the Lich-Gregoire antireflux technique and intraoperative double-J ureteral stenting (removed 4 weeks post-KT); the transurethral bladder catheter was usually maintained for 3–5 days.

After discharge, post-transplant care followed a standardized schedule, and every recipient was followed by the transplant center (Hub center) with at least 1 annual visit and by the local nephrologist (11 peripherical centers covering most of the Piedmont region) for their periodical follow-up.

All clinical and medical information (including donor data and immunosuppressive medications) was collected from patients' charts. Renal allograft function (eGFR) was estimated by the Chronic Kidney

Disease Epidemiology Collaboration (CKD-EPI) equation. We included eGFR values at discharge after transplantation and first year after transplant, considering a period after AGPN episodes of at least 2 weeks and with an eGFR documented stabilization in >2 tests in the absence of AGPN-induced acute kidney injury.

2.4 Outcomes

The primary purpose of this study was to evaluate the effect of AGPN on death-censored graft survival, stratifying for donor age to assess the potential impact of elderly donors.

Secondary purposes included identifying risk factors for AGPN, the impact of AGPN on patient survival rates, the possible modification in eGFR (available at discharge and first year after transplant), and the potential differences according to radiological presentation.

We subsequently compared death-censored graft survival rates and eGFR between KTR with and without AGPN. To discriminate at least the potential impact of donors in determining AGPN, we also investigated the AGPN rate in patients who received kidneys from the same donor (paired grafts).

2.5 Statistical methods

The distribution of continuous variables, overall and for subgroups, was analyzed with the Kolmogorov–Smirnov test. Based on their non-Gaussian distribution, we described age, eGFR, and follow-up with median and interquartile range (IQR).

Between-group comparisons of continuous variables were performed with the non-parametric Mann–Whitney test. To assess the effect of AGPN on the post-transplantation evolution of the eGFR, we compared eGFR at the first year vs. at discharge with the Wilcoxon signed-rank test.

To model the value of eGFR at the first year for patients with AGPN, we used linear regression with variables of interest with potential impact on AGPN severity (induction with ATG, multifocal presentation, abscedation, and donor age) as predictors. Considering the characteristics of the dependent variable, we used the *ln*-transformed eGFR at the first year to improve the accuracy of the linear regression model.

Categorical variables are presented as fractions, and Pearson's r, for small samples. Fisher's exact test was used to compare groups. The odds ratios (ORs) with a 95% confidence interval were used to measure relative risk.

Univariate survival analysis was performed utilizing the Kaplan–Meier method with the log-rank test to compare strata. The significance level for all tests was set at an α-value of <0.05.

Statistical analysis was performed with IBM SPSS Statistics for Windows, version 28.0.1α (IBM Corp., Armonk, NY, USA).

3 Results

3.1 Population characteristics

We analyzed 939 consecutive KTs, including 224 patients who received kidneys from the same donor (paired grafts). Among this population, 130 AGPN episodes in the first year after transplant were recorded based on the clinical criteria (5), but 21 of them (16.2%) were not confirmed by MRI and were analyzed separately.

Patient and donor characteristics stratified for AGPN occurrence are reported in Table 1.

Both groups have similar profiles, considering gender, patient and donor age, induction therapies, and rejection episodes. Worthy of mention, donor age was similar between groups (63 years [50–71] in AGPN and 60 [48–71] in non-AGPN, $p = 0.347$), with 449 of 897 (50.1%) KTs with a donor >60 years. Furthermore, ECDs [defined according to the Cristal City criteria (19, 20)] are equally distributed in both groups (41.3% in AGPN vs. 43.8% in non-AGPN, $p = 0.680$), reflecting our significant utilization with a preferential old-for-old allocation (21).

Patients in the AGPN group showed, as expected, a high percentage of positive urine culture (34.9% vs. 15.7%, $p < 0.005$), a high number of UTIs due to MDR bacteria (36.8% vs. 16.8%, $p = 0.022$), and a trend toward more recurrent episodes (71.1% of total positive urine cultures in the AGPN group vs. 55.1% in non-AGPN, $p = 0.063$).

Among potential risk factors, some patients experienced AGPN before double-J removal (20 of 108, 18.3%), but only UrS confirmed by antegrade pyelography appears to be significantly associated with AGPN (OR 2.9 [CI 95% 1.6 to 5.2]; $p = 0.001$).

3.2 Association between AGPN, patient and kidney survival, and graft function

Although AGPN has no apparent effect on both patient and death-censored kidney survival in the entire population (Figures 1A,B, respectively), KT patients who experienced AGPN with a donor age < 60 years had low death-censored graft survival (Figure 2).

Despite similar kidney function after transplant, AGPN was associated with a lower eGFR at the first year (median eGFR 40 mL/min/1.73 m^2 in the AGPN group vs. 52 mL/min/1.73 m^2, $p < 0.001$ with a ΔeGFR 5 mL/min/1.73 m^2 [−2–15] in non-AGPN vs. −1 [−6.5–8.5], $p < 0.001$, Figure 3).

Stratifying for donor age (Table 2 and Figure 4), this trend toward a significantly reduced allograft function was confirmed (ΔeGFR 6 mL/min/1.73 m^2 [−2–18] in non-AGPN vs. −1 [−8–11] in KTs with donors <60 years and 3 [−2–13] vs. −2 [−6–6.25] in donors ≥60 years).

3.3 Differences in AGPN groups stratified for clinical and radiological characteristics

AGPN episodes were therefore stratified according to donor age, clinical features (early [< 3 months post-KT] or late [3–12 months], solitary/recurrent), and, based on MRI evaluation, multifocal/unifocal, and with/without abscedation.

Early, solitary, multifocal, and non-abscessed AGPN cases were prevalent in our cohort (Table 1). No significant difference was observed in early vs. late, solitary vs. recurrent (apart from increased evidence of recurrent positive urine culture in recurrent AGPN), or in AGPN with or without abscedation (Supplementary Tables S1–S3).

The multifocal pattern demonstrates a different profile: KT patients who experienced a multifocal AGPN show a trend toward a younger age at transplant, received more frequent ATG at induction,

TABLE 1 Characteristics of the studied population according to AGPN occurrence.

	AGPN ($n = 109$)	non-AGPN ($n = 788$)	p
Women, *n* (%)	38 (34.9)	280 (35.5)	0.915
Age at KT, years (IQR)	63 (50–71)	55 (46–65)	0.945
Age ≥ 65, years (%)	30 (27.5)	198 (25.1)	0.639
Donor age, years (IQR)	63 (50–71)	60 (48–71)	0.347
Extended-Criteria Donors[a], *n* (%)	45 (41.3)	345 (43.8)	0.680
Previous KT, *n* (%)	21 (19.3)	101 (12.8)	0.074
Living donor, *n* (%)	10 (9.2)	56 (7.1)	0.434
Dual kidney transplantation, *n* (%)	4 (3.7)	24 (3)	0.766
Acute rejection episodes during the first year, *n* (%)	15 (13.8)	87 (11.3)	0.426
Induction immunosuppressive therapy			
ATG, *n* (%)	30 (27.5)	202 (25.6)	0.726
Basiliximab, *n* (%)	81 (72.5)	604 (74.4)	0.630
Ureteral Stenosis on KT, *n* (%)	17 (15.6)	48 (6.1)	**0.001**
UTI episodes			
Urinalysis with CFU of bacteria $>10^6$/ml, *n* (%)	38 (34.9)	107 (13.7)	<0.005
Recurrent urinalyses with positive urine culture, *n* (%)	27 (71.1)[b]	59 (55.1)[b]	0.063
Identification of MDR bacteria on positive urine culture, *n* (%)	14[c] (36.8)	18[d] (16.8)	**0.022**
AGPN Clinical Characteristics			
Transfer in the ICU, *n* (%)	3 (2.8)	/	
Use of vasopressors, *n* (%)	1 (0.9)	/	
Early/late, *n* (%)	87 (79.8) / 21 (19.3)	/	/
Solitary/recurrent, *n* (%)	88 (80.7) / 21 (19.3)	/	/
AGPN MRI Characteristics			
Multifocal/unifocal, *n* (%)	73 (67.0) / 36 (33.0)	/	/
Abscessed/not abscessed, *n* (%)	22 (20.2) / 87 (79.8)	/	/

AGPN, acute graft pyelonephritis; KT, kidney transplant; IQR, interquartile range; ATG, anti-thymocyte globulin; UTI, urinary tract infection; MDR, multidrug resistance; ICU, intensive care unit; MRI, magnetic resonance imaging.

[a] According to Crystal City criteria.

[b] Percentage of the total number of urinalysis with CFU of bacteria > 10^6/ml in each group.

[c] K. pneumoniae carbapenemase producing in 4 of 14, ESBL-producing E. coli in 7 of 14, MDR P. aeruginosa in 1 of 14, vancomycin-resistant Enterococcus faecium in 1 of 14, and others in 1 of 14.

[d] K. pneumoniae carbapenemase producing in 8 of 18, ESBL-producing E. coli in 6 of 18, vancomycin-resistant Enterococcus faecium in 2 of 21, and others in 2 of 21.

Bold values denote statistical significance at the $p < 0.05$ level.

and showed a lower donor age. Interestingly, despite a better eGFR at transplant, justified by the difference in donor ages, the eGFR tends to overlap among groups at first year (Supplementary Table S4).

Based on the differences observed in graft survival, we stratified our population for donor age classes (<60 and ≥ 60 years): AGPN with donors <60 years, apart from a younger age at KT (48 years [40–55] vs. 63 [55–69]), have similar characteristics (including gender, urinalysis with positive urine culture, and UrS) but have preferentially received ATG induction (40.4% vs. 17.7%, $p < 0.001$) and, interestingly, have more frequently experienced multifocal pattern (73.9% vs. 56.7%, $p = 0.026$) or abscedation (28.3% vs. 11.7%, $p = 0.074$) as detected in the MRI.

Considering that ATG induction has been associated with BK polyomavirus infection, which can increase the risk of graft damage and loss, we assess the number of BK virus nephritis (BKVN) in patients who lost their grafts (n = 30): three of them experienced BKN with similar distribution in AGPN and non-AGPN (1 of 7 [14.3%] vs. 2 of 23 [8.7%], respectively, $p = 0.564$).

We assessed a multiple linear regression model to better evaluate the impact of specific conditions on 1-year eGFR in patients with AGPN. A first analysis highlighted an asymmetric distribution of the residuals, violating the assumption of normality for linear regression. We, therefore, decided to use a logarithmic transformation of the response variable to improve our concerns.

Through linear regression, with a multifocal presentation, presence of abscedation, donor age > 60 years, and ATG at induction as predictors, we identified that the model explained more than 75% of the variation in log(eGFR) ($R^2 = 0.811$). The F statistic resulted significantly ($p < 0.001$), indicating that the model predicted eGFR at the first year better than the mean. All coefficients were significant, confirming that these variables contribute to the model. In particular, a multifocal presentation contributes more than donor age, ATG, and evidence of abscedation (Table 3).

To discriminate at least the potential impact of donors in determining AGPN, we also investigated the AGPN rate in paired

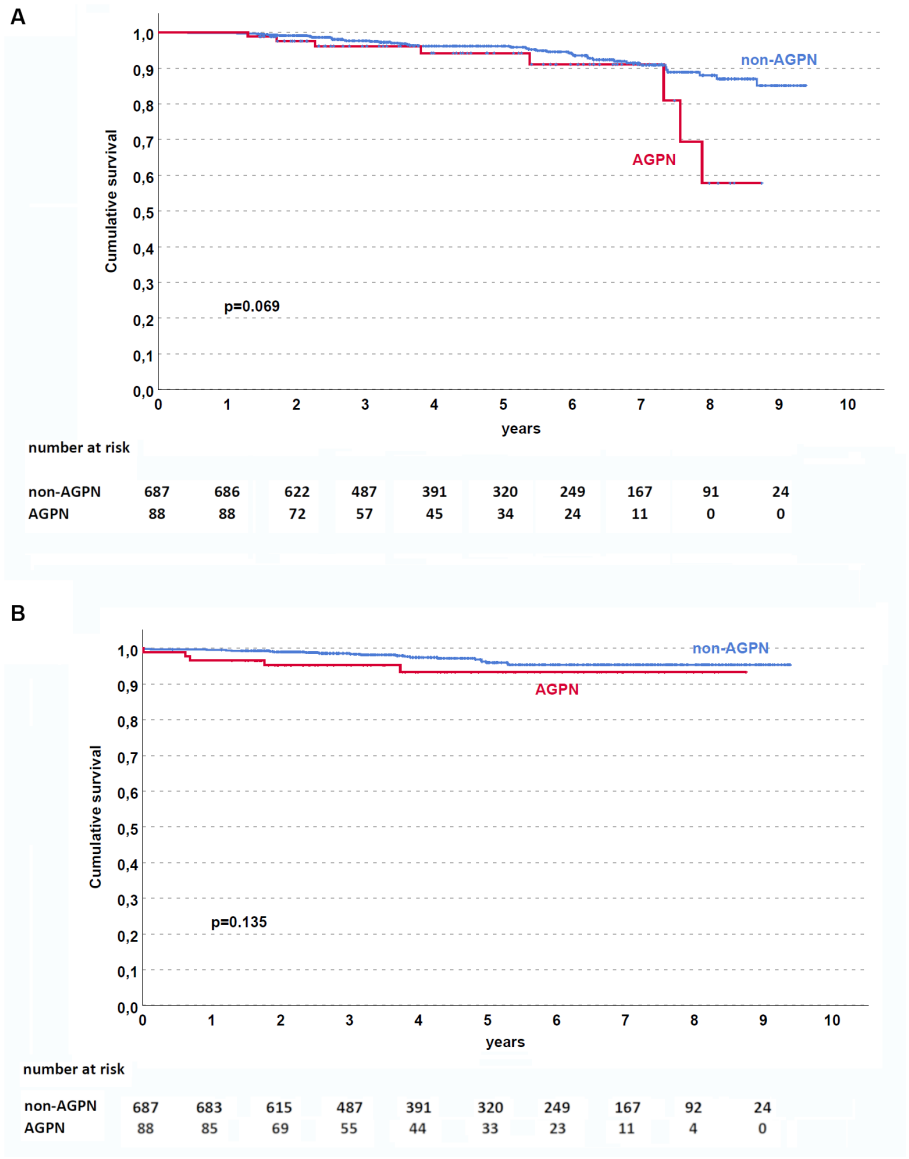

FIGURE 1
Kaplan–Meier curves in the studied population. AGPN and non-AGPN (excluding retransplant) had a similar patient **(A)** and death-censored graft **(B)** survival. AGPN, acute graft pyelonephritis.

kidneys. The absence of agreement (51 patients experienced AGPN but only in 4 cases did it occur in both recipients, Cohen's K coefficient = 0.116), also in early AGPN (45 episodes but only 3 in paired grafts, $K = 0.102$), suggests the lack of a donor effect in AGPN occurrence.

3.4 Characteristics of patients with a potential clinical diagnosis but without radiological signs of AGPN

As previously reported, 21 out of 130 patients with clinically-based AGPN showed no radiological signs of kidney involvement based on the MRI.

These patients had not developed severe infections or sepsis, had similar characteristics considering all examined previous variables but had significant evidence of urinalyses with positive urine cultures and, despite a slight increase during the first year, showed a reduced eGFR vs. the non-AGPN group (Supplementary Table S5).

4 Discussion

AGPN is one of the most frequent infections in KTs. In the past few years, this pathological process has been considered a relatively "benign" condition (22, 23). More recently, an increasing number of papers have highlighted the potential role of AGPN in determining reduced graft function and kidney survival (6, 7, 24–27).

One potential limitation of all previous studies in this field is the adoption of clinical criteria alone for AGPN diagnosis. However, as previously reported, especially regarding native kidneys,

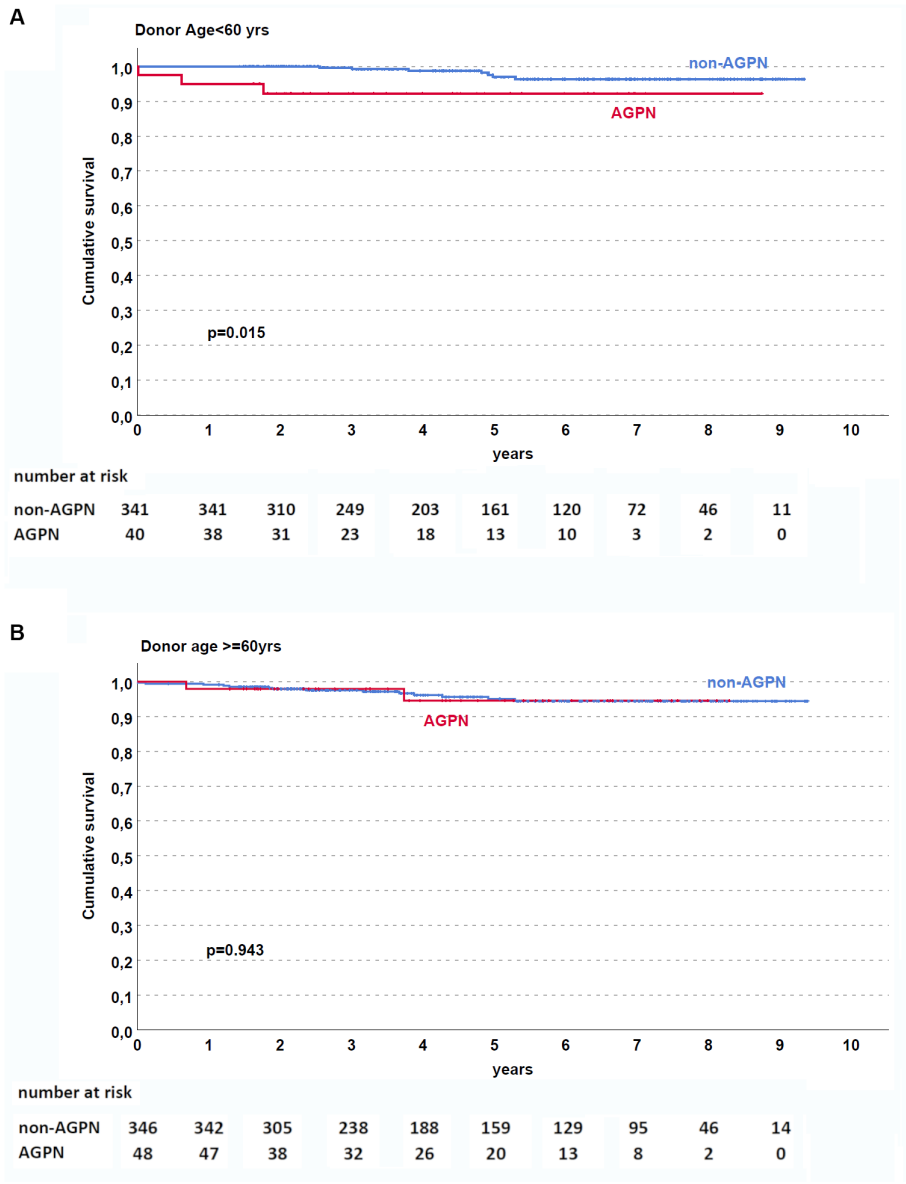

FIGURE 2
Kaplan–Meier curves according to donor age [**(A)** <60 years and **(B)** ≥ 60 years]. In patients (excluding retransplant) with donors <60 years, AGPN was associated with reduced death-censored graft survival. AGPN, acute graft pyelonephritis.

diffusion-weighted MRI with an apparent diffusion coefficient seems to be a reliable diagnostic tool with a very low false negative rate (18, 28, 29).

In our experience, MRI confirmation allows us to identify a significant percentage of patients with no parenchymal signs of infection (≈15%) and thus should be duly considered. On the one hand, this observation could be important in the transplant setting, where antibiotic overtreatment may favor MDR pathogen selection and rejection risk due to the potential minimization of immunosuppressive therapy after diagnosis. On the other hand, the analysis of the subgroup of KTs with clinical signs of AGPN and a negative MRI revealed a suboptimal kidney function associated with recurrent UTIs, suggesting, as previously described, a potential negative impact of UTIs by themselves on graft outcome and the need for continued surveillance of these patients (9, 10, 30).

Positive urinalyses and MDR detection are more common in AGPN, and this was the case in our population. At the same time, empiric and inappropriate antibiotic therapy is associated with a higher risk of bacteriemia due to MDR in SOTs (5, 13, 31), further corroborating our approach.

Many conditions that involve the urological tract are associated with UTIs and AGPN (e.g., bladder dysfunction, vesicoureteral reflux, and diabetes), although their impact has been debated (32–36). Our analysis identifies UrS as a significant risk factor for radiologically confirmed AGPN.

Similarly, Karam et al. highlighted a considerable incidence of AGPN among patients with UrS (29% vs. 14.4% in KTs without UrS,

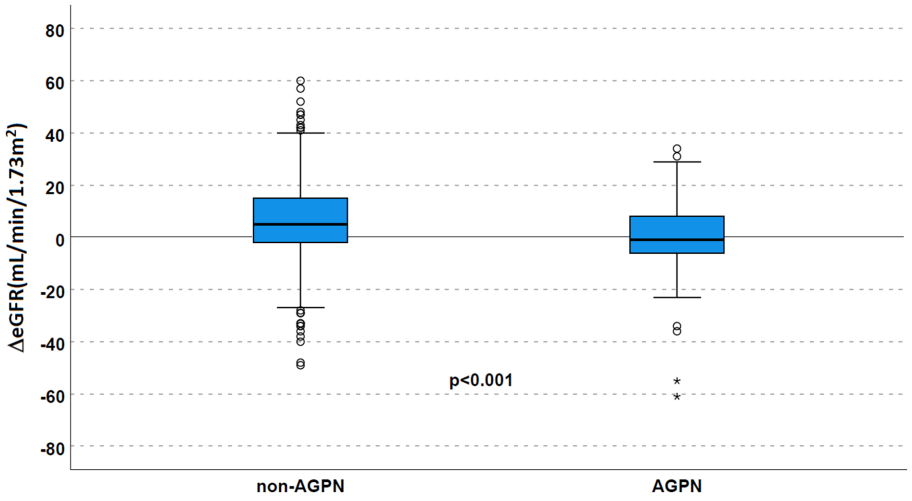

FIGURE 3
Renal function according to AGPN occurrence. ΔeGFR was reduced 1 year after transplant in patients who experienced AGPN. AGPN, acute graft pyelonephritis; eGFR: estimated glomerular filtration rate.

TABLE 2 ΔeGFR at transplant and the 1-year f/up in the studied population according to the AGPN occurrence.

	AGPN (n = 109)	non-AGPN (n = 809)	p
eGFR at transplant, mL/min/1.73m²	36 (26–61)	42 (32–59)	0.06
eGFR first year after KT, mL/min/1.73m²	40 (26–60.5)	52 (38–67)	**< 0.001**
ΔeGFR (overall population), mL/min/1.73m²	−0.2 [−6.5–8.5]	6 [−2–15]	**< 0.001**
Donor age < 60 years	−1 [−8–11]	6 [−2–18]	**0.012**
Donor age ≥ 60 years	−2 [−6–6.25]	3 [−2–13]	**0.002**

eGFR, estimated glomerular filtration rate; AGPN, acute graft pyelonephritis. Bold values denote statistical significance at the $p < 0.05$ level.

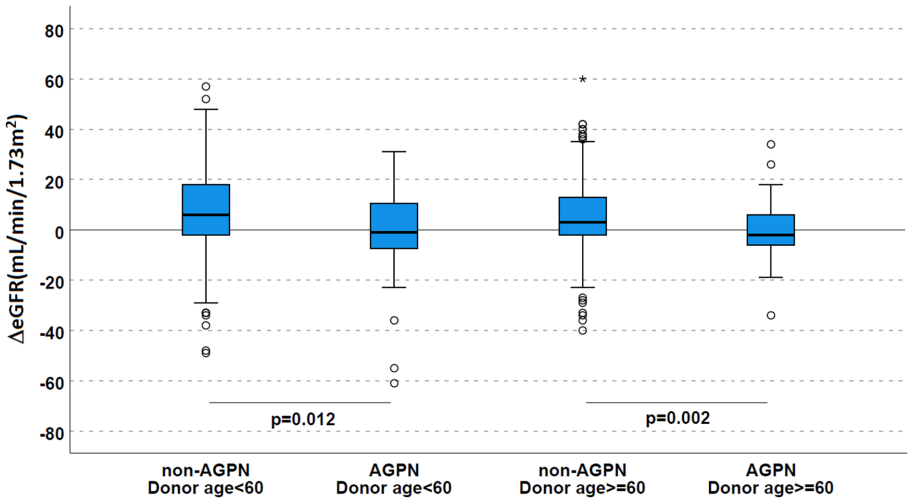

FIGURE 4
Renal function according to AGPN occurrence and donor age. ΔeGFR was reduced 1 year after transplant in patients who experienced AGPN, irrespective of donor age. AGPN, acute graft pyelonephritis; eGFR, estimated glomerular filtration rate.

$p < 0.05$) (17). UrS requiring a surgical approach determines a higher risk of AGPN, primarily when hydronephrosis is associated (17, 37). UrS and/or ureteral necrosis can lead to urine leakage into the abdomen and easier urinary tract contamination from intestinal bacteria. Besides, the surgical approach always represents a potential infectious risk, even more so among the immunosuppressed population.

TABLE 3 Linear regression analysis for a significant determinant of *ln*(eGFR) at first year.

	B	Standard error	Beta coefficient	*p*
Donor age ≥ 60 years vs. <60 years	1.791	0.248	0.364	**<0.001**
Multifocal presentation (yes vs. no)	2.211	0.262	0.487	**<0.001**
AGPN with abscedation (yes vs. no)	1.237	0.376	0.150	**0.001**
ATG at induction (yes vs. no)	1.169	0.350	0.165	**0.001**

AGPN, acute graft pyelonephritis; ATG, anti-thymocyte globulin. Bold values denote statistical significance at the $p < 0.05$ level.

An important topic of our study is the specific correlation of AGPN with eGFR post-KT. We found that patients with AGPN experienced a reduction in eGFR at the first year, irrespective of donor age. However, this condition determined an inferior death-censored graft survival in patients with donors <60 years; this subgroup concurrently experienced more frequent multifocal presentation and abscedation and preferentially received ATG induction. Although the correlation between high immunosuppression (i.e., after acute rejection episodes) and increased infection (including AGPN) rates is well established (8), the characteristics of different populations, especially with elderly donors, have not been intensively investigated.

Recent studies that evaluated the impact of AGPN on eGFR showed negative effects on graft survival and eGFR, but in populations with a limited number of old recipients/donors [recipients and donor age 52.6 [40.15–60.7] and 53 [41–62] in Maanaoui et al. (6), and 51.0 ± 14.1 and 52.1 ± 16.4 in Pacaud et al. (7)]. Additionally, neither of them routinely prescribed radiological confirmation for an AGPN diagnosis. Our population reflects our allocation policy with a homogenous clinical and therapeutical approach (20, 21), probably emphasizing the niche of KTs that developed increased organ damage and impairment of reserve graft function after "severe" AGPN (abscessed or multifocal). This consideration is highlighted by the multivariate linear regression model, where multifocal presentation (with a strong coefficient), donor age, abscedation, and ATG induction are independent predictors of eGFR at the first year. These data, combined with the evidence that multifocal presentation is more common among patients with ATG induction and young age, suggest that these KTs could be more susceptible to this severe presentation and that patients with multifocal features could be treated more intensively and actively monitored to reduce the potential impact on eGFR. Since abscedation and multifocal presentation are unrelated and abscedation does not seem to be influenced by induction therapy, this pattern could depend more on local conditions (i.e., the specific pathogen involved).

Our study has some limitations (retrospective design, absence of routine post-AGPN protocol biopsies, and availability of limited eGFR time-points). We are also aware that MRI assessment is expensive, and its availability broadly differs among centers. However, we suggest that it offers some advantages over CT scans (especially for radiation exposure) and better reproducibility than contrast-enhanced ultrasonography, also depicting some patterns (multifocal involvement/evidence of abscedation) that may be related to adverse outcomes requiring careful management with eventually prolonged therapy and surveillance.

Additionally, our real-life analysis of a population of recipients with a significant percentage of elderly recipients/donors may have identified a niche group of KTs requiring prompt and effective therapy to respond to AGPN episodes and avoid renal scarring development and long-term allograft dysfunction.

5 Conclusion

AGPN, influenced by multifocal presentation, ATG induction, donor age, and abscedation, affects kidney function and significantly impacts allograft survival in KTs with donors <60 years.

Although we are aware of limited availability and costs, radiological confirmation may help in this setting to establish the appropriate antibiotic therapy, avoid overtreatment, and prevent the potential risk of allograft dysfunction.

Data availability statement

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

Ethics statement

The studies involving humans were approved by Comitato Etico Interaziendale A.O.U. Città Della Salute e Della Scienza di Torino - A.O. Ordine Mauriziano - A.S.L. Città di Torino. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

RT: Conceptualization, Formal analysis, Writing – original draft. GC: Formal analysis, Investigation, Writing – original draft. AM: Data curation, Formal analysis, Methodology, Validation, Visualization, Writing – review & editing. GA: Writing – review & editing, Formal analysis. FF: Writing – review & editing, Data curation, Methodology, Software, Visualization. CD: Supervision, Writing – review & editing. EG: Supervision, Writing – review & editing. MV: Supervision, Writing – review & editing. RF: Supervision, Writing – review & editing, Investigation, Methodology. AB: Supervision, Writing – review & editing. PG: Supervision, Writing – review & editing. CC: Supervision, Writing – review & editing. RC: Supervision, Writing – review & editing. FM: Supervision, Writing – review & editing, Formal analysis. SC: Supervision, Writing – review & editing. FR: Supervision, Writing – review & editing. PF: Supervision, Writing – review & editing. LB: Conceptualization, Data curation, Formal analysis,

Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1342992/full#supplementary-material

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Acute post-renal kidney graft dysfunction due to cytomegalovirus-positive nephrogenic adenoma—case report and review of the literature

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Tissue-invasive cytomegalovirus (CMV) disease represents a well-recognized complication after kidney transplantation. However, direct involvement of the urogenital tract and CMV-ureteritis occur less frequently. Nephrogenic adenomas are benign lesions of the urinary tract preferentially reported in kidney transplant recipients. We herein report a second case of a 33-year-old male kidney transplant recipient with acute post-renal allograft dysfunction due to CMV-positive ureteral nephrogenic adenoma. A causal connection might be suspected but remains to be proven.

KEYWORDS

case report, nephrogenic adenoma, cytomegalovirus, kidney transplantation, allograft dysfunction, post-renal, infection, urologic complication

Introduction

Transplantation is the treatment of choice for patients with end-stage kidney disease (1). Given current highly effective immunosuppressive regimens, infectious complications represent a main cause for morbidity and mortality in patients after solid organ transplantation (2). Infections with cytomegalovirus (CMV) are among the most common opportunistic infections occurring in kidney transplant recipients and negatively affect transplant outcome (3). CMV-induced asymptomatic viremia and systemic disease are well recognized, as is tissue-invasive disease with classical involvement of the upper and lower gastrointestinal tract, the lungs or the liver (4). However, direct involvement of the kidney graft or the urogenital tract is rare.

Nephrogenic adenoma is a particular, uncommon benign lesion of the urinary tract with a wide range of histopathological characteristics mimicking malignant neoplasms (5). Nephrogenic adenomas mainly arise in the bladder while other locations in the urinary tract are less frequent (6). In kidney transplant recipients, the occurrence of nephrogenic adenoma in the bladder has been reported with incidences ranging from 0.53 to 4.3 per 100 transplants (7, 8). Despite various hypotheses, the underlying pathogenesis for the development of nephrogenic adenoma has not been completely elucidated to date.

Herein, we report a rare case of a kidney transplant recipient with acute post-renal allograft dysfunction due to CMV-positive ureteral nephrogenic adenoma and discuss a potential link between both conditions.

Case description

A 33-year-old male of West-African descent with end-stage kidney disease due to hypertensive nephropathy received a kidney transplant from a deceased donor 6 years after initiating hemodialysis treatment. His past medical history was remarkable for hypertensive cardiopathy, chronic hepatitis B, and latent tuberculosis, for which treatment had been completed 2 years before transplantation.

Transplant allocation parameters included a kidney donor profile index of 4%, 0/8 HLA matches, and intermediate CMV and EBV-risk-constellations (donor+/recipient+). Transplant surgery was performed (left donor kidney into right iliac fossa) with a cold ischemia time of 11 h and a warm ischemia time of 27 min. The immunosuppressive regimen included basiliximab as induction therapy as well as cyclosporin A, mycophenolate mofetil (MMF) and prednisolone as maintenance therapy. According to local practice, a preemptive approach was followed using regular monitoring of CMV viremia without prophylactic antiviral therapy.

The immediate postoperative course was complicated by delayed graft function requiring continued hemodialysis treatment. On postoperative day 2, magnetic resonance imaging (MRI) of the kidney graft showed subtotal stenosis of the transplant artery at the outflow of the right common iliac artery due to dissection of the right common iliac artery and kidney graft infarcts. Therefore, explantation, thromboendarterectomy of the right common iliac artery, ventral reconstruction of the common iliac artery using a pericard patch and re-implantation of the allograft was performed on the same day. Postoperative duplex ultrasound showed restored graft perfusion. Subsequently, graft function slowly recovered allowing discontinuation of hemodialysis therapy on postoperative day 13 and stabilization of graft function at a serum creatinine level of 270 µmol/l corresponding to an estimated glomerular filtration rate (eGFR) of 26 mL/min/1.73 m^2 according to chronic kidney disease epidemiology (CKD-EPI) formula (Figure 1).

Two months post-transplant during a regular visit to the transplant outpatient clinic, increasing CMV viremia of 1940 copies/ml was detected after low grade viremia at <500 copies/ml had been weekly monitored since a month post-transplant. At this time, valganciclovir at therapeutic dosage was started. Only 6 days later, a rise in serum creatinine to 341 µmoL/L was noted as well as new-onset microhematuria that had been retrospectively present since the preceding week. Duplex ultrasound of the kidney graft newly revealed hydronephrosis grade III. Consequently, urgent percutaneous nephrostomy was placed leading to a prompt fall in serum creatinine. Three weeks after treatment start, CMV viremia was undetectable and valganciclovir was stopped. Due to this satisfying response to valganciclovir, MMF was maintained at the same dose of 2 g per day (Figure 1). BK viremia was undetectable throughout follow-up.

Three months after nephrostomy placement, further urologic work-up by antegrade pyelography was performed revealing a stenotic ureteral lesion in proximity to the bladder. However, an attempt to insert a ureteral stent during the same session was unsuccessful. Therefore, definitive surgical treatment was undertaken consisting of secondary uretero-ureterostomy with the ipsilateral native ureter. Subsequently, the nephrostomy could be removed, and the serum creatinine remained stable at 193 µmoL/L (Figure 2).

The histological examination of the resected transplant ureter showed presence of a PAX8-positive cell proliferation with surrounding fibrosis (Figure 3) consistent with nephrogenic adenoma. There were no signs of malignancy. Surprisingly, several cells showed cytopathic changes characteristic for CMV (Figure 4) and immunohistochemistry was positive for cytomegalovirus (CH2- and DDG-antibodies, Dako, dilution 1:400, pre-treatment H2 30 95; Figure 5). In the simultaneously taken kidney graft sample, signs of acute tubular injury without further anomalies were seen; however, tissue sampling was limited. CMV immunohistochemistry and SV40 staining, as BK-virus marker, were negative.

In the presence of CMV-positive nephrogenic adenoma, another course of valganciclovir at therapeutic dosage was introduced for 6 weeks. Immunosuppressive therapy was maintained unchanged (Figure 1). The following clinical course was unremarkable with a serum creatinine value measured at 136 µmol/l at last follow-up and disappearance of hematuria after the last urologic intervention.

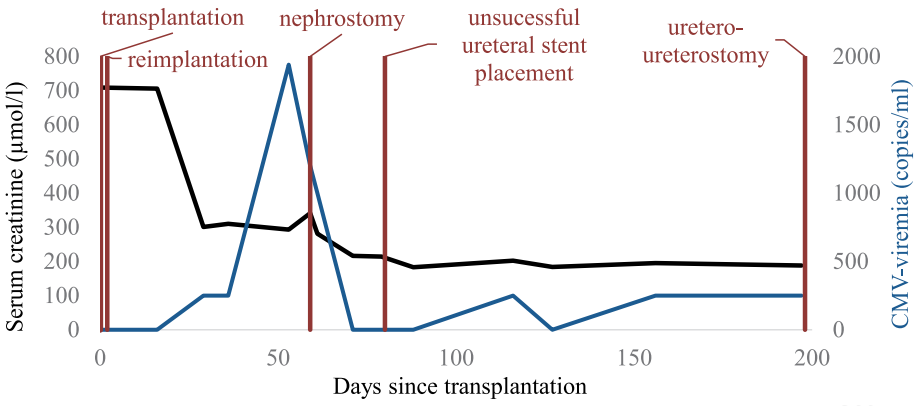

FIGURE 1
Timeline after kidney transplantation. CMV, cytomegalovirus.

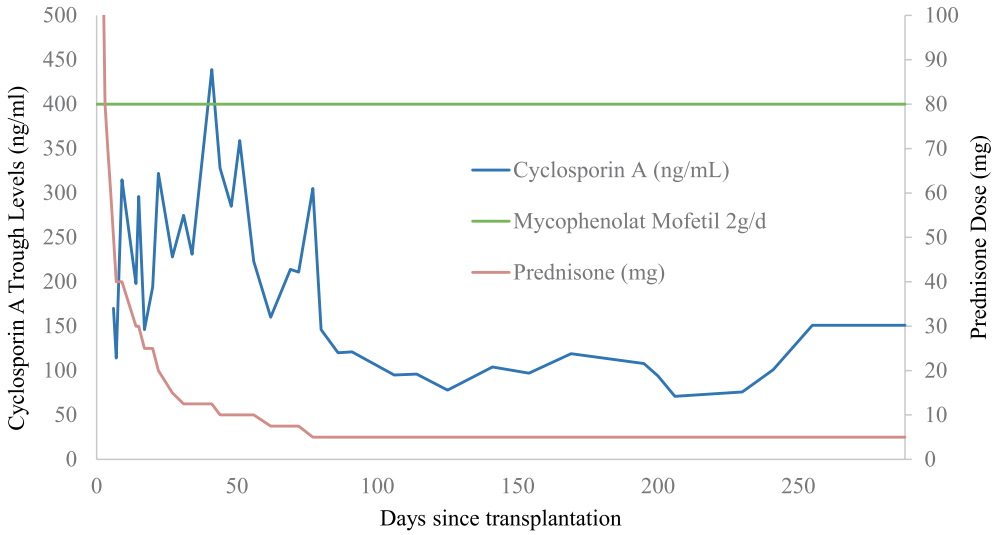

FIGURE 2
Immunosuppressive regimen and cyclosporin A trough levels after transplantation.

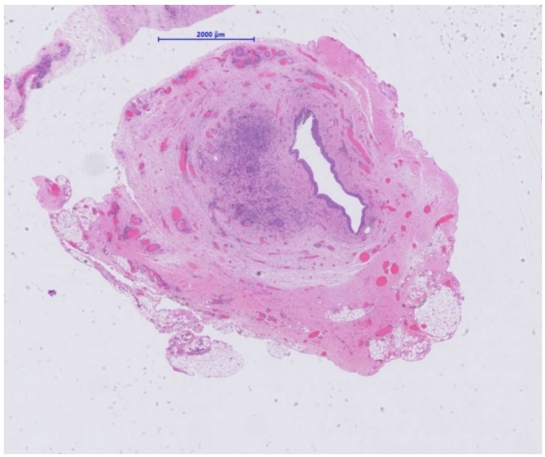

FIGURE 3
Resected ureter segment. Narrowed lumen in transplant ureter due to the presence of a cellular proliferation (H&E, x 10).

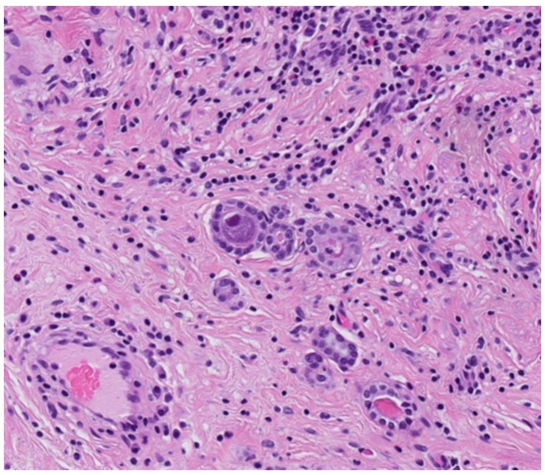

FIGURE 4
Resected ureter segment. Microglandular proliferation of a nephrogenic adenoma with typical cytopathic appearance of a CMV-infected cell (H&E, x 200).

Discussion

We present the case of a kidney transplant recipient with acute post-renal kidney graft dysfunction due to CMV-positive nephrogenic adenoma of the ureter. To the best of our knowledge, this is the second described case of ureteral nephrogenic adenoma with CMV superinfection to date.

Acute kidney graft dysfunction has been estimated to occur with an incidence of 21% in the first 6 months after kidney transplantation and adversely affects patient and transplant outcomes (9, 10). Causes for acute kidney graft dysfunction include transplant-specific etiologies such as acute rejection, BK virus nephropathy and calcineurin inhibitor toxicity in addition to classical causes of acute kidney injury. In a single center cohort of 326 kidney transplant recipients, urinary tract obstruction accounted for 10% of cases of acute kidney injury in the early post-transplant period (9). Both CMV ureteritis and nephrogenic adenoma represent possible, albeit infrequent causes for post-renal acute kidney graft dysfunction.

CMV ureteritis is a rare manifestation of CMV-related tissue-invasive disease that has been increasingly recognized in kidney transplant recipients during the last decades and been linked to the progressive use of mycophenolate in the transplant setting (11–15). Its main manifestations include mild fever, urinary obstruction and kidney impairment. Risk factors for the development of CMV ureteritis are acute allograft rejection, the use of depleting immunosuppression or MMF as well as the absence of prophylactic antiviral therapy (11, 12). In our patient, use of an MMF-based immunosuppression and lack of antiviral prophylaxis following the preemptive therapy approach might have favored the occurrence of CMV-associated tissue-invasive disease.

Nephrogenic adenoma of the urinary tract may present with various symptoms. According to a single center retrospective analysis of 32 cases of nephrogenic adenoma, symptoms were present in 72% of patients including hematuria, urinary symptoms or incontinence, flank pain and hydronephrosis (6). In our patient, new-onset microhematuria was retrospectively noted 1 week before acute worsening of graft function together with the finding of hydronephrosis. There were no urinary symptoms nor painful graft site.

Until now, the pathogenesis of the development of nephrogenic adenoma remains incompletely understood. Several hypotheses have been put forward including the development from remnant mesonephric tissue, the development as metaplastic response to local trauma, irritation, inflammation or immunosuppression as well as the development from shed, secondarily implanted renal tubular cells (16). Indeed, in a landmark study in 24 kidney transplant recipients, bladder nephrogenic adenoma has been shown to derive from the kidney graft (i.e., donor) using fluorescence *in situ* hybridization studies of sex chromosomes (17). However, controversial data exist outside the transplant setting (16).

In our patient, nephrogenic adenoma of the ureter was found to be CMV-positive. We are aware of four previously reported cases of CMV-positive nephrogenic adenomas in kidney transplant recipients (18–21); while three of them affected the bladder, only one case involving the transplant ureter has been described so far (Table 1). Most of the cases were diagnosed within 1 year post-transplant, all of them by histological analysis. In the majority of general cases of nephrogenic adenoma in kidney transplant recipients published so far, CMV testing has not been reported (Table 2) (7, 8, 17, 22–36). On one hand it may be speculated whether CMV-induced local inflammation may have predisposed to the development of nephrogenic adenoma. CMV encodes several proteins inhibiting the assembly and trafficking of cellular proteins, which participate in immune recognition (e.g., major histocompatibility complex 1 and major histocompatibility complex 2). Consequently, CMV hides infected cells from adaptive immunity (37). This immune evasive capability not only helps CMV to persist within its host cells, but may further may predispose to the formation of metaplasia such as nephrogenic adenoma. In addition, sequential surgical procedures may have played a causative role in our case. Thus, the explantation and reimplantation of the allograft due to the artery dissection may have led to substantial shedding of tubular epithelial cells into the ureter and bladder of our patient finally leading to the formation of nephrogenic adenoma (17). On the other hand, nephrogenic adenoma *per se* may have favored CMV reactivation. Indeed, CMV reactivation secondary to inflammatory stimuli has been suggested previously (38). Interestingly, nephrogenic adenoma of the bladder positive for BK polyomavirus has similarly been reported in a kidney transplant recipient (34). According to the histological findings, the authors suggested BK virus contributed to cell atypia, but was not a causative factor for the development of nephrogenic adenoma.

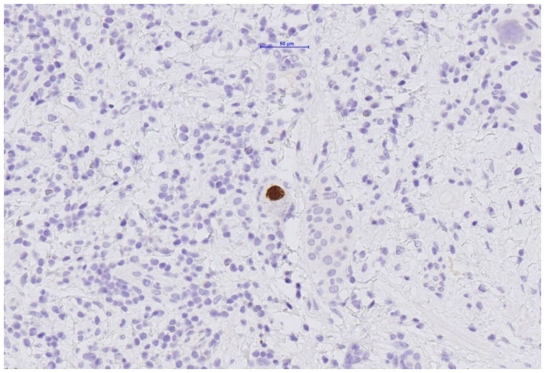

FIGURE 5
Resected ureter segment. Cells within nephrogenic adenoma with positive immunohistochemic staining for CMV.

Currently supported preventive strategies for CMV in kidney transplant recipients include prophylactic and preemptive therapy approaches (39). Preemptive CMV therapy includes regular monitoring of CMV viremia and start of antiviral therapy in case of viral replication at pre-specified levels. In patients with high-risk constellation (donor +/ recipient -) and after induction with depleting agents, a prophylactic approach may be chosen (40). However, the diagnosis of CMV-related tissue-invasive disease requires detection of CMV in the tissue by histology (cytopathic changes) or immunohistochemistry (39). In addition, CMV-related tissue-invasive disease may occur in the absence of CMV viremia as has also been reported for CMV ureteritis (14). Fortunately, in our patient, local symptoms were accompanied by a simultaneous rise in CMV viremia leading to prompt start of antiviral treatment, although being stopped after viremia was undetectable. However, the clinical course of our patient might advocate for a lower viremia threshold for instauration of antiviral therapy. Indeed, during the month preceding the acute rise in CMV viremia, low-grade viremia (< 500 copies/ml) had been present.

Management of the patient with ureteral nephrogenic adenoma reported by Hung et al. included resection of the lesion and

TABLE 1 Reported cases of nephrogenic adenoma with CMV-infection after kidney transplantation.

Author	Time after kidney transplantation	Symptoms	Location	Detection of CMV	Therapy
Beaudry 1983 (18)	5.33 years	Gross hematuria	Over 50% of bladder surface including the site of the reimplanted ureter	Biopsy and serum	Withdrawal of azathioprine
Buzelin 1988 (19)	2.4 years	Gross hematuria, dysuria	Bladder	Biopsy	Resection
Redman 2000 (20)	1 year	Vesical calculi	Bladder next to the ureteroneo-cystostomy	Biopsy	Resection
Hung 2001 (21)	3 months	Ureteral obstruction, gross hematuria	Ureter	Biopsy	Resection, Valganciclovir, withdrawal of azathioprine
Our case	2 months	Ureteral obstruction, hematuria	Ureter	Biopsy and serum	Valganciclovir and resection

TABLE 2 Previous cases of nephrogenic adenoma after kidney transplantation.

Year	Author	Number of Cases	Supposed precipitating factors	CMV testing	Total
1975	Gordon et al. (22)	1	Impaired immunologic surveillance	Not reported	1
1982	Behesti et al. (23)	1	Renal transplantation, UTI	Not reported	2
1988	Gonzalez et al. (24)	8	Renal transplantation	Not reported	10
1992	Zeidan et al. (25)	2	Renal transplantation, transurethral resection of the prostate	Not reported	12
1995	Colombo et al. (26)	5	Mechanical trauma and recurrent UTI	3 with postoperative systemic CMV infection, 2–4 years before nephrogenic adenoma	17
1996	Fournier et al. (8)	9	Ureterovesical anastomosis, chronic prostatitis, vesicorenal reflux, cyclophosphamide, condyloma	No viroid inclusions in biopsy	26
1997	Tse et al. (27)	7	Immunosuppression, ureterovesical anastomosis, recurrent UTI, changes of JJ stents	Not reported	33
1998	Banyai-Falger et al. (7)	7	Recurrent UTI, surgical procedure of renal transplantation	2 with CMV disease, unrelated to nephrogenic adenoma	40
1998	Pycha et al. (28)	12	Renal transplantation, inflammation	Not reported	52
2002	Whang et al. (29)	1	Kidney-pancreas transplantation with drainage of the pancreas into the bladder	Not reported	53
2002	Mazal et al. (17)	29	Shedding of renal tubular cells due to trauma, infection and/or immunosuppression	Not reported	82
2008	Kim et al. (30)	1	UTI and bladder stones	Not reported	83
2009	Ladenheim et al. (31)	1	Renal transplantation	Not reported	84
2013	Voss et al. (32)	1	Renal transplantation	Not reported	85
2014	Kuzaka et al. (33)	2	Renal transplantation, immunosuppression, reoperation	Not reported	87
2015	Alexiev et al. (34)	1	BK Virus infection	Not reported	88
2017	North et al. (35)	1	Recurrent UTI	Not reported	89
2020	Kahn et al. (36)	1	Surgery	Not reported	90

CMV, cytomegalovirus; UTI, urinary tract infection; JJ, double J.

pyeloplasty, intravenous ganciclovir treatment for 2 weeks and withdrawal of the antimetabolite azathioprin (21). The optimal duration of antiviral treatment for CMV-related tissue-invasive disease is not known. However, longer therapy courses are generally admitted in these cases (39). Therapy of nephrogenic adenoma usually involves endoscopic resection of the lesion with variable reported recurrence rates (6, 41). In our patient, valganciclovir treatment was re-started after diagnosis of CMV-related tissue-invasive disease for a total of 6 weeks without concomitant change in immunosuppression. Given the shortness of the lesion-free transplant ureter, surgical reconstruction after ureter resection involved proximal uretero-ureterostomie between transplanted and patient ureter.

In conclusion, CMV-ureteritis and nephrogenic adenomas are rare causes for acute post-renal kidney graft dysfunction. Diagnosis of tissue-invasive CMV disease requires histological evidence of CMV at the affected site. To the best of our knowledge, this is the second reported case of ureteral nephrogenic adenoma with CMV superinfection in a kidney transplant recipient. A causal link might be suspected but remains to be proven (42, 43).

Data availability statement

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

Ethics statement

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

NH: Investigation, Visualization, Writing – original draft, Writing – review & editing. MM: Resources, Writing – review & editing. L-YM: Supervision, Investigation, Writing – original draft, Writing – review & editing.

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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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The use of extended-release tacrolimus twice a day might be beneficial for selected kidney transplant recipients: a case report

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The calcineurin inhibitor tacrolimus, which is available as an immediate- or extended-release formulation, is the standard-of-care immunosuppression after kidney transplantation with low rejection rates, especially in the first year after transplantation. However, its highly variable metabolism rate, narrow therapeutic window, and nephrotoxic side effects require close drug monitoring and individual dosing. Here, we describe first the application of extended-release tacrolimus (ER-Tac) twice daily with beneficial effects in a kidney transplant recipient under extensive therapeutic drug monitoring. A 47-year-old female kidney transplant recipient, who was identified as a fast metabolizer for tacrolimus, presented with declining allograft function and low tacrolimus through levels over time and 8 years after a second kidney transplantation despite the administration of high doses of ER-Tac once daily. Therefore, the area under the concentration–time curve (AUC) showed exceedingly high blood levels of ER-Tac. The latest biopsy of the kidney transplant showed arteriolar hyalinosis with pole vessel stenosis as a sign of chronic transplant vasculopathy and transplant glomerulopathy as a sign of chronic humoral rejection. After the exclusion of other options for immunosuppressive therapy due to the patient's high immunological risk, the patient was switched from ER-Tac once daily to ER-Tac twice daily. After switching to ER-Tac twice daily, the AUC for oral tacrolimus decreased and the transplant function improved despite higher tacrolimus trough levels and a lower total dose administered. This case highlights the importance of careful therapeutic drug monitoring with the performance of an AUC in the follow-up management of kidney transplant recipients.

KEYWORDS

tacrolimus, kidney transplantation, calcineurin inhibitor toxicity, extended-release tacrolimus, prolonged-release tacrolimus

1 Introduction

The calcineurin inhibitor (CNI) tacrolimus either as prolonged-, extended-, or immediate-release administration is part of the standard-of-care immunosuppressive therapy after kidney transplantation (1). Therefore, the acute rejection rates in tacrolimus-based immunosuppressive therapy, especially in the first year after kidney transplantation, are lower in contrast to other immunosuppressive regimes (2). However, a typical side effect of CNIs like tacrolimus is nephrotoxicity due to vasoconstriction. Histological lesions such as arteriolar hyalinosis can be associated with chronic CNI nephrotoxicity (3). Furthermore, the highly variable metabolism rate of tacrolimus and its narrow therapeutic window require close drug monitoring and individual dosing (4). Fast tacrolimus metabolism is associated with reduced kidney transplant function and survival, the tacrolimus metabolism rate being defined as the drug trough concentration (C) normalized by the corresponding daily tacrolimus dose (D) (5, 6). Therefore, a C/D ratio of <1.05 ng/mL*1/mg indicates fast tacrolimus metabolism (6).

Tacrolimus is available as an immediate-release formulation, which must be given twice a day, or as an extended- or prolonged-release formulation, which should normally be given once daily (1). The application of extended-release tacrolimus (ER-Tac) twice a day with a possible positive effect, especially in "fast tacrolimus metabolism," has not been described so far (7).

2 Case description

We report the case of a 47-year-old female kidney transplant recipient who presented with declining allograft function 8 years after a second kidney transplantation and the resulting unusual application of extended-release tacrolimus twice daily (BID).

Kidney transplantation had previously been performed after HLA-incompatible living donation with a donor-specific antibody (DSA), specifically anti-HLA DQ7, HLA-mismatch of 1-1-2, and 82% panel-reactive antibodies. The initial immunosuppression included rituximab, plasmapheresis, and anti-thymocyte globulin (ATG) due to high immunological risk, as well as immediate-release tacrolimus (IR-Tac), mycophenolate mofetil, and prednisolone in the follow-up. Over time, the pre-known DSAs were detectable with high signal intensity (MFI approximately 20.000) for years despite the administration of tacrolimus, high-dose antimetabolite [mycophenolate mofetil (CellCept) 1 g twice daily], and persistent steroid administration (prednisolone 5 mg once daily) in this patient.

The dosage of mycophenolate mofetil and prednisolone was continued at this dose due to the immunological risk and was not changed over time.

To reduce peak levels of calcineurin inhibitors, IR-Tac twice a day was switched to extended-release tacrolimus (ER-Tac) once daily during long-term follow-up (8). After that, ER-Tac trough levels were in the lower range despite the administration of high doses (12 mg daily), a normal BMI (20 kg/m^2) with a body weight of 60 kg, and correct administration, which was critically assessed by anamnesis regarding medication intake and eating behavior as well as review of concomitant medication. A C/D ratio of <1.05 ng/mL*1/mg revealed fast tacrolimus metabolism in this patient. According to the relatively high dose, the area under the concentration–time curve (AUC) showed exceedingly high blood levels of ER-Tac (Figure 1A).

There was a history of a previous graft biopsy 7 years after transplantation in this patient which revealed a mild cellular reaction (BANFF borderline), discrete signs of glomerulitis (without C4d positivity in immunohistochemistry), and transplant glomerulopathy (verified by electron microscopy) according to the BANFF lesion scores (t1, i2, v0, g1, ptc0, ci1, ct1, cv2, cg1b, mm1, ah1, aah0, ti2, and i-IFTA1). Based on these findings, the patient was treated with high-dose steroids and immunoglobulins.

Over time, the patient presented with declining eGFR and increased proteinuria. An indication biopsy performed again showed arteriolar hyalinosis with pole vessel stenosis as a sign of chronic transplant vasculopathy, which is likely aggravated by calcineurin inhibitor toxicity, as well as further evidence of transplant glomerulopathy, which is likely associated with chronic (non-active) antibody-mediated rejection (ABMR) in the absence of histological signs of acute rejection (9).

Switching to a selective co-stimulation blockade of T-cell activation with belatacept or to a mammalian target of rapamycin (mTOR) inhibitor-based regimen was not considered a suitable option because of the patient's high immunological risk (2, 10).

To reduce the progress of worsening allograft function due to calcineurin inhibitor toxicity and to achieve an optimized therapeutic drug level, the administration of ER-Tac twice daily in adjusted dosage was prescribed after discussion of all possible options of immunosuppressive therapy and after informing the patient in detail about the unusual use. After switching to ER-Tac twice daily (BID), the AUC for oral tacrolimus (Figure 1A) and the transplant function fortunately improved despite higher tacrolimus trough levels and a lower total dose administered (Figure 1B). The patient tolerated the non-standard application of ER-Tac (BID) with no side effects, no negative effects on glucose hemostasis monitored by fasting glucose, and no doubts about her adherence. Rather, her general condition improved against the background of having at least temporarily decelerated the rapid function decline of the kidney transplant. The transplant function is still stable 3 years after switching to ER-Tac twice daily (as of March 2024).

3 Discussion

To our knowledge, this is the first described case in the application of ER-Tac twice a day with beneficial effects in a kidney transplant recipient under extensive therapeutic drug monitoring.

The use of prolonged- or extended-release formulations of tacrolimus, usually taken once a day, is a part of the clinical standard of care for kidney transplant recipients (1). Recently, a meta-analysis indicated that the conversion from IR-Tac twice daily to ER-Tac once daily may decrease serum creatinine in kidney transplant recipients with a follow-up duration of more than 48 weeks, but at the same time, eGFR remained unchanged (11). At least many randomized controlled studies could show that the administration of ER-Tac once daily with less peak levels (and therefore in total reduced maximum plasma concentrations) does not appear to have an impact on either the efficacy or safety of this formulation and is an effective

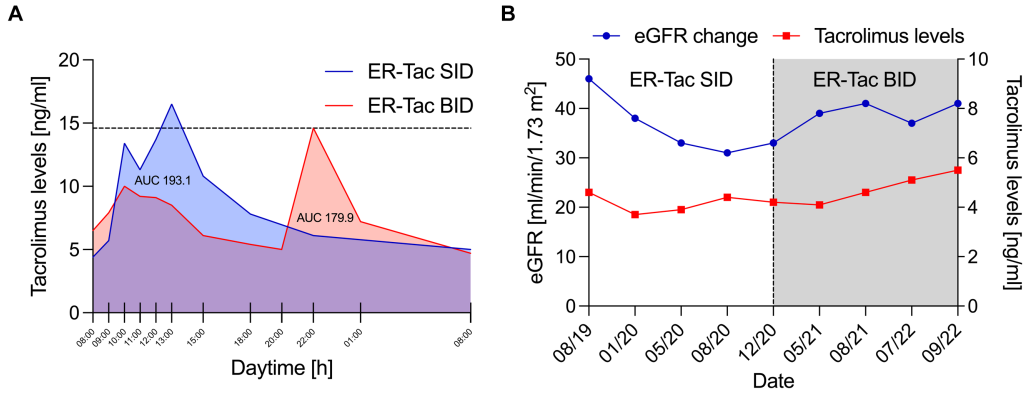

FIGURE 1
(A) Serum levels of tacrolimus [ng/ml] over 24 h. Blue line: Serum levels of extended-release tacrolimus (ER-Tac) once daily (SID, 12 mg); red line: serum levels of extended-release tacrolimus (ER-Tac) twice daily (BID, 2 × 5 mg). The AUC (area under the concentration–time curve) was calculated using PRISM by GraphPad. The time points used to calculate the AUC are marked on the *x*-axis. **(B)** Estimated glomerular filtration rate (eGFR) [ml/min/1.73m^2] according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (blue line) and extended-release tacrolimus (ER-Tac) trough levels over time (red line). The black dashed line marks the switch from ER-Tac once daily (SID) to ER-Tac twice daily (BID).

immunosuppressant treatment in kidney transplant recipients (7, 8, 12, 13). However, the nephrotoxicity of tacrolimus according to its peak levels and frequently unfavorable AUC with a risk of developing transplant glomerulopathy remains a limiting factor for graft survival, particularly in fast metabolizers (14, 15). This can be observed in immediate and extended-release formulations of tacrolimus (5, 6, 15).

Concerning therapeutic drug monitoring, the AUC is considered a better surrogate marker of systemic tacrolimus exposure than the maximum plasma concentration and is strongly associated with clinical outcomes (7). Furthermore, it is essential to identify the high peak levels of tacrolimus, which could cause renal toxicity. However, the determination of the AUC (including maximum concentration) for oral tacrolimus remains challenging and time-consuming in the clinical routine. Therefore, we performed only one AUC under each formulation for ER-Tac. Approaches of finger prick measurements might be a future option to easily include AUC in routine follow-up management of kidney transplant recipients (16). Other considerations in therapeutic drug monitoring include measuring tacrolimus concentrations in intracellular rather than whole blood. This is because most of the tacrolimus measured in whole blood is bound to erythrocytes and plasma proteins and thus represents the pharmacologically inactive fraction (17).

However, the off-label use of ER-Tac twice a day suggests critical discussion. *First,* in this case, after conversion to ER-Tac twice daily, trough levels increased (with two peaks), but the 24 h total AUC decreased (as seen in Figure 1A). This could be related to the improved transplant function with better eGFR. These potential beneficial effects must be weighed against the risk of long-term under immunosuppression and our patient's history with histologically detected transplant glomerulopathy and preexisting DSAs (18). According to general data, chronic rejection is the major cause of death-censored transplant failure after kidney transplantation in the long-term follow-up (19). *Second,* several investigations revealed significantly higher peak levels and AUC of IR-Tac after the morning dose compared to after the evening dose, suggesting a circadian dependence in the clinical pharmacokinetics of tacrolimus (20). In the present case, the peak level in the evening was higher during the administration of ER-Tac twice daily, which could be due to the retard formulation. However, this requires further observation. *Third,* the application of ER-Tac once daily may improve patient adherence, which is an independent risk factor for the development of *de novo* DSAs (21). This advantage could be lost when taking ER-Tac twice a day. Therefore, the possibility of switching to the more frequent ER-Tac twice a day should be weighed carefully against possible patient's non-adherence. Fourth, the absolute effect of the intervention seems quite modest. The reduction in AUC was in line with the dose reduction, although the switch to ER-Tac twice daily was accompanied by higher trough levels of tacrolimus. The improvement in renal function is probably due to the lower AUC, but other factors such as adherence, favorable hydration state or daily blood pressure, physical activity of the patient, or hyperfiltration of the kidney transplant might be involved. *Finally,* based on our observation, we hypothesize a particular advantage for high metabolizers taking ER-Tac twice daily. Thus, a further limitation of our case report is the absence of data on our patient's specific CYP genotyping. However, the C/D ratio for IR-and ER-Tac strongly suggests fast tacrolimus metabolism in our patient (6). Our case report should focus on transplant recipients requiring high doses of extended- or prolonged-release tacrolimus, which are likely to result in high peak levels when taken as a single dose. Distributing the administration into two doses can result in lower peak and higher trough levels, thereby avoiding unnecessary dose increase and toxicity.

In conclusion, the administration of ER-Tac twice daily (BID) might be beneficial for selected patients with fast tacrolimus metabolism. However, in this special application, careful therapeutic drug monitoring including AUC is necessary and the transplant community should critically discuss this off-label drug use. As of today, the patient was converted 3 years ago and the kidney transplant function is stable, but the follow-up course of the transplant under ER-Tac twice a day needs to be further monitored closely.

Data availability statement

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

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

LF: Formal analysis, Writing – original draft. LK: Data curation, Writing – review & editing. KN: Formal analysis, Methodology, Writing – review & editing. TS: Visualization, Writing – review & editing. MJS: Funding acquisition, Supervision, Writing – review & editing. DK: Supervision, Writing – review & editing, Funding acquisition. BM: Writing – review & editing, Supervision. MS: Writing – review & editing, Formal analysis, Methodology, Software. MF: Conceptualization, Supervision, Writing – review & editing. SK: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft.

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Glossary

Ah	arteriolar hyalinosis
aah	hyaline arteriolar thickening
ABMR	antibody-mediated rejection
ATG	anti-thymocyte globulins
AUC	area under the concentration–time curve
BID	bis in die
BMI	body mass index
C	concentration
Cg	glomerular basement membrane double contours
ci	interstitial fibrosis
ct	tubular atrophy
cv	vascular fibrous intimal thickening
D	dose
DSA	donor-specific antibody
eGFR	estimated glomerular filtration rate
ER-Tac	extended-release tacrolimus
g	glomerulitis
g	gram
HLA	human leukocyte antigen
IR-Tac	immediate-release tacrolimus
i	interstitial inflammation
i-IFTA	inflammation in the area of IF/TA
MFI	mean fluorescence intensity
mg	milligram
mm	mesangial matrix expansion
mTOR	mammalian target of rapamycin
ptc	peritubular capillaritis
SID	semel in die
t	tubulitis
ti	total inflammation
v	intimal arteritis

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